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Cationic and Non-ionic Surfactant Pre-treatment of PET to Enhance the Bacterial Biodegradation of PET

Seyedehazita Ahmaditabatabaei[a,*], Hafiz M.N. Iqbal[b], Zeynab Moinipoor[a], Godfrey Kyazze[a], Tajalli Keshavarz[a]

[a]School of Life sciences, College of Liberal Arts and Sciences, University of Westminster, London W1W 6UW, UK
[b]Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey 64849, Mexico
w1745896@my.westminster.ac.uk

Plastic wastes and their persistence in the environment impose prominent problems around the globe. Among different types of plastics, synthetic ones such as polyethylene terephthalate (PET) are more difficult to degrade. This is due to the high ratio of aromatic terephthalate units in the PET structure. Amongst different approaches for the degradation of plastics, biodegradation is considered increasingly as a preferred method due to it being environmentally friendly. However, the biodegradation efficiency is not high. One of the reasons for this is that the temperature required for bacterial growth is much less than the glass transition temperature of PET. To overcome the shortcoming, complementary techniques have been suggested to enhance biodegradation. Amongst the techniques, pre-treatment of PET has shown promise. To this effect, physical (e.g., use of UV radiation), chemical (e.g., alkaline treatment) and biochemical methods (use of surfactants) have been adopted with varying success. This paper reports the effects of two surfactants, a cationic surfactant dodecyl trimethylammonium bromide (DTAB), and a non-ionic surfactant Dodecyl polyethylene oxide-23 ether (Brij-35) for pre-treat the PET. Two techniques were used to study the outcome of the aforementioned treatments on PET and its biodegradation: FTIR and biofilm assay. DTAB treatment in the presence of the bacterial culture *Ideonella sakaiensis* (*I. sakaiensis*) in Yeast Extract-Sodium Carbonate and Vitamins (YSV) medium depicted notable potential for enhancement of the degradation of PET. This was verified by changes in PET carbonyl groups. So, it is possible that other mechanisms could be involved in addition to the already suggested alteration in electrical charges.

1. Introduction

Today, plastic pollution of land and oceans is one of the main concerns of both scientists and the public. The majority of plastics are mineral-based and non-biodegradable. Besides, plastics as serious environmental pollutants are mostly single-use ones. Examples of single-use plastics are plastic bottles, plastic bags, and plastic packaging. From 1950 to 2017, the production of these plastics increased from 2×10^6 t to 400×10^6 t worldwide (PlasticsEurope, 2018). However, since the end of the 1900s the popularity of plastic has come to scrutiny due to the concerns about plastic waste; plastic wastes that are being accumulated in the landfills, and in the oceans through the spread of smaller pieces of plastic (Geyer et al., 2017).
Most consumer products such as packaging bags, bottles, medicine jars, rope, clothing, foil and films are made from Polyethylene Terephthalate (PET). PET is derived from crude oil. It is the most common thermoplastic polymer in the polyester family. PET monomer repeating unit is $C_{10}H_8O_4$.
In the above context and considering the excessive use of single-use plastics (Rivard et al., 1995), degradation of PET is the focus of this paper.
Several routes are practiced for tackling the plastics wastes; these include disposal (landfill, open-air), incineration, recycling and biodegradation. Biodegradation of plastics is the most environmentally friendly method by where enzymes, microbes, and their combination (Biocatalysis) is utilised. In this paper, PET treatment by surfactants is presented as a method for facilitating consecutive microbial degradation.

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Biodegradation process can be divided into four steps including biodeterioration, bio-fragmentation, assimilation and mineralization. Biodeterioration of plastics is the process in which microorganisms degrade the substance and this process maybe assisted by abiotic activities such as chemical, mechanical, light and thermal factors leading to smaller fractions (Amobonye et al, 2021). Bio-fragmentation is the next stage. In this step, microorganisms secrete the catalytic agents (enzymes and free radicals) that break down the polymer to its monomers (Shah et al, 2008). In assimilation, the degraded molecules of plastic may integrate into a chemical structure through microbial activity. Mineralisation is the last stage during which the plastic monomers are converted to CO_2 and H_2O (Pathak and Navneet, 2017).

