INTRODUCTION

A biofilm can be defined as cells immobilized on a substratum, which are often embedded in a matrix of bacterially produced extracellular polymeric substances (EPS) (Campbell, 2000). The formation of a mature biofilm occurs as follows: transport of microorganisms to a surface, initial microbial attachment, formation of microcolonies, and formation of mature biofilm(Sauer and Camper, 2001). Biofilms occur extensively in aquatic systems, where they are implicated in industrial concerns such as corrosion of surfaces or spoilage of food, and where they are also implicated in drug resistance and dental caries (Auerbach and *al*.,2000).

Controlling biofilms is not an easy task. Indeed, bacteria in a biofilm acquire an extreme resistance to antimicrobial agents as sanitizers and to heat. So it is more difficult to kill bacteria embedded in biofilm than the same cells in a free floating state (Cooper and Schraft, 1998). According to Campbell (2000), "the study of biofilms in the food industry has begun only recently compared with other industries, possibly because it was assumed that the cleaning and sanitizing procedure performed in modern food plants would prevent their formation. However, biofilms are still observed in the food industry on rough surfaces, in end sections. In spite of automated cleaning-in-place, however, biofilms still persist in certain areas in food plants."

So attachment surfaces play a critical role in the formation of biofilm and it is important to identify and elucidate general principles that apply to mechanisms of adhesion. 2 theorical approaches have been applied to studies of bacterial attachment to evaluate and understand the interactions that control adhesion: "these 2 approaches are the DLVO theory, which takes into account the attractive van der Waals interactions and repulsive electrostatic interactions, and thermodynamic models, in which the adhesive interaction is treated as an equilibrium process and is described in terms of the surface free energies of the bacterium, substratum and separating liquid"(Fletcher, 1996).

Pseudomonas putida is chosen for this study: it is a free-living saprophytic organim in soil where plays an important role in decomposition, biodegradation, and the carbon and nitrogen cycles. This bacterium is interesting in food industry because it causes important food spoilage and it is capable to metabolize a wide variety of carbohydrates, proteins, and lipidsin food. Moreover Pseudomonas putida is found widely in the environment. Spoilage can typically occur in eggshells, fish, meat, milk, cheese: it will grow rapidly using glucose first and then aminoacids which involves changes in odor, texture, color, and sliminess. So because it is an aerobic bacterium, it is preferable to store products in vaccum-package (Bibek, 2000). But this process of storage does not give a good image of the product to consummers. So the best solution is to act on surfaces touching food to reduce spoilage on food.

The strain of *Pseudomonas putida* used in this study was genetically engineered to express green fluorescent protein (GFP) and will autofluoresce with an emission maximum at 510 nm. It was demonstrated that GFP, a kind gift from the jellyfish *Aequorea victoria*, is a naturally fluorescent, nontoxic protein that requires no substrate but fluoresces simply in response to blue light. GFP is resistant to bleaching and is therefore ideal for long time-course analysis (Haseloff and *al*., 1999).

The goal of this study is to understand initial microbial attachment for formation of a mature biofilm during the first hour after adding cells. Initial attachment in done on self-assembled monolayers (SAMs) formed on the adsorption of alkanethiols to the surface of gold and are well-ordered surfaces that permits have constant surface (Mrksich and Whitesides, 1996). The study is done on 2 different kinds of SAMs: one made with dodecanethiol to obtain an hydrophobic surface and one done with mercaptoundecanol to obtain an hydrophilic surface. So this project will permit know if there is a difference of initial bacterial behaviour (attachment, detachment, and movements of bacteria) on hydrophilic or on hydrophobic surfaces during one hour after adding cells.

MATERIALS AND METHODS

Bacterial culture

Pseudomonas putida GFP 9 was maintained on tryptic soy agar (TSA) plates. Stock cultures of the organisms were frozen at -80°C in a 50% glycerol solution made with phosphate buffered saline (PBS).

For all adhesion experiments, cultures were grown in advance in tryptic soy broth (TSB) at 30°C for 24h without shaking. Cultures were harvested by centrifugation (3000g for 10 minutes), and washed with PBS. The bacteria were resuspended in PBS to an optical density of 0.3 OD ± 0.05 at 600nm. The exact number of bacteria was determined by plate counting. The washed bacteria were used during the day.

BATH (hydrophobicity of bacteria)

The bacterial adherence to hydrocarbon (BATH) test was performed in accordance with the procedure used by Bellon-Lafontaine et al. (1996). Basically, 0.2 mL of certified hexadecane was added to 1.2 mL of a cell suspension containing ~ 10^9 CFU/mL in PBS. The mixture was vortexed for 90 seconds, to mix the phases, and allowed to stand for 15 minutes to ensure complete separartion of the phases. A 1 mL sample was then removed from the aqueous phase, the optical density measured at 400 nm, and the percentage of cells bound to the organic phase calculated using the following formula:

$$\% \text{ adherence} = (1-A/A_0)x100\%$$

where A_0 is the OD_{400} of the cell suspension before mixing and A is the OD_{400} after mixing.

