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ORIGINAL ARTICLE

Cell surface hydrophobicity: a key component in the degradation of polyethylene succinate by *Pseudomonas* sp. AKS2

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Keywords
biofilm, bioremediation, cell surface hydrophobicity, polyethylene succinate, *Pseudomonas*.

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Abstract

Aim: Polyethylene succinate (PES) contains hydrolysable ester bonds that make it a potential substitute for polyethylene (PE) and polypropylene (PP). Towards bioremediation of PES, we have already reported that a new strain of *Pseudomonas*, *Pseudomonas* sp. AKS2, can efficiently degrade PES and hypothesized that cell surface hydrophobicity plays an important role in this degradation process. In this study, our efforts were targeted towards establishing a correlation between cell surface hydrophobicity and PES degradation.

Methods and Results: We have manipulated cell surface hydrophobicity of AKS2 by varying concentrations of glucose and ammonium sulphate in the growth medium and subsequently examined the extent of PES degradation. We observed an increase in PES degradation by AKS2 with an increase in cell surface hydrophobicity. The increased surface hydrophobicity caused an enhanced biofilm formation on PES surface that resulted in better polymer degradation.

Conclusion: The current study establishes a direct correlation between cell surface hydrophobicity of an organism and its potential to degrade a nonpolar polymer like PES.

Significance and Impact of the Study: Cell surface hydrophobicity manipulation can be used as an important strategy to increase bioremediation of nonpolar polymer like PES.

Introduction

Waste generated from chemosynthetic polymers has resulted in worldwide environmental problem as most of these polymers are nonbiodegradable. Biodegradable polymers are a possible solution to this problem. Polyethylene succinate (PES), which contains hydrolysable ester bonds, is a good example of such a synthetic polymer. In addition to the presence of ester bonds, PES exhibits physical properties that are very similar to polyethylene (PE) and polypropylene (PP), and thus, PES has the potential to be a substitute for PE and PP in the plastic industry (Fujimaki *et al.* 1995). The presence of a hydrolysable ester bonds makes PES susceptible to microbial breakdown. Existing literature has documented the isolation of micro-organisms capable of degrading PES efficiently (Tansengco and Tokiwa 1998; Tezuka *et al.* 2004; Hoang *et al.* 2007; Ishii *et al.* 2007). Towards this direction, we have also reported the isolation of a new mesophilic strain, *Pseudomonas* sp AKS2, that is capable of degrading PES efficiently (Tribedi *et al.* 2012) wherein we demonstrated that AKS2 has high cell surface hydrophobicity. We hypothesized that this high surface hydrophobicity plays an important role in PES degradation by allowing better attachment of cells to the hydrophobic polymer surface (Tribedi *et al.* 2012).

Attachment of bacteria to a polymer is the initial step for biofilm formation where the cell surface hydrophobicity greatly modulates the attachment properties of the organism (Gilan *et al.* 2004). In biological systems,

hydrophobic interactions are considered to be the strongest long-range noncovalent interactions and are accepted as a major determining factor in microbial adhesion to surfaces (Van Loosdrecht *et al.* 1987). Because PES is a nonpolar polymer and the bacterial cell surface of AKS2 is also hydrophobic, hydrophobic interaction may play a key role in the process of microbial attachment to polymer surface.

In our previous report, we showed that the presence of glucose in the conditioned media significantly reduced the extent of PES degradation (Tribedi *et al.* 2012). This is consistent with reports from other groups that showed cell surface hydrophobicity of *Pseudomonas* sp. is modulated significantly under the conditions of carbon and nitrogen stress in growth media (Sanin *et al.* 2003). Similarly, Czaczyk *et al.* (2008) also showed that the cell surface hydrophobicity of *Bacillus* sp. reduced significantly with the simultaneous reduction in optimal nitrogen concentration. In the present study, we have modulated the cell surface hydrophobicity of AKS2 by manipulating nutrient concentration of the growth media and we have targeted our efforts to establish a positive correlation between cell surface hydrophobicity and PES degradation.

Materials and methods

Bacterial strain and culture condition

Pseudomonas sp. AKS2 was previously isolated from Kolkata municipal solid waste disposal ground (Kolkata, India) as a potential degrader of PES (Tribedi *et al.* 2012). AKS2 was grown in 50 ml of basal media [200 mg $MgSO_4{\cdot}7H_2O$, 100 mg NaCl, 20 mg $CaCl_2{\cdot}2H_2O$, 10 mg $FeSO_4{\cdot}7H_2O$, 0·5 mg $Na_2MoO_4{\cdot}2H_2O$, 0·5 mg $Na_2WO_4{\cdot}2H_2O$, 0·5 mg $MnSO_4$, 1·6 g K_2HPO_4 and 200 mg KH_2PO_4 (per litre of distilled water)] supplemented with different amounts of glucose and $(NH_4)_2SO_4$, as per the experimental requirement, and incubated at 30°C for 3 days. Thereafter, 50 mg of sterile PES films was aseptically added in each experimental set and further incubated at 30°C for different lengths of time in shaking condition.