Microbial degradation of plastics by bacteria, fungi and algae has been reported (Wei and Wierckx, 2021). Over the last 20 years, increasing reports on the ability of microbes degrading petroleum-based plastics has been documented. For example, *Pseudomonas mendocina* (*P. mendocina*) reported by Lee et al. (2021), *I. sakaiensis* isolated and introduced by Yoshida et al. (2016) can degrade PET. *Pestalotiopsis microspore* are able to degrade polyurethane (PUR) (Verstraeten et al., 2021). *Rhodococcus* (Xu et al., 2021), *Enterococcus faecalis, Pseudomonas putida* (Jadaun et al., 2022) and *Salmonella enterica* (Dawan et al., 2022) degrade polystyrene (PS) (Ghosh et al., 2013).

Among the bacteria, *I. sakaiensis* has shown good promise for PET degradation. It can provide PET hydrolytic enzymes PETase and MHETase. Both enzymes act in their specific roles, with PETase being responsible for hydrolytic conversion of PET into oligomers that include MHET as the main component. MHETase further hydrolyses MHET into PET monomers, terephthalic acid (TPA), and ethylene glycol (EG) (Yoshida et al., 2016). Also, an extracellular cutinase from *P. mendocina* can degrade the PET and produce TPA and EG (Wilkes and Aristilde, 2017).

PET, due to its hydrocarbon bonds and ester groups, has a high resistance to degradation (Aziz et al., 2020). The hydrocarbon bonds have resistance to degradation by bacteria due to the lack of water in their structure. Ester groups are non-degradable because of the presence of aromatic groups in their structure. The high ratio of aromatic terephthalate units reduces chain mobility, which leads to a reduction in degradation. In addition, surface hydrophobicity affects their resistance to degradation by microorganisms (Wei and Zimmermann, 2017).

The pretreatment of PET is used to overcome the difficulty of biodegradation. Pre-treatment occurs through using acid, alkaline, temperature, UV (Falkenstein et al., 2020) and surfactants to enhance the subsequent degradation process (Shah et al., 2008). This report has focused on investigating the effects of surfactant pre-treatment on PET. Pre-treatment approaches can change the physicochemical properties of the surface of the PET, such as hydrophobicity and enhancing the bacterial adhesion to the surface. This improves the exposure of PET pieces to the degradative enzymes.

Surfactants are amphiphilic molecules that have both hydrophilic and hydrophobic segments in their molecular structure. This property can help in biodegradation through surface modification of plastics. The hydrophobic part of the surfactant can attach to the surface of the plastic while the hydrophilic part attaches to the water molecule and enhances the surface hydrophilicity (Kaczorek et al., 2010).

An anionic surfactant (Sodium dodecyl sulfate) as a pre-treatment for PET pieces in the presence of *I. sakaiensis* was studied by Furukawa et al. (2018). The anionic surfactants can accelerate the hydrolysis reaction on PET. This acceleration has been attributed to the positive electrical charge of PETase and the negative charge from the anionic surfactant. However, there has been a lack of investigation on cationic and nonionic surfactants on PET in the presence of *I. sakaiensis.* This was investigated in this paper.

2. Material and methods

2.1 Microorganisms

Two microorganisms; *I. sakaiensis* strain 201-F6T (NBRC 110686) and *P. mendocina* NCIMBCH 50 were used during the experiments. *P. mendocina* strain was available in the School of Life Sciences culture collection of the University of Westminster. *I. sakaiensis* strain 201-F6T (NBRC 110686) was purchased from the National Institute of Technology and Evaluation (NITE) Biological Resource Centre (NBRC), Japan.

2.2 Chemicals and reagents

All chemicals used for media preparation were ordered from Sigma-Aldrich. Two surfactants were ordered from Fisher Scientific.