Self assembled monolayer (SAM) preparation

SAM was formed from adsorption of long-chain alkanethiols on a layer of gold. Alkyl disulfide monolayers could be produced on gold substrates thanks to a strong affinity between sulfur and gold (van der Waals interactions). “A benefit of using gold as the substrate was that gold did not oxidize readily in air so the preparation of alkylthiol SAM could be performed in ambient conditions” (A. MacDairmid, 2001).

Gold evaporation (A. MacDairmid, 2001)

Gold films are made by evaporating gold onto cleaved mica in high vacuum. Mica was used as the substrate because it is arranged in layers of atomically flat sheets which can be easily cleaved using adhesive tape to yield a clean, flat surface. The evaporating chamber was pumped to a base pressure of ~ 10^{-3} atm using a rotary vane roughing pump and a diffusion pump with a liquid nitrogen trap (Appendix 1).

Once the base pressure was attained, the 2.5 cm of 1mm gold wire was melted in a tungsten wire basket positioned 5 cm below the mica surface. The mica was placed on a stage and held in place by a clipped glass slide which kept a heating filament in contact with the back of the mica. Prior to evaporation the mica was heated to 130°C using a 2.5 A current supplied by a DC constant current power source to expel water trapped between mica layers. Indeed, trapped water can destroy the film during the rapid heating of the flame annealing process. Once the mica was heated for 6 hours, the tungsten basket was heated from an AC power supply. A shutter shielded the bare mica from evaporation until a stable gold flux was achieved. The mica was coated with gold for a period of ten minutes. The deposited film was allowed to cool under vacuum before it was removed for use. The evaporation process produced a 2 cm x 3 cm gold film that could be cut into smaller pieces to fit into the Petri dishes for thiol modification. Each piece of gold was passed through a flame.

Thiol modification

The thiol solutions were prepared at a concentration of 1mM. This corresponds to an addition of 0.002g of 11-mercapto-1-undecanol ($C_{11}H_{24}OS$) or 2,4µL of 1-dodecanethiol ($C_{12}H_{25}SH$) to 10 mL of ethanol (Laura Pedri, 2003).Before each experiment, glass ware was rinsed with distilled water followed by ethanol. Gold supports were placed in these solutions and kept for 24h at room temperature. The samples were then removed from the solution and rinsed in copious amounts of ethanol to remove any unbound thiol.

Modification verification

Modification of the surface was verified using contact angle measurements as follows: A small deionised water droplet (5µL) was placed onto the gold film. A photograph of the droplet was taken and the contact angle was measured. 3 drops of distilled water were then pipetted onto the surfaces, and a Sony DSC-P30/P50 digital camera was used to take photographs of the drops. Contact angles were determined from the photographs using a protractor and a ruler. The final results were determined by averaging the results from separate trials for each surface. These trials were repeated 3 times. The higher contact angle for dodecanethiol can be attributed to the hydrophobic character of the methyl groups in the tail region of the molecule. The contact angle for mercaptoundecanol was lower due to the presence of hydrophilic groups.

On a first experiment, SAMs were produced and kept to a box and used when it was necessary for experiments. So for each experiment (contact angle measurements and

adhesion of bacteria), SAMs did not have the same age. For bacterial adhesion experiment, SAM was glued on slip 24h in advance to avoid moving.

On a second experiment, SAMs were used one hour after they had been prepared (time required that glue was dried). So each SAM used had the same age. To study the evolution of hydrophobicity and hydrophilicity during the time, contact angle measurements were done at different time.

Confocal microscope

"The confocal microscope is an established tool in many fields of biomedical research where its major application is for improved light microscopic imaging of cells within fluorescently-labeled tissues" (S.W. Paddock, 1999). The confocal microscope has several advantages over a traditional microscope: it reduces chances of artifacts from the techniques of specimen preparation, prolongs the time of fluorescence of bacteria, and out of focus light is eliminated. (Appendix 2) Indeed, the illumination in a confocal microscope is achieved by scanning one after one focused point and thus avoids a long excitation of fluorescent molecules. Also, the confocal microscope allows observing and saving a focus point over time and this particular feature was used to observe the formation of a biofilm.

The confocal laser scanning microscope used was Olympus Fluoview model FV-300 with the microscope BX51.The objective 60X PLAPO was used in immersion oil. The analysis program was Fluoview version 4.0. Thanks to GFP present in *Pseudomonas putida*, no dye was required and argon laser (488 nm) was sufficient (blue laser intensity: 10%). Indeed, GFP has the advantage to function in living bacteria and eliminating the need for dye fixation and its associated artifacts. The experimental conditions were channel 1, PMT: 702v, gain 1.0x, and offset 0%.