Evaluation of bacterial cell surface hydrophobicity

An aliquot of AKS2 saturated culture was inoculated into different conical flasks containing 50 ml of basal media supplemented with different concentrations of glucose and ammonium sulphate and incubated at 30°C for 3 days. Thereafter, cells were harvested from each experimental set and cell surface hydrophobicity was examined by bacterial adhesion to hydrocarbon (BATH) assay as described previously (Rosenberg *et al.* 1981). In brief, harvested cells were washed with sterile water and resuspended in phosphate urea magnesium (PUM) buffer [K_2HPO_4 (17 g l^{-1}), KH_2PO_4 (7·26 g l^{-1}), urea (1·8 g l^{-1}) and $MgSO_4{\cdot}7H_2O$ (0·2 g l^{-1})] such that OD_{400} becomes 1·0–1·2. An aliquot of this suspension was added equally to several tubes to which increasing volumes (ranging from 0 to 0·2 ml) of *n*-hexadecane were added. Tubes were then shaken for 10 min and allowed to stand for 15 min to complete the phase separation. The OD_{400} of the aqueous suspensions was measured. Cell-free PUM buffer was served as the blank. The formula for measuring cell surface hydrophobicity is as follows:

$$\text{Cell surface hydrophobicity (in \%)} = 100\times\{(\text{initial OD} - \text{final OD})/\text{initial OD}\}$$

Adhesion and biofilm formation on polystyrene surface by crystal violet assay

Adhesion and biofilm formation of AKS2 on polystyrene surface were monitored by performing crystal violet (CV) assay as described previously (Pompilio *et al.* 2008). Overnight saturated cultures of AKS2 were inoculated in basal media containing different concentrations of glucose and ammonium sulphate and incubated at 30°C for 3 days. After the incubation, *c.* 10 000 cells of AKS2 from each growth medium were separately added into sterile 96-well flat-bottomed polystyrene tissue culture plates. After 6 h (adhesion assay) or 24 h (biofilm formation assay) of incubation at 30°C, culture broths (planktonic cells of AKS2) from each well were discarded. Wells were then washed with Milli Q water twice, and to each well, 200 μl 0·4% CV was added and incubated for 15 min at room temperature. CV solution was discarded from each well, and then, the wells were washed with sterile water twice to remove any unabsorbed CV from the wells. Thereafter, 200 μl of 33% acetic acid was added to each well to dissolve the CV adsorbed into bacterial biofilm, and the amount was measured by determining the absorbance at 630 nm.

PES degradation assay

An aliquot of saturated culture of AKS2 was inoculated into different conical flasks containing 50 ml of sterile basal media supplemented with varying concentrations of glucose and ammonium sulphate and incubated at 30°C for 3 days. In each conical flask, sterile PES film weighing 50 mg was aseptically added and further incubated at 30°C for different lengths of time. Degradation of PES films under varied experimental conditions was examined by weight loss and scanning electron microscopic analysis as described previously (Tribedi *et al.* 2012). In brief, PES

films were recovered from each conditioned medium, and adhered bacterial cells, if any, were washed off from the PES surface with 2% (v/v) sodium dodecyl sulphate (SDS) solution and further washed with distilled water. The dry weight difference between initial weight and final weight indicates the extent of PES utilization by the bacteria. On the other hand, the same PES films that were recovered under varied conditions were examined under scanning electron microscope (SEM). For this purpose, the recovered PES films were cut into small strips, coated with gold and thereafter examined under SEM (X6000).

Microscopic observation of bacterial attachment to PES film

Cell surface hydrophobicity of AKS2 was manipulated by growing the cells in media containing varied amounts of glucose and ammonium sulphate as described in previous section. Thereafter, equal weight (50 mg) of sterile PES films was added into each growth medium and further incubated at 30°C for another 4 days. PES films were collected from each growth medium and observed under either fluorescence microscope or scanning electron microscope to examine the extent of microbial attachment to PES film. For fluorescence microscopy, recovered PES films were incubated with acridine orange solution (4 μg ml^{-1}) prior to observation under fluorescence microscope. For SEM analysis, the PES films were fixed with 2·5% glutaraldehyde for 1 h after its removal from the growth medium. Films were then dried in vacuum and coated with gold. Adhered cells were examined under SEM ($\times$ 10 000).

Estimation of bacterial population on PES film surface

The population density of AKS2 on PES film surface was determined indirectly by measuring the concentration of extractable protein from the PES film as the amount of extractable protein is directly proportional to the number of adhered micro-organisms (Tribedi and Sil 2013a). To extract the protein from the micro-organisms adhered to PES films, PES films were recovered from the growth media at regular time interval, washed with water and then boiled for 30 min in 3 ml of 0·3 N NaOH. The suspension was then centrifuged, and the protein concentration of the supernatant was determined by the Lowry method (Lowry *et al.* 1951).