2.3 Plastic

PET sheets (amorphous, 0.25 mm thickness), were purchased from Goodfellow Cambridge Ltd. The PET sheet was cut to 2 × 2 mm pieces. Then, they were sterilized using 70 % (v/v) ethanol and were dried under an aseptic environment overnight.

2.4 Media and growth conditions

I. sakaiensis was grown in HPP as a complex medium. HPP medium contained (g/L): 5 peptone from casein; 5 BBL polypeptone; 2 yeast extract, and 1 $MgSO_4.7H_2O$. The Nutrient Broth complex medium was used for *P. mendocina* and comprised (g/L): 1 D (+) glucose; 3 yeast extract; 15 peptone and 6 NaCl. M9 minimal medium and YSV was used as defined media. M9 minimal medium included (g/L): 4 glucose; 0.246 $MgSO_4$; 0.044 $CaCl_2$; 0.001 biotin; 0.001 thiamine; 100 (mL/L) M9 minimal salts solution (10X) and 10 (mL/L) trace element solution (100X). The M9 minimal salts solution (10X) contained (g/L): 75.2 $Na_2HPO_4.2H_2O$; 30 KH_2PO_4; 5 NaCl and 5 NH_4Cl. The trace element solution for M9 minimal medium contained (g/L): 5 EDTA; 0.83 $FeCl_3.6H_2O$; 0.084 $ZnCl_2$; 0.013 $CuCl_2.2H_2O$; 0.010 $CoCl_2.2H_2O$; 0.010 H_3BO_3 and 0.0016 $MnCl_2.4H_2O$. YSV consisted of (g/L): 0.1 yeast extract; 1 ammonium sulfate; 0.2 sodium hydrogen carbonate; 2.5 thiamine HCl; 0.05 biotin; 0.5 vitamin B12; 1 $FeSO_4.7H_2O$; 1 $MgSO_4.7H_2O$; 0.1 $CuSO_4.5H_2O$; 0.1 $MnSO_4.5H_2O$ and 0.1 $ZnSO_4.7H_2O$. The inoculum was incubated at 30 °C, 180 rpm overnight. Then, the OD adjusted to 0.125 based on McFarland standard (~ 1.5×10^8 cells).

2.5 Surfactant pre-treatment

PET pieces (150) were pre-treated with 15 mL surfactants (DTAB and Brij-35, 0.005% (w/v)) separately overnight. The treatments were carried out under stagnant (no stirring) conditions. After the treatment, the PET pieces were washed gently using 5 mL distilled water under aseptic conditions. The washing was repeated twice.

2.6 Fourier-transform infrared spectroscopy (FTIR)

FTIR was used as an analytical method to investigate the alteration of PET functional groups before and after surfactants treatment. PET samples were placed directly onto the diamond crystal. The force gauge was chosen at 113 units and the infrared absorption spectrum was measured between the wavelength region of 4,000 – 450 cm^{-1}. The spectral resolution was 4 cm^{-1} and spectra were collated based on 5 scans.

2.7 Biofilm assay

The 6-well plates were used for the investigation of biofilm formation on PET pieces. At first, each one of the three media HPP, YSV and M9 was transferred to six 6-well plates aseptically. Then, 10 pieces of pre-treated PET were added to each well of the 6-well plate related to a specific medium. The non-treated PET in the presence of *I. sakaiensis* or *P. mendocina* in each medium was used as a control. Finally, the turbidity adjusted (for consistency) inoculum was added to all wells of all plates and the plates were placed in the incubator at 30 °C. The experiment continued for one week. The PET samples were taken from the 6-well plates and transferred to the 96-well plates with different columns representing different PET treatments. Subsequently, the planktonic cells were removed by gently adding 100 μL distilled water to each well and discharging the whole liquid from the well. This process was repeated twice to ensure no planktonic cells were left on the PET pieces in the wells. Next, 200 μL Crystal violate (0.1 % w/v) was added to each well and incubated at room temperature for 10 minutes. Then, the liquid was removed from the wells and the PET pieces were washed twice with 200 μL distilled water. Finally, the plate was taken place at room temperature overnight to become dry. In the final step of the biofilm assay, 200 μL of 30 % (v/v) acetic acid was added to dissolve the crystal violate. The solution was mixed gently with a pipette tip to reach a homogenous colour. The optical density (OD) was read at 570 nm, a 30 % (v/v) acetic acid solution was used as blank.