150 µL of washed cells in PBS were placed on the SAM and surrounded by an imaging chamber (PCl-2.0, *Grace bio-labs*). A cover slip was glued 24h before on the imaging chamber. Presence of bacteria was first verified by epifluorescence microscopy. Then,the argon laser (fast scan speed in XY-mode) was used the movethe Z-stage to the SAM-level where bacteria were attaching. Every effort was made to keep this set-up as brief as possible to permit observation of bacterial adhesion shortly after addition of cells to the chamber. After the initial set-up, XYT was launched with an interval scanning of 30 seconds for one hour. The pictures were saved as single-tiff in two different colors (for superimposition of images) and as multi-tiff. Each image had a 55,555 μm^2 dimension.

Analysis programs

Everytime, analysis were done on a part of SAM of 55,555 μm^2 dimension.

To define biomass, a Matlab program calculates the percentage of place occupied by bacteria in an image. One caracteristic example was chosen for each kind of SAM.

To know exactly how many bacteria were present on each image, Image pro permit to dilate pixels and thus count bacteria easier even bacteria which were not on the focus. The results were reported on graph. An average of all graphs obtained from same aged SAMs was done and standard deviation was mentinned to be able to compare evolution of number of adhered bacteria following the case.

Time of bacteria attachment was determined like this: 30 bacteria were taken at random in the image “time 15 minutes after adding cells” until “time 63 minutes after adding cells” to have the same interval time because each experiment did not start at the same moment after adding cells. The duration of attachment of each bacterium was noted.

Another method was to superimpose two images of different color and different time (interval of 2 minutes) during the interval time 2 minutes to 36 minutes after adding cells. Then number of bacteria which attach, detach, and move were determined by comparing 2 superimposed images.

RESULTS

BATH test

Results found after BATH test were as follows:

OD_{400} of the cell suspension before mixing $A_0 = 0.475$

OD_{400} of the cell suspension after mixing $A = 0.461$

According to the formula % adherence = $(1-A/A_0)x100\%$, there was 2.95% of adherence.

So adherence of *Pseudomonas putida GFP 9* on a hydrophobic component (hexadecane) was low meaning that this bacterium had not good affinity to a hydrophobic component and its surface was hydrophilic.

Contact angle measurements on SAMs

For a first series of experiments, different aged SAMs were used.

	Average	St.Deviation	Average	St.Deviation	Average	St.Deviation
SAM made with mercaptoundecanol	40.3°	2.9	30.8°	2.5	38.8°	0.8
SAM made with dodecanethiol	82.8°	6.4	96.8°	0.7	104.6°	0.8
Series	1		2		3	

Table 1: Contact angle measurements on 3 different series of SAMs

First, contact angle averages of SAMs made with mercaptoundecanol were smaller than contact angle averages of SAMs made with dodecanethiol. This difference was statistically significant (p=). Thus, SAMs made with mercaptoundecanol could be considered a hydrophilic surface and SAMs made with dodecanethiol could be considered a hydrophobic surface.

The high standard deviations indicated that for series 1, the surfaces were not very uniform in both cases, but specially for hydrophobic surfaces. But on the other hand, the

surfaces of series 3 were quite uniform. So, according to SAM chosen surface was not as much uniform in all cases.

Also, for an hydrophilic surface, contact angles could vary between ~ 30° and ~ 40° and for an hydrophobic surface, between ~ 80° and ~ 100°.

So for a conclusion thanks to contact angle measurements, there were a lots of variations between two same kinds of surfaces and might be because SAMs had not the same age.

A second experiment was conducted to determine the influence of time on contact angle measurements and consequently on changes of SAMs over the time.

	Average	St.Deviation	Average	St.Deviation	Average	St.Deviation
SAM made with mercaptoundecanol	27.8°	1.36	32°	1.41	42.3°	2.06
SAM made with dodecanethiol	98.3°	0.41	99.5°	1.12	98.6°	3.83
Time	0 week		+ 1 week		+ 3 weeks	

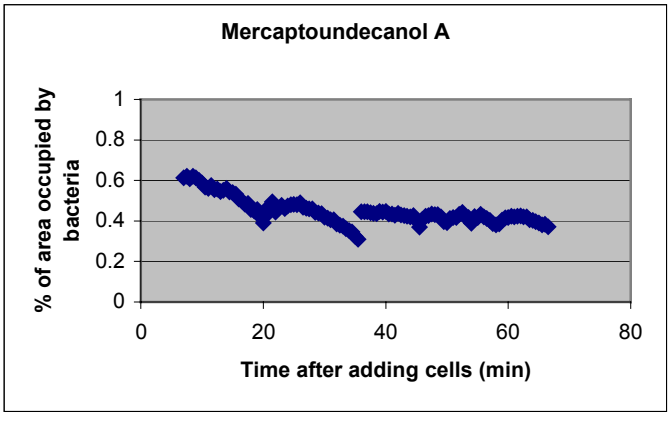
Table 2: Contact angle measurements at different time on the same SAM