Microbial activity of the bacterial biofilm on PES film

Microbial activity of the bacterial biofilm on PES surface was determined by following the protocol as described previously (Gilan *et al.* 2004; Balasubramanian *et al.* 2010; Tribedi and Sil 2013a). To examine the metabolic activity of biofilm on PES surface, PES films were removed from each conditioned medium at regular time interval and then added to a flask containing 60 ml of 60 mmol l^{-1} sodium phosphate buffer, pH 7·6. Fluorescein diacetate (FDA) was added to a final concentration of 10 μg ml^{-1}. The flask was shaken at 30°C for 15 min, and 1 ml aliquot was withdrawn. These samples were centrifuged at 6000 ***g*** for 5 min, and the absorbance of the supernatant was measured at 494 nm. Samples without FDA served as blanks, and a sample of PES films from a sterile basal medium served as a control.

Extraction and measurement of exopolysaccharides

Exopolysaccharides were measured as described previously (Tribedi and Sil 2013b). In brief, the biofilm on PES surface was extracted by scrapping in sterile water. This biofilm suspension was centrifuged at 3500 ***g*** for 20 min at 4°C. The supernatant was collected. The pellet was treated with 10 mmol l^{-1} EDTA, vortexed for 15 min and recentrifuged to extract cell-bound exopolysaccharides (EPS). The supernatant was collected and mixed with the previous supernatant. The pooled supernatant was then mixed with 2·2 volume of chilled absolute ethanol, incubated at −20°C for 1 h and centrifuged at 3500 ***g*** for 20 min at 4°C. The pellet containing EPS was dissolved in sterile water and measured by phenol–sulphuric acid method (Dubois *et al.* 1956).

Statistical analysis

Experimental results were subjected to statistical analysis of one-way analysis of variance (ANOVA). Correlation coefficient between the variables was measured by using Minitab 16 software (Minitab Inc., State College, PA, USA). The relationship between cell surface hydrophobicity, adhesion, biofilm formation and PES degradation was analysed by constructing contour plot by using Minitab 16.

Results

Nutrient composition of growth media modulates cell surface hydrophobicity of AKS2

It is known that bacteria under carbon or nitrogen stress alter their cell surface hydrophobicity (Sanin *et al.* 2003). To examine the effect of nutritional stress on cell surface hydrophobicity of AKS2, cells were grown in media containing varying concentrations of glucose and ammonium sulphate and incubated at 30°C for 3 days. After the incubation, cell surface hydrophobicity was measured. An

increase in cell surface hydrophobicity was observed with the reduction in glucose concentration in the growth media wherein ammonium sulphate concentration remained unchanged at 0·2% (Fig. 1). The lowest concentration of glucose (0·05%) tested yielded the highest cell surface hydrophobicity. Subsequently, this concentration of glucose was maintained constant while varying the concentration of ammonium sulphate. Under these conditions, a reduction in cell surface hydrophobicity of AKS2 was observed with a decrease in ammonium sulphate concentration in the medium (Fig. 1). Thus, the cell surface hydrophobicity of AKS2 can be changed by changing the concentrations of glucose and ammonium sulphate in the growth medium. In subsequent experiments, we have only used those concentrations of glucose and ammonium sulphate that cause a significant difference in cell surface hydrophobicity of AKS2.

Cell surface hydrophobicity exerts a positive effect towards AKS2-mediated PES degradation

To establish the role of cell surface hydrophobicity towards PES degradation by AKS2, first we have modulated the cell surface hydrophobicity of AKS2 to different extents by allowing it to grow in basal media containing varying concentration of glucose and ammonium sulphate for 3 days at 30°C. Subsequently, equal weight (50 mg) of sterile PES films was added to each experimental set and

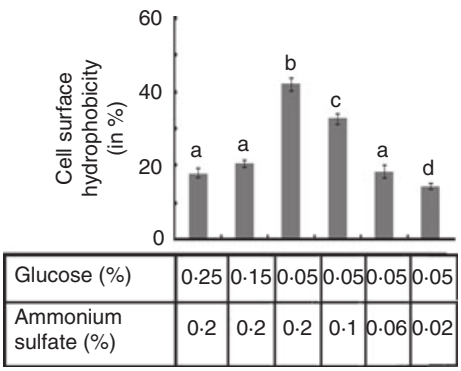

Figure 1 Manipulation of glucose and ammonium sulphate concentration in the growth medium alters cell surface hydrophobicity of AKS2. Equal numbers of AKS2 cells were inoculated in basal media containing varying concentrations of glucose and ammonium sulphate and incubated at 30°C for 3 days. After the incubation, cells were harvested from each conditioned medium and cell surface hydrophobicity was examined by bacterial adhesion to hydrocarbon (BATH) assay. The result represents the average of three independent experiments. Error bars indicate standard deviation (±SD). Statistical significance between the groups was evaluated by ANOVA at 5% level. Mean values with same letters are not significantly different between the treatments.