3. Result and discussion

The aim of FTIR analysis in this paper is to interpret the alteration of functional groups' intensity, specifically an increase of O-H and a decrease of other functional groups (C-H, C=O, C-O) intensities to investigate the most efficient treatment to degrade the PET. The main focus of this paper is on the C=O bond due to the ester cleavage in the PET degradation (Roberts et al., 2020) which leads to BHET and MHET production. The FTIR was carried out on the treated and non-treated PET samples (total of 6 samples) with $P < 0.001$.

The investigation of biofilm formation on PET pieces is important as it increases the efficacy of the enzymatic process. The biofilm formation was carried out on treated and non-treated PET samples (total of 6 samples) with $P < 0.001$. In this context, the potential correlation between the biofilm formation and transmittance

percentage of functional groups is investigated in this study. It is expected that more biofilm formation, at least to a certain degree, would lead to a decrease in the functional groups' (C-H, C=O, C-O) intensity and an increase in the O-H group which represents the formation of water.
Figure 1a shows, the PET molecular structure. During the hydrolysis reaction, the ester bonds become weaker and finally break down which leads to the presence of hydroxyl group (O-H) and carboxylic acid groups (COOH) in the spectrum. Carboxylic acid contains the carbonyl group and hydroxyl group. Figure 1b illustrates the degradation products of PET and the presence of the hydroxyl group during this process.

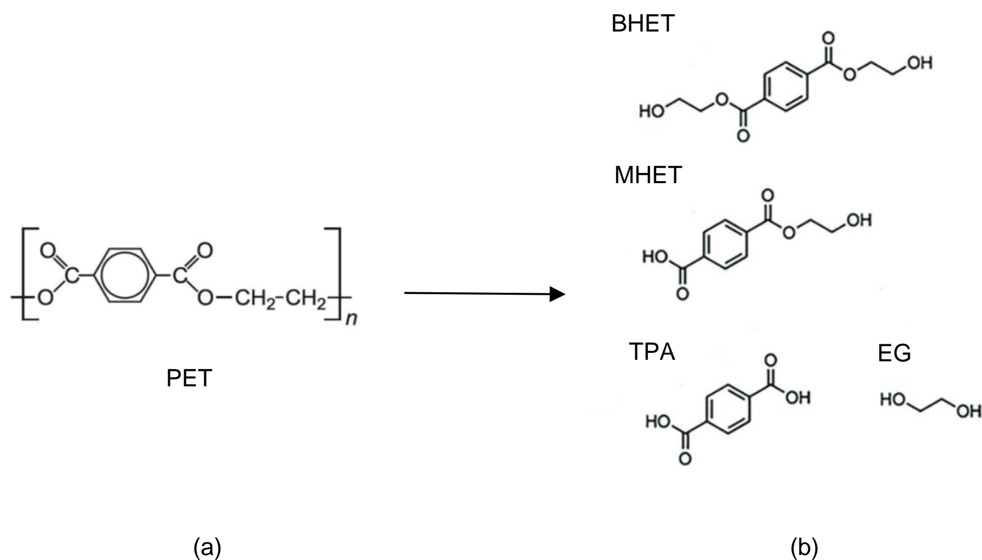

Figure 1: a) PET molecular structure. b) The PET sample after hydrolysis reaction has broken down to Bis (2-hydroxyethyl) terephthalate (BHET), Mono (2-hydroxyethyl) terephthalate MHET, Terephthalic acid (TPA) and ethylene glycol (EG) (adapted from Roberts et al., 2020).