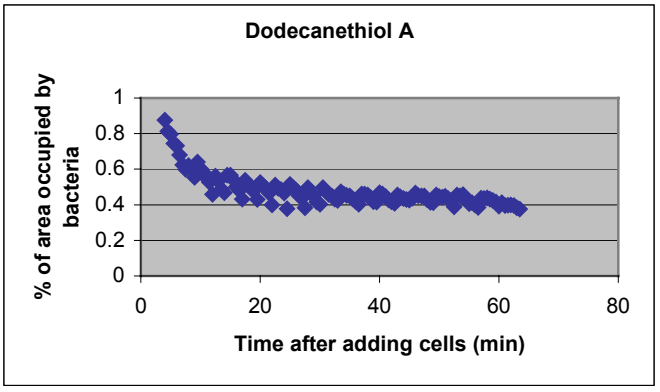
First, even during the time contact angle measurements indicated that there were an hydrophilic surface and an hydrophobic one .However, SAMs made with dodecanethiol had not their contact angle changing during the time whereas SAMs made with mercaptoundecanol that means that globally their hydrophobicity stayed constant. SAMs made with mercaptoundecanol became less and less hydrophilic during the time: 27.8° to 42.3° after 3 weeks.

Then, the increase of standart deviation showed hydrophobic or hydrophilic surfaces became less uniform during the time.

Biomass of bacteria during 60 minutes

Initially, the image-analysis program COMSTAT was used to determine biomass over time. Biomass corresponded to the percentage of total area occupied by luminescent spots on the SAM which should be bacteria.





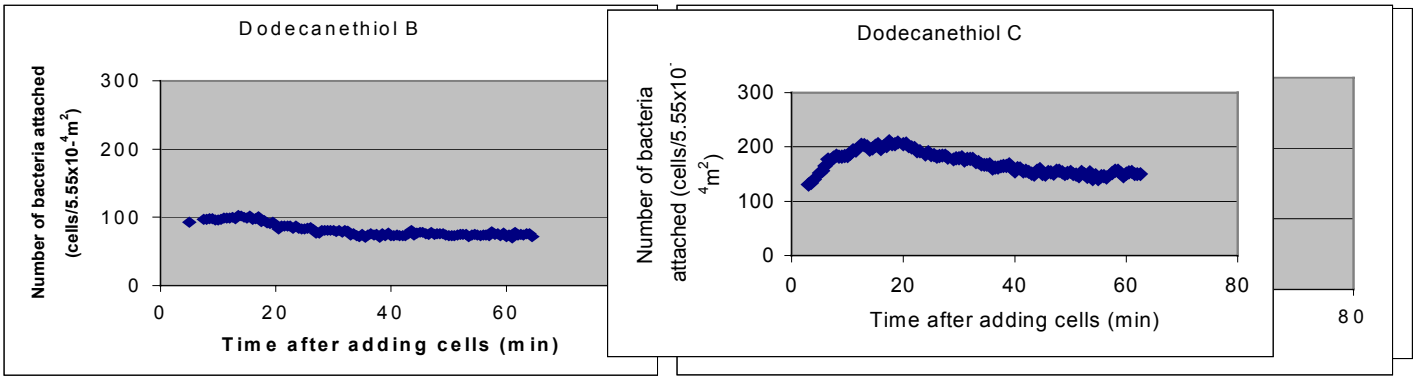
Graph 1: Percentage of area occupied by bacteria on a part of SAM (55,555 μm^2) over time for an hydrophilic and an hydrophobic surface.

Dodecanethiol A means SAM made with dodecanethiol and mercaptoundecanol A SAM made with mercaptoundecanol.

In both case, the room occupied by bacteria decreased during the time and this decrease was not even. There were a lots of jumps on the curves at different places. The 2 graphs shown are representative of other graphs obtained.

Adhesion of bacteria during 60 minutes

A first series of experiments was performed on different aged SAMs. Graph 2 shows the most caracteristic graphs. The number of bacteria attached to the SAM was determined by Image Pro program for analysing images.



Graph 2: Adhesion of bacteria on SAMs during the time, after some minutes adding cells. The little variations were due to attachment and detachment of bacteria. Each experiment did not start at the same time.

For dodecanethiol B, the number of adhered bacteria was quite constant over time even if it decreased a little bit 20 minutes after adding cells. The number of attached bacteria was contained between 115 and 65.

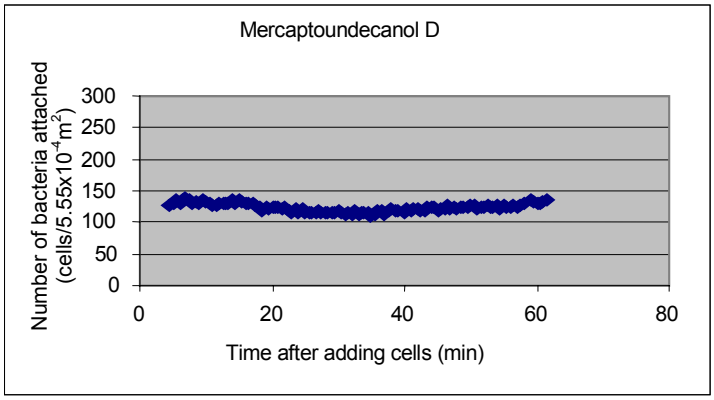
For dodecanethiol C, the number of adhered bacteria varied during the time. Twenty minutes after adding cells, the number of attached bacteria increased up to 215. Then, there was a decrease of the number of bacteria, until becoming constant with about