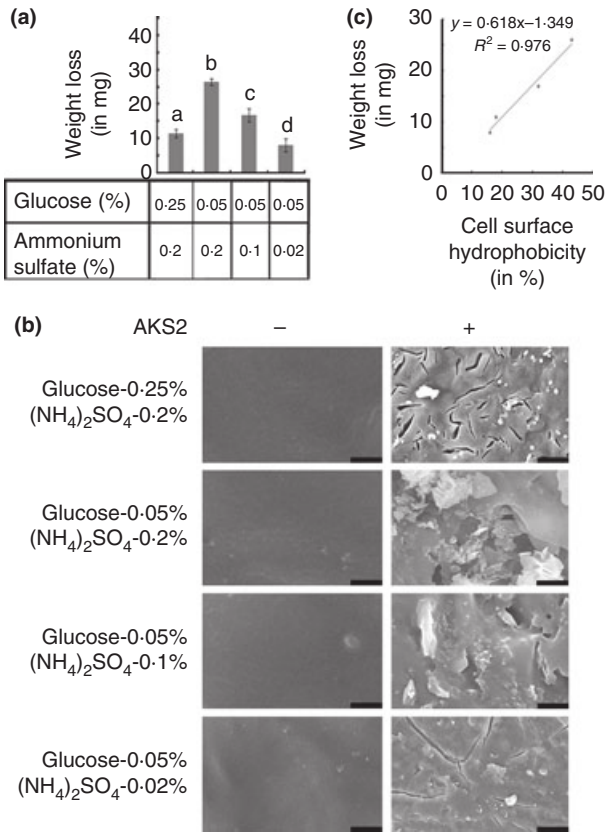

Figure 2 Cell surface hydrophobicity of *Pseudomonas* sp. AKS2 positively influences PES degradation. (a) Weight loss of PES films by AKS2 grown under different nutritional conditions. PES films that were treated with AKS2 for 12 days in different growth media were recovered and washed with 2% SDS. Dry weights of these films were taken. Three replicates have been used for each experimental set, and the result represents the average of these three replicates. Error bars indicate standard deviation (±SD). Statistical significance between the groups was evaluated by ANOVA at 5% level. Mean values with different letters are significantly different between the treatments. (b) Scanning electron micrographs of PES films. After 12 days of treatment, PES films were removed, washed with 2% SDS and examined under SEM. The figure is representative of images obtained from 20 different fields and from three independent experiments. The bar in the figure represents 5 μm. (c) Plot of cell surface hydrophobicity *vs* PES degradation by AKS2.

further incubated at 30°C for another 12 days. At the end of this incubation period, degradation of PES was examined by measuring the weight loss of the films. We observed an increase in PES degradation by AKS2 with a decrease in glucose level in the media wherein ammonium sulphate concentration was kept constant at 0·2% (Fig. 2a). On the contrary, when the ammonium sulphate concentration was lowered at constant level of glucose in the growth media (0·05%), a gradual reduction in PES degradation by AKS2 was observed (Fig. 2a). To further confirm this observation, we examined the surface