The obtained results from the FTIR spectra of surfactant treatments on PET pieces in the presence of *I. sakaiensis* culture in HPP medium indicate no changes on PET pieces. The biofilm formation results related to each treatment in the presence of *I. sakaiensis* in HPP medium indicate higher optical density records from Brij-35 and DTAB-treated PET pieces.
Based on the results acquired from FTIR spectra of surfactant treatments on PET pieces in presence of *I. sakaiensis* culture in YSV, it can be interpreted that DTAB and Brij-35 have a higher impact on PET pieces in a defined medium. However, the non-treated PET pieces show lower transmittance percentage rather than in complex medium (HPP). This is expected as *I. sakaiensis* grows well planktonically with a rich medium such as HPP, so there is little tendency for the microbe to use any other difficult nutrient source of carbon for assimilation (such as PET). In a defined medium, the lack (or low concentration of readily available carbon source) derives the cells towards other possible source of carbon, in this case, PET. The cells form a biofilm, and through the enzymatic process, assimilate the carbon source in the PET backbone. Based on the transmittance percentages obtained from FTIR, the study demonstrates a correlation between the pre-treatment of PET pieces with surfactants and the energy needed to break down the PET bonds. Both DTAB (cationic) and Brij-35 (nonionic) surfactants lead to an increase of transmittance percentage of the carbonyl group which suggests lower energy to break down. Besides, in the spectrum of both surfactants-treated PET pieces, the O-H group can be observed. In the YSV medium, the main biofilm formation is linked to DTAB and Brij-35-treatment, respectively. Both biofilm formation and transmittance percentages of functional groups have the same pattern.
The results from the different treatments using *I. sakaiensis* culture in M9 minimal medium, confirm that DTAB treatment has the most impact on PET pieces. In the M9 minimal medium, the biofilm formation shows the superior result for DTAB treatment. Brij-35-affected samples follow the same pattern as DTAB. Comparing the biofilm formation results and transmittance percentages, it can be interpreted that in DTAB treatment and under circumstances of this experiments, there is a direct relationship between increased transmittance and biofilm concentration.

The obtained results from the FTIR spectra of surfactant treatments on PET pieces in the presence of *P. mendocina* culture in the HPP medium indicate no changes on PET pieces. For the YSV medium, the Brij-35 treatment has a notable effect on PET pieces. In M9 minimal medium, DTAB has notable effect on PET pieces. The biofilm formation results show that DTAB provides a better possibility to form the biofilm on PET pieces. Also, the more biofilm formation related to the higher transmittance percentages.