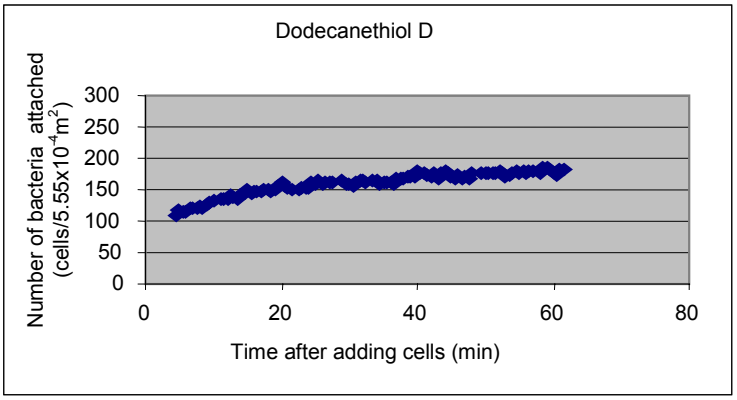
150 bacteria. However the number of bacteria at the beginning was lower than the number at the end.

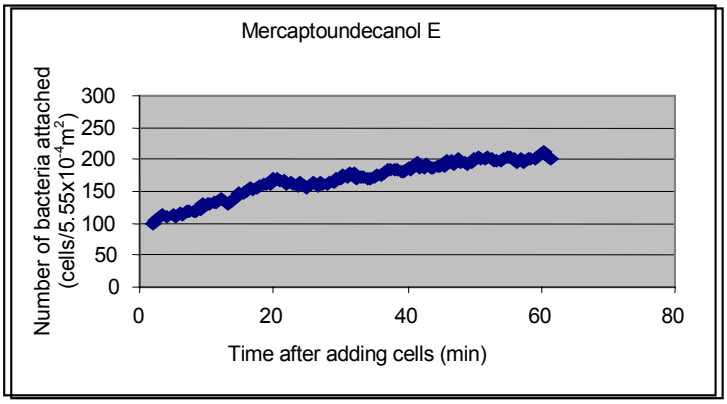
For mercaptoundecanol B, 7 minutes after adding cells the number of adhered bacteria was 160 and 1 hour after the number was 70. So, the number of bacteria decreased dramatically during this time. five minutes after adding cells, the number of bacteria was 145 and 2 minutes after the number was 160 then this number decreased.

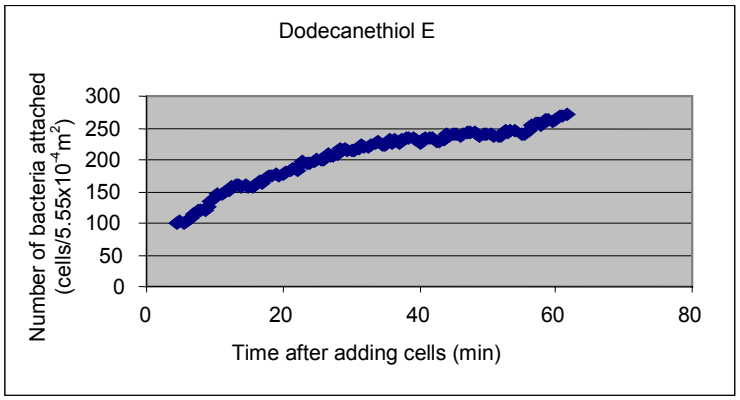
For mercaptoundecanol C, the behaviour of the curve was different. Seven minutes after adding cells the number of adhered bacteria was also 160. Then it increased up to 190 and afterwards, the number decreased. The number of attachedbacteria at the beginning and at the end were the same and this number was 150, much higher than in mercaptoundodecanol B.

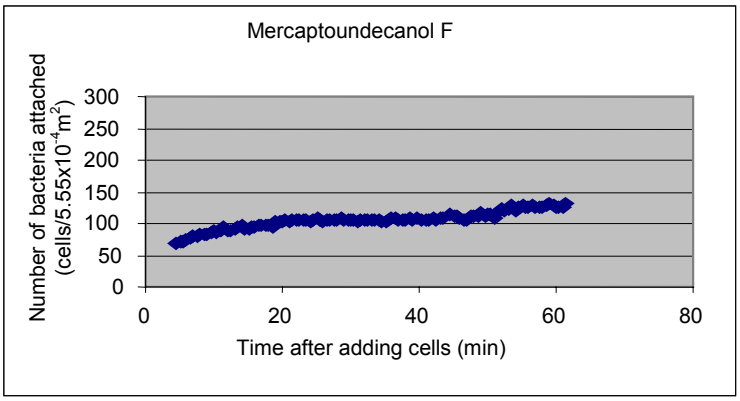
In all further experiments, SAMs used had the same age: between 2h and 4h old.