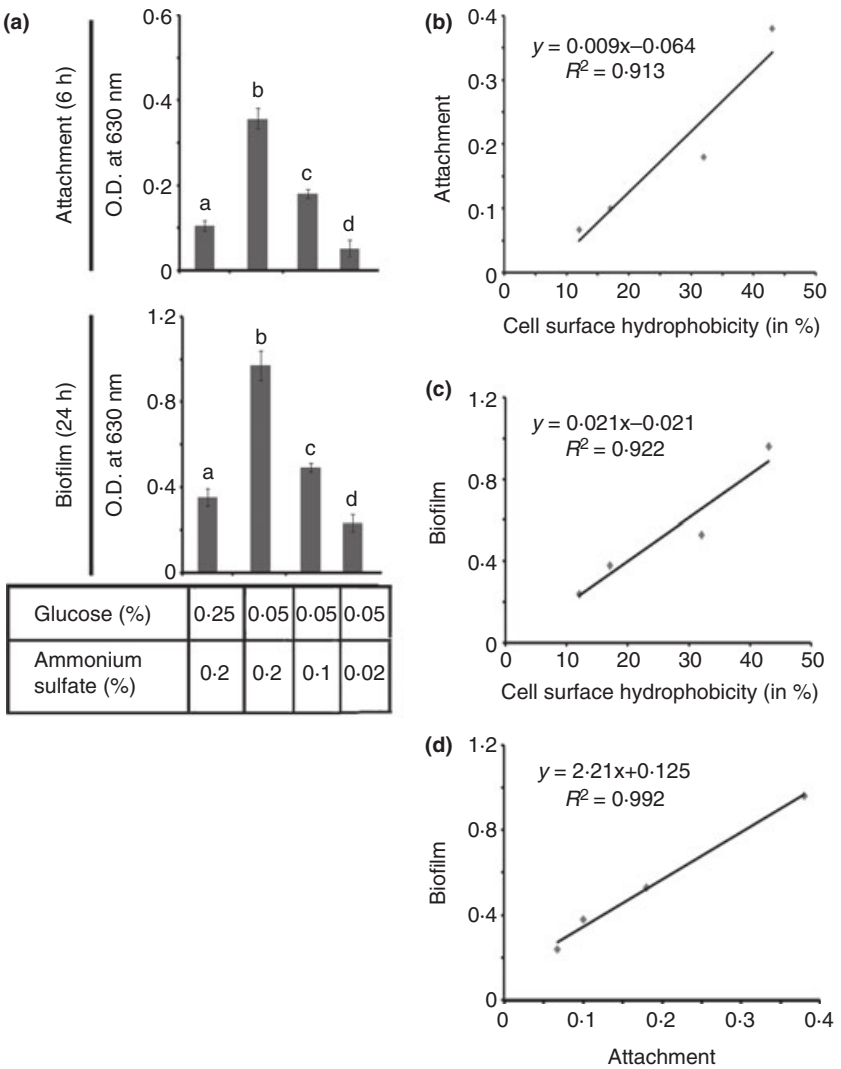

Figure 3 Effect of cell surface hydrophobicity of AKS2 on adhesion and biofilm formation to polystyrene plate. Equal numbers of AKS2 were inoculated in media containing varying concentrations of glucose and ammonium sulphate and incubated at 30°C for 3 days. Thereafter, *c.* 10 000 cells of AKS2 were transferred from each experimental set to fresh separate 96-well plates and incubated for either 6 h or 24 h for examining cellular adhesion and formation of biofilm, respectively. (a) crystal violet (CV) assay profile. After the incubation, wells were washed and CV assay was performed for cellular adhesion and biofilm formation. Three replicates have been used for each experimental set, and the result represents the average of these three replicates. Error bars indicate standard deviation (±SD). Statistical significance between the groups was evaluated by ANOVA at 5% level. Mean values with different letters are significantly different between the treatments. (b) Plot of cell surface hydrophobicity *vs* cellular adhesion to polystyrene surface. (c) Plot of cell surface hydrophobicity *vs* biofilm formation on polystyrene surface. (d) Plot of AKS2 attachment *vs* biofilm formation on polystyrene surface [Correction added on <25 November 2013>, after first online publication: <Equation in Fig. 3d has been changed from "$y = 2{\cdot}21x{-}0{\cdot}125$" to "$y = 2{\cdot}21x{+}0{\cdot}125$">].

topology of PES films, recovered after differential treatment, by scanning electron microscopy. Consistent with the weight loss data, we observed varying degree of cracks and pits on PES films obtained from different growth media and the greatest extent of such change in surface topology was observed on PES film incubated in medium containing 0·05% glucose and 0·2% ammonium sulphate (Fig. 2b). These results establish a linear correlation between cell surface hydrophobicity of AKS2 and its ability to degrade PES as the change in PES degradation by AKS2 and cell surface hydrophobicity follow the similar pattern with the change in glucose and ammonium sulphate concentrations in the growth medium (compare Figs 1 and 2a,b). To further validate this relationship, weight loss of PES films *vs* cell surface hydrophobicity was plotted, which yielded a straight line (Fig. 2c). It confirms the linear correlation between cell surface hydrophobicity of AKS2 and its PES degradation potential.

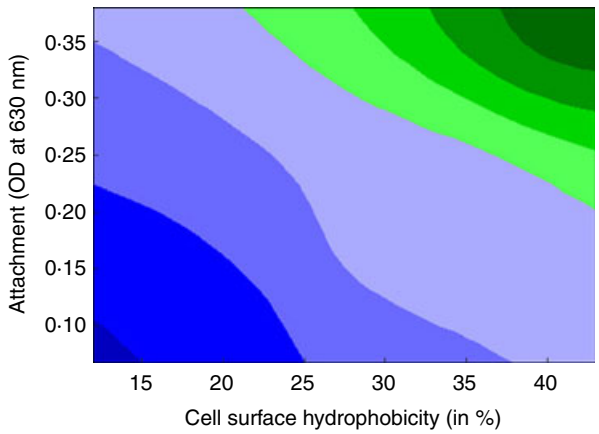

Figure 4 Contour plot of biofilm *vs* cellular attachment and cell surface hydrophobicity. The plot was constructed using Minitab 16 software. Different colors represent different degrees of biofilm formation on polystyrene surface. (■)<0·3; (■) 0·3–0·4; (■) 0·4–0·5; (■) 0·5–0·6; (■) 0·6–0·7; (■) 0·7–0·8; (■) 0·8–0·9 and (■) >0·9.