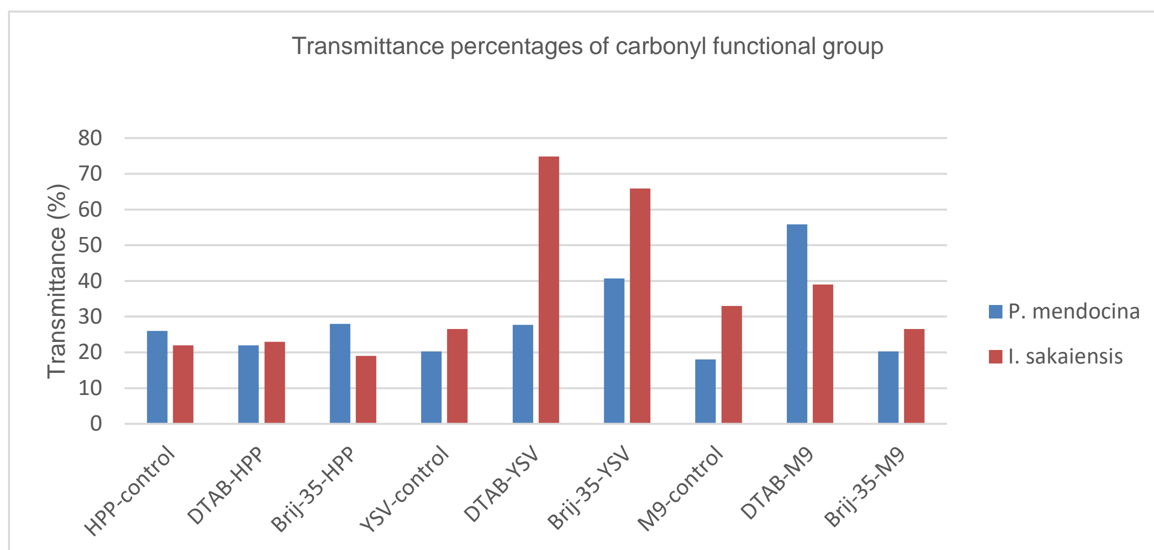

Figure 2: Comparing the carbonyl functional groups related to DTAB and Brij-35-treated PET pieces in the presence of I. sakaiensis and P. mendocina in defined media (YSV and M9) and complex medium (HPP) after 1 week. The non-treated PET in each medium in the presence of I. sakaiensis or P. mendocina used as a control.

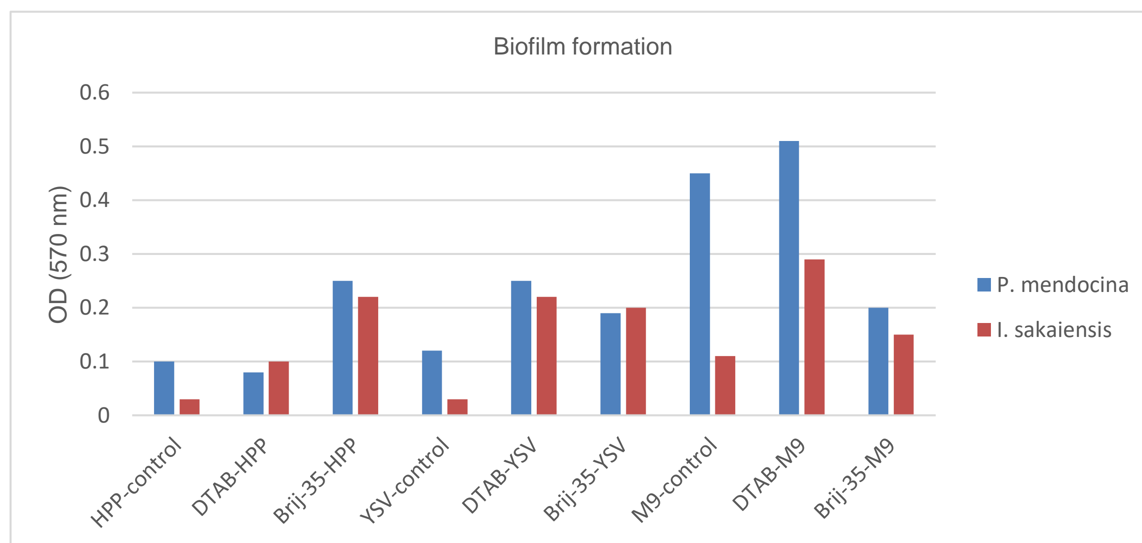

Figure 3: Comparing the biofilm formation related to DTAB and Brij-35-treated PET pieces in the presence of I. sakaiensis and P. mendocina in defined media (YSV and M9) and complex medium (HPP) after 1 week. The non-treated PET in each medium in the presence of I. sakaiensis or P. mendocina used as a control.

4. Conclusion

It can be concluded that DTAB treatment of PET in the presence of *I. sakaiensis* in the YSV medium can notably enhance the degradation of PET. Also, Brij-35 in YSV medium can pre-treat the PET and make it ready for bacterial adhesion with a slight difference compared to DTAB. However, higher biofilm concentration of *I. sakaiensis* was formed with DTAB-treated PET in M9 minimal medium. Also, in the presence *P. mendocina*, DTAB treatment in M9 medium and Brij-35 in YSV medium seem to be the most effective treatments. The biofilm formation in the presence of *P. mendocina* confirms similar result as FTIR transmittance percentages. DTAB in M9 and Brij-35 in YSV generate higher concentration of biofilm.

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