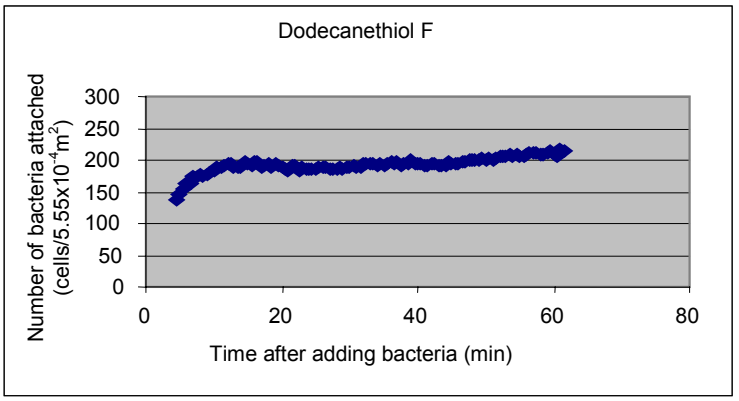


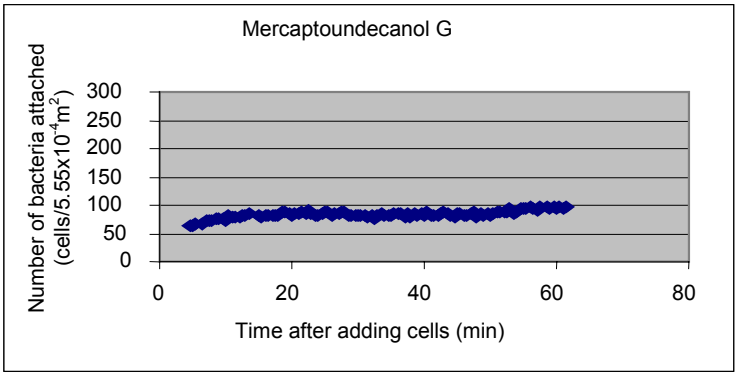




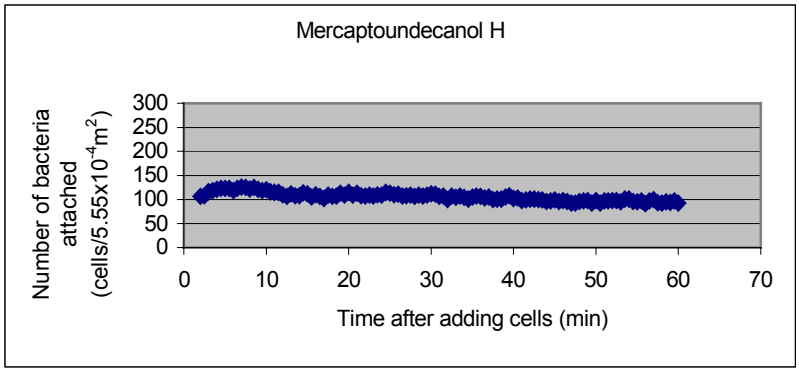








Graph 3: Adhesion of bacteria on same aged SAMs during the time after 2 minutes adding cells. Each experiment started at the same time excepted for mercaptoundecanol. Dodecanethiol E and dodecanethiol F were obtained using the same bacteria e.g.same concentration ($3{,}8x10^9$ CFU/mL) and same time of incubation. The concentrations of bacteria were unknown for dodecanethiol D and mercaptoundecanol G and H because PBS used for dilutions was contamined.



For mercaptoundecanol D, 100 bacteria had adhered 2 minutes after adding cells. Then, the adhesion increased a little bit (up to 140) and the number stayed constant around 120. And it increased slightly again.

For mercaptoundecanol E, the number of adhered bacteria started also at 100 but the increase of adhered bacteria was considerable (up to 220 bacteria). However, the concentration of bacteria ($2{,}16x10^8$ CFU/mL) was slightlyhigher than for mercaptoundecanol D ($0{,}92x10^8$ CFU/mL).

For mercaptoundecanol F, the number of adhered bacteria 2 minutes after adding cells was low (70). However 1 hour later this number had increased to 130. The concentration of bacteria ($1{,}21x10^9$ CFU/mL) was higher than for mercaptoundecanol E.

For mercaptoundecanol G, the evolution of the number of adhered bacteria over time was similar to mercaptoundecanol F except that the maximum of bacteria was 100.

For mercaptoundecanol H, there was an increase of adhered bacteria at the beginning, followed by a decrease thereafter. It was the same phenomenom seen for mercaptoundecanol D. But here, the decrease continued even though it was small .