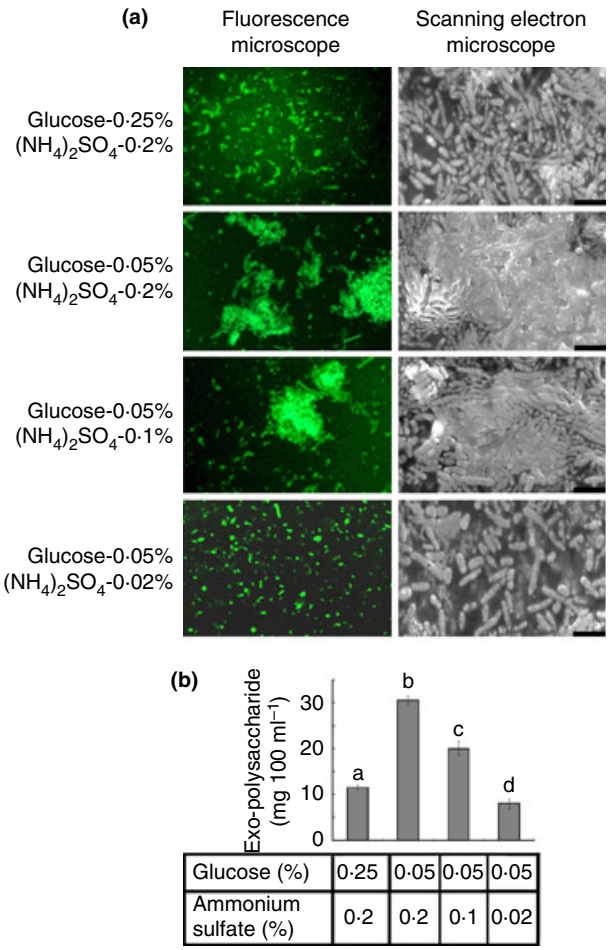

Figure 5 Effect of cell surface hydrophobicity of AKS2 on cellular attachment to PES surface. (a) Fluorescence and SEM micrographs. PES films that were treated with AKS2 for 4 days in different growth media were recovered and either examined under fluorescence microscope after staining with acridine orange or viewed under SEM (×10 000) after gold coating. The figure is representative of images obtained from 20 different fields and from three independent experiments. The bar in the figure represents 2 μm. [Correction added on <21 November 2013>, after first online publication: <Top right image has been replaced>] (b) Exopolysaccharides (EPS) secretion profile. EPS was extracted from PES films that were treated with AKS2 for 12 days in different growth media and measured by the conventional phenol–sulphuric acid method. The result represents the average of three independent experiments. Error bars indicate standard deviation (±SD). Statistical significance between the groups was evaluated by ANOVA at 5% significance level. Mean values with different letters are significantly different between the treatments.

Cell surface hydrophobicity positively influences AKS2 adhesion and biofilm formation on PES film

Biofilm, an assemblage of the microbial cells that are cooperatively attached to a particular surface, improves bioremediation potential of an organism (Gilan *et al.* 2004; Balasubramanian *et al.* 2010; Tribedi and Sil 2013b). It is possible that an alteration in cell surface hydrophobicity can modulate the formation of AKS2 biofilm on polymer surface by altering their attachment to polymer surface. Literature documents that cell surface hydrophobicity of a bacterium positively influences attachment and subsequent biofilm formation on polystyrene surface (Pompilio *et al.* 2008). Therefore, it is possible that change in cell surface hydrophobicity may also influence AKS2 attachment and biofilm formation on polystyrene surface. To investigate this, equal numbers of AKS2 cells, grown in media containing varying concentration of glucose and ammonium sulphate for 3 days at 30°C, were added separately into 96-well polystyrene plates and incubated at 30°C for 6 and 24 h. After the incubation, CV assay was performed. Results of CV assay obtained after 6 h indicate bacterial attachment on polystyrene, while results of the 24-h incubation indicates biofilm formation on polystyrene. The results showed varied degree of attachment of cells to polystyrene surface (6 h) and subsequent biofilm formation (24 h) by AKS2 with change in media composition and these variations followed similar pattern for both the attachment and biofilm formation (Fig. 3a). In the previous section, we established that cell surface hydrophobicity of AKS2 changes with the change in media composition, and again, this pattern is similar to that observed for attachment and biofilm formation of this strain on polystyrene surface (compare Figs 1 and 3a). The attachment and biofilm formation on polystyrene surface by AKS2 reached maximum when the cell surface hydrophobicity of AKS2 was maximum under our experimental conditions (Figs 1 and 3a). These results indicate that cell surface hydrophobicity of AKS2 positively modulates the

