Bacterial immobilization by adhesion onto agave-fiber/polymer foamed composites.

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1. Abstract

Adhesion of *Pseudomona putida* F1 onto agave-fiber/recycled-polyethylene foamed composites was studied under different controlled conditions during batch experiment. Factors such as pH, contact time and temperature, were controlled. Adhesion was verified by scanning electron microscopy (SEM). Experimental data analysis showed that adhesion is an irreversible process which follows a first-order kinetics model. In addition to the obvious reuse of waste material, the amount of bacteria adhered onto the agave-fiber/polymer foamed composites suggested that this material could be used as support for bacterial immobilization to be applied in biotechnological processes.

2. Introduction

Millions of tons of synthetic polymers are discarded each year. On the other hand, another specific pollution problem taking place in South-west Mexico is the *Agave*-fiber wasted throughout the process of Tequila. Thus the recycling of polymers and *Agave*-fiber become a very important aspect and it is necessary to find alternatives for the reprocessing of these materials. One of these alternatives is the production of natural fiber composites which is an emerging area in polymer science. Nowadays the main application of natural fiber composites is the production of materials for construction like panels, doors, windows, furniture and decorative pieces [1]. In this research we propose, as an alternative to these applications, to use these natural fiber composites as a less expensive support for immobilization of enzymes, bacteria and other biopolymers with applications to biological processes. There are some studies of bacterial adhesion onto cellulose which establish that bacterial attachment onto fibers are manly function of temperature, ionic strength, contact time and cell concentration. Bacterial adhesion is a complex problem involving interactions at the cell surface level and it may come partially from non-specific acid–base hydrophobic attraction [2, 3, 4].

3. Experimental conditions

3.1 Microorganism and media

The microorganism used in this research was *Pseudomona putida* F1 ATCC 700007, which was activated in nutrient broth (Difco 0003) and stored frozen in 20% glycerol at -20ºC. The growth medium was the reported by Rochex et al. [2].

3.2 Composite preparation

The materials used to produce the composites were recycled high-density polyethylene (HDPE), fibers of *Agave Tequilana* Weber var. Azul, obtained from residues of a local tequila company and azodicarbonamide (ACA) as blowing agent from Sigma-Aldrich (USA). Fibers from *Agave Tequilana* Weber var Azul were washed and afterwards they were chemically treated (digestion) with a 20% (wt/wt) NaOH solution and a temperature of 170ºC during 90 minutes in order to eliminate the lignin present in the fibers. Once the fibers were treated, composite preparation was the next step. Foamed composites extrusion was carried out in a single-screw extruder Haake Rheomex 254 controlled by a Haake Rheocord 9000 control system. An amount of 0.5% of ACA (wt/wt) was added to a blend of 30% fiber and 70% recycled polymer and extruded with a screw speed of 30 rpm. The temperature profile from the hopper to the die of the extruder was set to 150/160/170/180ºC. After the extrusion process, the foamed composites were pelletized.

3.3 Adhesion experiments and SEM observations

Freshly prepared bacteria were suspended in a 500mL Erlenmeyer flask with 100 mL of 10mM NaCl in order to obtain the desire concentration. For each experiment two flasks were used, one “sample” flask containing 3g of composite pellets and the other one without composite called “blank flask”. The technique of serial dilution was used to determine bacterial adhesion. The difference between the numbers of suspended cells in both flasks was reported as the number of adhered bacteria (A_B). The effect of *pH* on the adhesion of *P. putida* F1 onto foamed composites was also investigated adjusting it in the range 5–9 with HCl or NaOH as needed. The effect of the time of contact (t_c) between composite and *P. putida* F1 in suspension was analyzed as well as the effect of the experimental temperature (T), which was pre-set at 25, 30 and 40ºC. Each experiment for all the conditions studied was performed in triplicate. Finally, SEM observations were carried out via a HITACHI Tabletop Scanning Electron Microscope TM-1000.

4. Results and discussion

4.1. Effect of contact time and temperature on adhesion

Figure 1 shows the number of adhered bacteria with respect to contact time (t_c). From these results, it is clear that adhesion depends on the contact time and the temperature. A maximum number of adherent bacteria of $1x10^9$ cfu/g composite was reached for both temperatures of 30 ºC and 40 ºC however the rate of adhesion was greater for 40ºC than for 30ºC, attaining the stationary phase at five and seven hours respectively. For 25ºC a maximum value of 6×10^8 cfu/g composite was observed which is considerably smaller than the value reached for 30 ºC and 40ºC experiments. The rate of the adhesion also decreased attaining the stationary phase after 10 hours. The adhesion process is well described by a first-order irreversible kinetic model described as follows (Figure 1):

$$\ln\left[\frac{C_C}{C_{C,0}}\right] = -k_a C_{S,0} t_c . \qquad [1]$$

where C_C is the cell concentration, $C_{C,0}$ is the initial cell concentration, $C_{S,0}$ is the active sites concentration on the composite surface and k_a is the rate constant for adsorption. These kinetic results are in agreement with previous studies of bacterial adhesion [5, 6, 7].