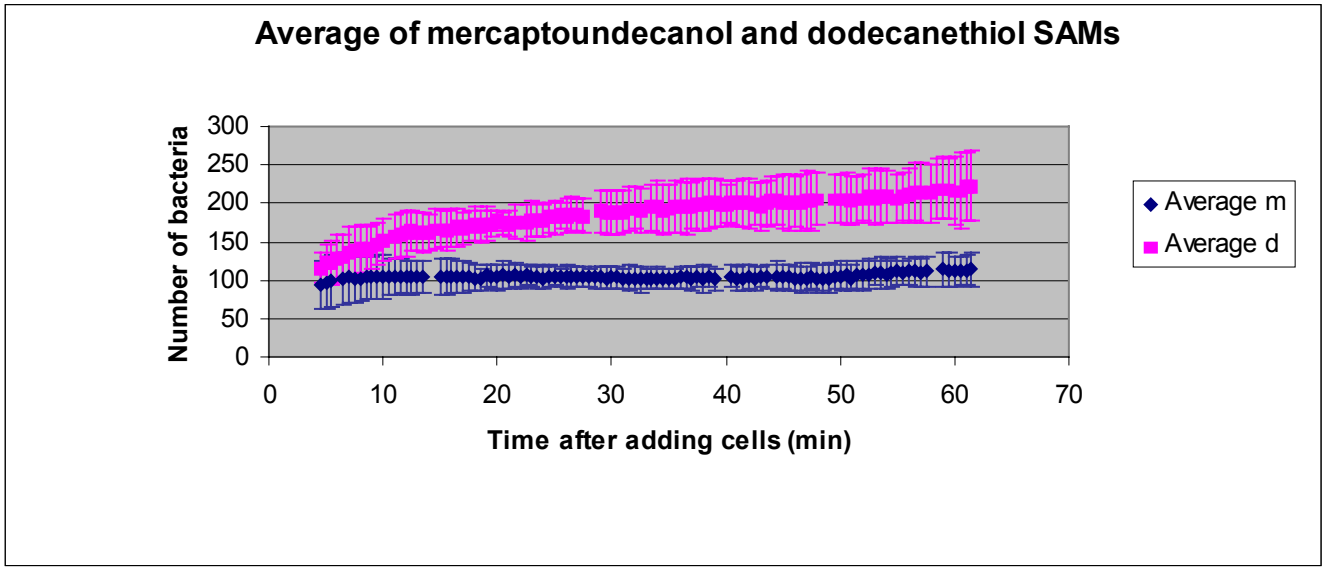
When all graphs obtained from mercaptoundecanol SAMs were compared, mercaptoundecanol E seemed different because of its high number of adhered bacteria.

But this experiment had been done under the same conditions as all the others and the reason for this observation is not known.

For dodecanethiol D, the number of adhered bacteria started around 100 then increased until 180. The concentration of bacteria was exactly the same as for mercaptoundecanol G.

For dodecanethiol E, the increase of the number of adhered bacteria was large, yet at the beggining there were only about 100 bacteria, but the attached cells increased continuously to approximately 280.

For dodecanethiol F, the concentration of bacteria ($3{,}8 \times 10^9$ CFU/mL) was exactly the same as dodecanethiol E. However at the beginning there were 80 adhered bacteria and at the end 210. During the 10 first minutes, the increase was large followed by a stagnation of the number of bacteria.



Graph 4: Average of graphs obtained from mercaptoundecanol SAMs and graphs obtained from dodecanethiol SAMs. Standard deviation was mentionned by vertical bars. Mercaptoundecanol E was not used. Some pictures could not be analysed. This explains that the curves are not continuous.

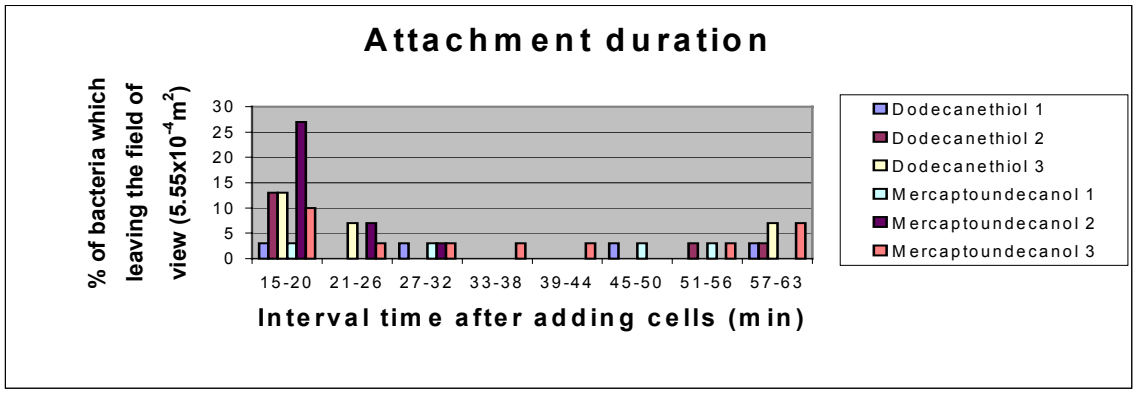
To allow a comparaison between the two types of SAMs, averages of all experiments are shown in graph 4.