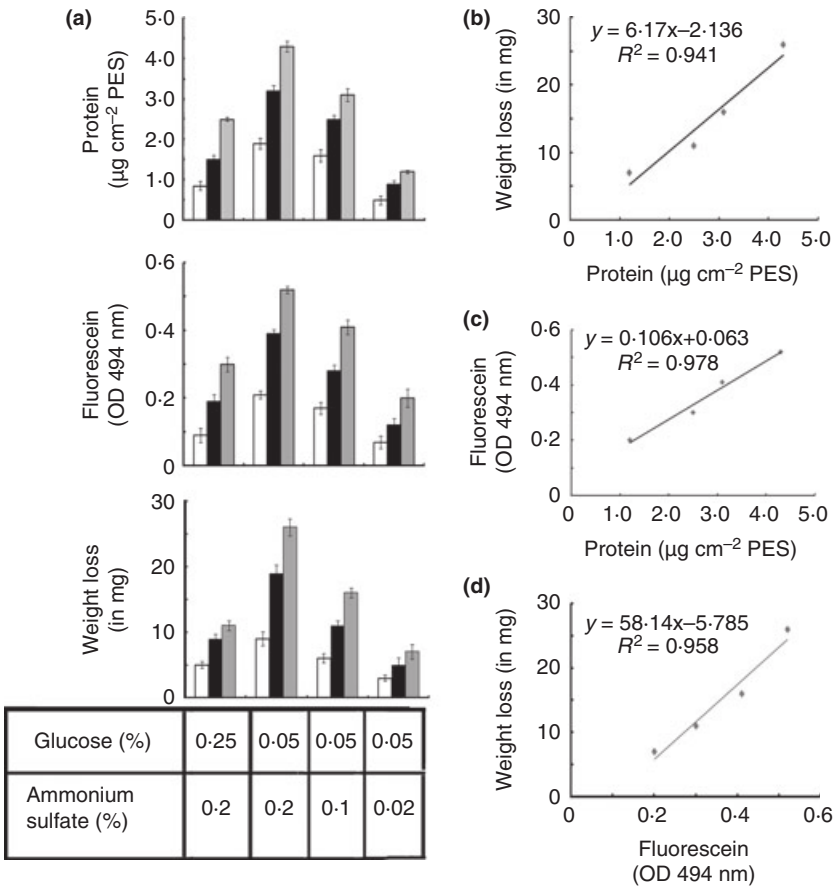

Figure 6 Effect of cell surface hydrophobicity on cellular adherence, biofilm formation on PES surface and PES degradation. (a) Microbial biomass, metabolic activity and PES degradation profile. PES films that were treated with AKS2 for different days in different growth media were recovered, and adhered microbial biomass (upper panel), microbial activity (middle panel) and PES degradation (lower panel) were examined. Three replicates have been used for each type of experimental set. Error bars indicate standard deviation (±SD). (b) Plot of cellular attachment *vs* PES degradation. (c) Plot of cellular attachment to PES film and microbial activity on PES surface. (d) Plot of microbial activity *vs* PES degradation. (▒) 12 day; (■) 8 day and (□) 4 day.

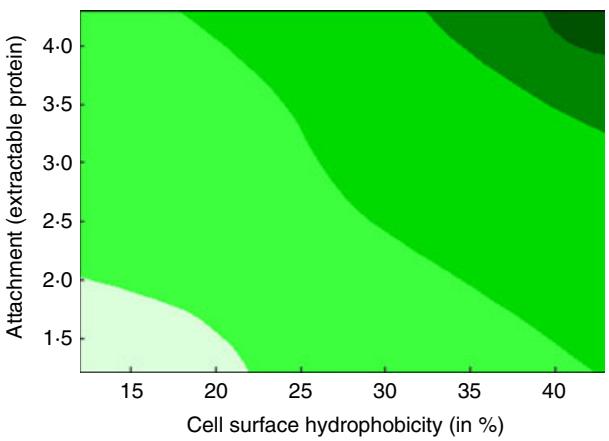

Figure 7 Contour plot of PES degradation *vs* cellular attachment and cell surface hydrophobicity. The plot was constructed using Minitab 16 software. Different colors represent different degrees of PES degradation. (▒)<10; (▒) 10–15; (▒) 15–20; (▒) 20–25 and (▒) >25.

attachment and biofilm formation on polystyrene surface. To further test the possible correlation between cell surface hydrophobicity, attachment and biofilm formation, we have plotted these variables against each other as indicated in Fig. 3b,c and d. For all the cases, we obtained straight lines indicating a linear correlation between cell surface hydrophobicity, cellular attachment to polystyrene surface and biofilm formation by AKS2 (Fig. 3b,c and d). To better understand the correlation between cell surface hydrophobicity, attachment and biofilm formation by AKS2 on polystyrene surface, we constructed a contour plot (Fig. 4). Consistent with our expectation, contour plot demonstrated that cell surface hydrophobicity of AKS2 positively influences the attachment and formation of biofilm on polystyrene surface.

Because PES, like polystyrene, is also nonpolar in nature, similar effects may also be observed. Thus, to verify whether the cell surface hydrophobicity of AKS2 influences the attachment and biofilm formation on PES surface, PES films, having equal weight, were added to AKS2 cultures grown for 3 days in basal media supplemented with varying concentrations of glucose and ammonium sulphate and incubated at 30°C for different lengths of time. After 4 days of incubation, a batch of PES films were removed from each growth medium and were either examined under fluorescence microscope after staining with acridine orange or viewed by scanning electron microscope after coating with gold. Both the results showed variations in