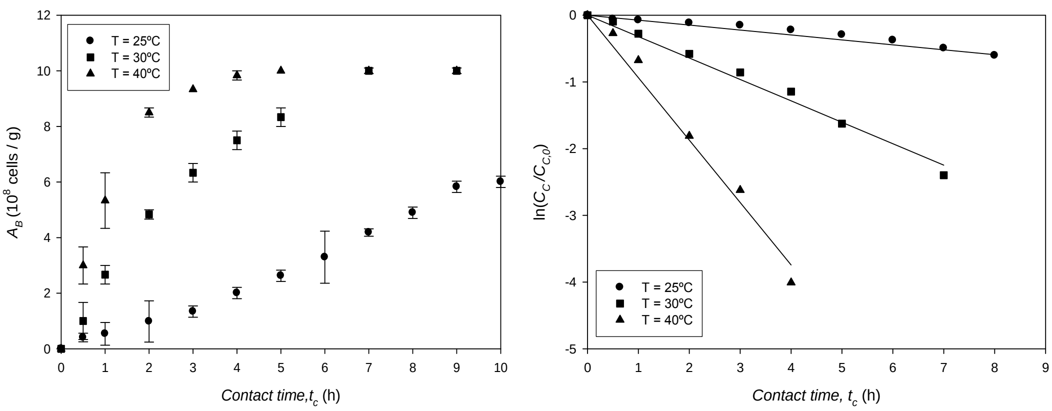

Figure 1. Bacterial adhesion onto foamed composite as function of contact time at different temperatures (left) and adhesion kinetics (right).

4.2 Effect of pH on adhesion

The *pH* range studied was from 5 to 9, since an extreme *pH* value (< 4 or > 9) inhibits microbial growth and biosynthesis of extracellular polymers necessary to biofilm formation

[8]. It is clear from the results shown in Figure 2 for an initial biomass concentration of 3.3×10^{7} cfu/mL, that bacterial adhesion increases as *pH* decreases from 9 to 5. Liu [6] reported similar results for bacterial adhesion to thermoplastics. Rochex et al. [2], reported that the isoelectric point occurs at *pH* = 3, below this value the net surface charge will be positive and above will be negative. In this sense, in our *pH* range, the surface is negatively charged and as the *pH* decreases, the negative charge density of the bacterial surfaces should decrease because of decreased deprotonation of carboxyl, phosphate and amine groups which result in a decreased electrostatic repulsion between bacteria and the support favoring the adhesion phenomena [6].

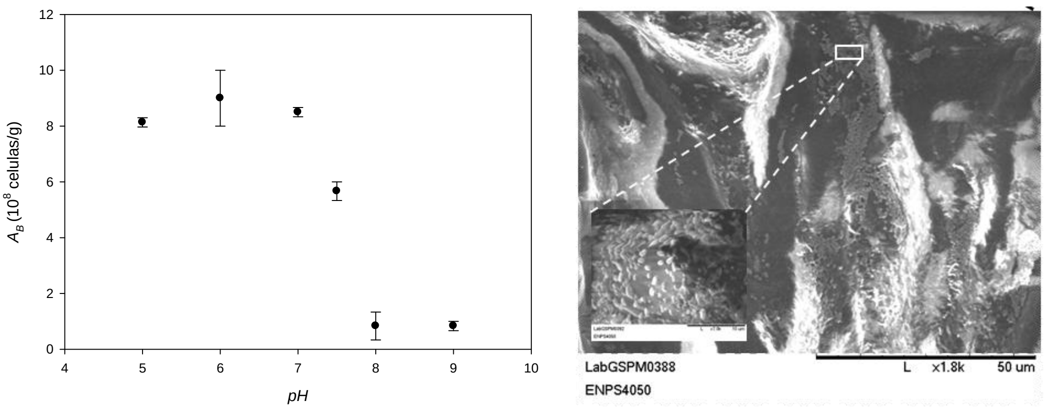

Figure 2. Bacterial adhesion onto foamed composite as function of pH (left). SEM micrographies of adhered bacteria (right).

5. Conclusions

In this study was found that the adhesion process is well described by a first-order irreversible kinetic model. The results suggested that the rate of adhesion is strongly affected by the temperature. Maximum numbers of adhered bacteria were found for *pH* values between 5 and 7. Based on the results, agave-fiber/recycled polyethylene foamed composites are able to be used as support for bacterial immobilization with potentially applications on biotechnological processes.

6. References

[1] Nabi Saheb D. and Jog J.P., 1999. *Adv. Polymer. Tech.* 18(4), 351-363.

[2] Rochex A., Lecouturier D., Pezron I., Lebeault J.M., 2004. *Appl. Microbiol. Biotechnol.* 65(6), 727-733.

[3] Pezron I., Rochex A., Lebeault J.M., Clausse D., 2004. *J. Dispersion Sci. Technol.*, 25 (6), 781-787.

[4] Lahtinen T., Kosonen M., Tiirola M., Vuento M., Oker-Blom C., 2006. *J. Ind. Microbiol. Biotechnol.* 33(9), 734-740.

[5] Korber D.R., Lawrence J.R., Caldwell D.E., 1994. *Appl. Environ. Microbiol.* 60(5), 1421-1429.

[6] Liu Y., 1995. *Colloids Surf. B. Biointerfaces.* 5(5), 213-219.

[7] Yang S.T., Lo Y.M., Chattopadhyay D., 1998. *Biotechnol. Prog.* 14(2), 259-264.

[8] Czaczyk K., Myszka K., 2007. *Polish J. Environ. Stud.* 16(6), 799-806.