the degree of AKS2 attachment on PES surface with the change in medium composition, and the pattern for variation in attachment with the medium composition appeared similar to the pattern observed for cell surface hydrophobicity (compare Figs 1 and 5a). The maximum attachment on PES surface was formed by AKS2 grown in the presence of 0·05% glucose and 0·2% ammonium sulphate, which is the optimum concentration for obtaining highest cell surface hydrophobicity under the experimental conditions tested thus far (Figs 1 and 5a). These results clearly demonstrate that cell surface hydrophobicity of AKS2 positively influences the attachment of AKS2 to PES film. The scanning electron micrograph in particular shows the formation of multiple layers of AKS2 on PES surface prominently in media containing 0·05% glucose and 0·2% ammonium sulphate wherein the cell surface hydrophobicity reaches its maximum indicating the development of biofilm by AKS2 at this medium composition (Fig. 5a). Secretions of exopolysaccharides (EPS) from organisms stabilize the network of biofilm, and thus, EPS measurement is also considered to be an assay for biofilm formation. Therefore, to further validate biofilm formation by AKS2, we measured EPS secretion, and consistent with our expectation, we observed maximum EPS secretion by AKS2 cells that were grown in media containing 0·05% glucose and 0·2% ammonium sulphate (Fig. 5b).

The extent of biofilm formation on PES film with time was further measured by determining the amount of total extractable protein and metabolic activity (Gilan *et al.* 2004; Balasubramanian *et al.* 2010; Tribedi and Sil 2013b). As extracted protein is directly proportional to the number of attached bacteria, the result indicates the extent of bacterial population on PES surface. The metabolic activity of the bacterial biofilm on polymer surface was determined by measuring the hydrolysis of fluorescein diacetate (FDA) to fluorescein. Metabolically active cells release an array of hydrolytic enzymes that can cleave FDA. Thus, the rate of FDA hydrolysis indicates the degree of viability and metabolic potential of biofilm (Killham and Staddon 2002; Teng *et al.* 2010). Consistent with previous observations, we observed a significant variation in both the amount of protein extraction and metabolic activity on PES surface with progression of incubation and with the change in composition of the growth media (Fig. 6a; upper and middle panels). We observed the highest level of protein and maximum FDA activity for AKS2 cells that were grown in media containing 0·05% glucose and 0·2% ammonium sulphate. These results demonstrated that AKS2 cells developed maximum biofilm on PES surface at a media composition wherein cellular surface hydrophobicity is the maximum.

To investigate the effect of adhered microbial population on PES degradation, we examined the extent of PES degradation by AKS2 with the progression of time. We observed that PES degradation by AKS2 with time followed the pattern observed for bacterial load and metabolic activity (Fig. 6a, compare lower panel with upper and middle panels). To better understand this relationship, we plotted the extent of attachment of AKS2 to PES film *vs* PES degradation and obtained a straight line (Fig. 6b). Thus, the result showed a linear correlation between cellular attachment to PES surface and PES degradation by AKS2. Similarly, we also established a linear correlation between cellular attachment and microbial activity, as well as microbial activity and PES degradation (Fig. 6c,d). Thus, these results indicate that an increase in the attachment of AKS2 to PES surface positively correlates with both the higher metabolic activity and enhanced PES degradation. We constructed a contour plot that showed that cell surface hydrophobicity positively influences microbial adherence, which leads to an increased PES degradation (Fig. 7). Taken together, the results demonstrate that an increase in cell surface hydrophobicity of AKS2 resulted in an enhanced microbial adherence to PES surface, which lead to the increase in PES degradation by *Pseudomonas* sp. AKS2.

Discussion

In this study, we report varying degree of PES degradation by a mesophilic bacteria *Pseudomonas* sp. AKS2 under different nutritional conditions. Our results showed an alteration in AKS2 cell surface hydrophobicity with the change in concentrations of glucose and ammonium sulphate in the growth media. Subsequently, we observed a linear correlation between PES degradation ability of AKS2 and its cell surface hydrophobicity. Because PES is nonpolar in nature, an organism with higher cell surface hydrophobicity is likely to utilize the polymer more efficiently because cell surface hydrophobicity favours closer association between organism and PES. Our result also showed that increased cell surface hydrophobicity promotes efficient biofilm formation by AKS2 on PES film. It has been found in the literature that micro-organisms often form biofilm on polymer surface, which results in better remediation (Gilan *et al.* 2004; Balasubramanian *et al.* 2010). This better remediation by biofilm may be attributed to the high microbial biomass and enhanced adaptation ability of the cells in biofilm compared to planktonic cells (de Carvalho 2012; Dash *et al.* 2013). In agreement with this, our result showed maximum microbial biomass and highest level of metabolic activity when AKS2 formed maximum biofilm on PES surface. In the current study, we have also observed that AKS2 secreted exopolysaccharides (EPS) during biofilm formation and this EPS secretion is considered to be a stabilizing network of biofilm. Thus, AKS2 adapts to the

PES film by increasing metabolic potential as well as secreting EPS. Therefore, the maximum PES degradation by AKS2 could be attributed to this physiological adaptation as a result of biofilm formation on PES surface, and this biofilm formation is dependent on cell surface hydrophobicity. Thus, this study reveals that cell surface hydrophobicity plays a vital role in the bioremediation process of nonpolar polymers like PES by promoting cellular attachment and subsequent formation of biofilm on polymer surface.

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Conflict of interests

The authors declare no conflict of interests.

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