Except at the beginning, these 2 curves were significantly different. Indeed, between 4 and 12 minutes after adding cells there, was a zone where the 2 curves were very close. Thus, we could conclude that at the beginning, the number of adhered bacteria was similar for the 2 kinds of SAMs. But then, adhesion was higher on the dodecanethiol SAM than on the mercaptoundecanol SAM. For the curve corresponding to the average of mercaptoundecanol SAMs, there was a slight increase at the beginning, but then the number of adhered bacteria stayed constant. On the other hand, for the curve corresponding to average of dodecanethiol SAMs, there was a continuous increase in cell numbers. This increase was more pronounced at the beginning.

Overall, more bacteria attached overtime on SAMs made with dodecanethiol than on SAMs made with mercaptoundecanol. This distinction was evident after only 12 minutes.

Attachment and detachment of bacteria

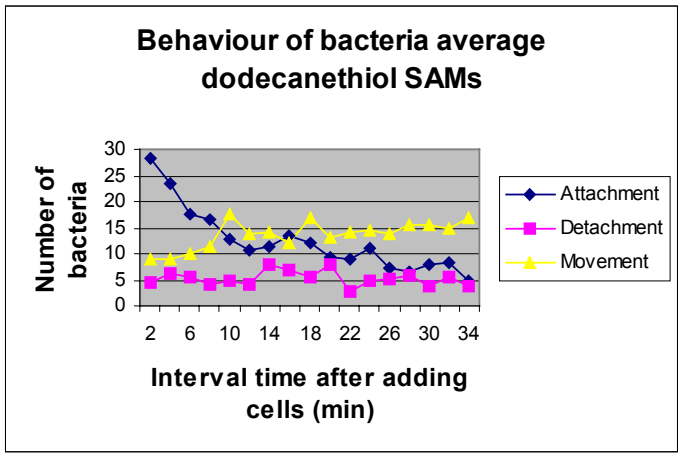
In a first experiment, which was done on different aged SAMs, only the duration of attachment was studied.

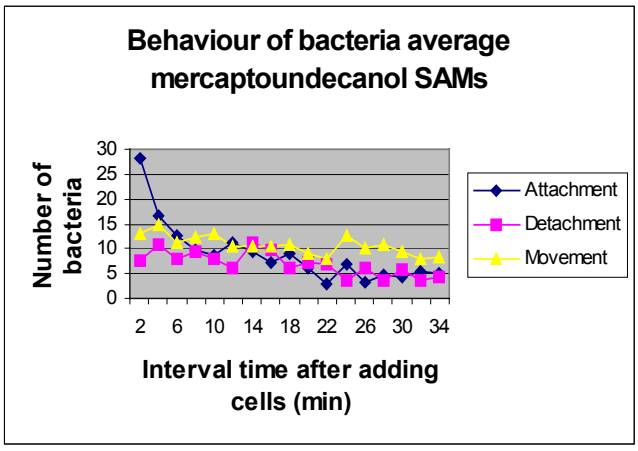


Graph 5: Attachment duration of bacteria on the 2 kinds of SAM

There were no differences in attachment duration of bacteria to SAMs made with mercaptoundecanol or dodecanethiol. However, a larger percentage of bacteria detached at the beginning, (within 26 minutes after adding cells) than during the later stages of the experiment.

A second experiment focused on studing attachments, detachments, and movements of bacteria during the first 36 minutes after adding cells.





Graph 6: Average of attachments, detachments, and movements of bacteria on SAMs made with dodecanethiol or with mercaptoundecanol. Average of mercaptoundecanol D, F, and H and dodecanethiol D, E, and F.

Attachment of bacteria on hydrophobic surfaces was very high between 2 and 4 minutes after adding cells and until 14 minutes the decrease was rectiligne with a decrease of ~ 33%. After the decrease was less high. On the other hand, on hydrophilic surfaces the attachment was the same as on hydrophobic surfaces at the biginning but the decrease was rapidly higher (~ 33% after 10 minutes). Moreover, during the time the number of bacteria which attached was lower than on hydrophobic surfaces.

The number of bacteria which detached from an hydrophobic surface was rather regular around 5 bacteria in a 2 minute interval. However, the number of bacteria which detached was always lower than the number of bacteria which attached. On hydrophilic surfaces, detachment of bacteria was more numerous (~ 10 every 2 minute internal time) and after 26 minutes this number decreased to 5 bacteria per 2 minutes. Also, after 8 minutes after adding cells, the number of bacteria which detached was similar the number of bacteria which attached.

For the movements, bacteria moved more on hydrophilic surfaces at the beginning than on hydrophobic surfaces. Then, 10 minutes after adding cells, movements of bacteria were higher on hydrophobic surfaces.

DISCUSSION

Bacterial adhesion involved lots of experiments and different parameters had been studied like bacterialstrain or surface composition. The goal of this study was to determine if the surface composition influenced bacterial adhesion.

So surface was an important point which should be the same for each experiment and could be modified in an hydrophilic or an hydrophobic surface. The contact angle measurements on SAMs corresponded well with previous results meaned that SAMs made with mercaptoundecanol was an hydrophilic surface and SAMs made with dodecanethiol was an hydrophobic surface. But contact angle measurements permited to understand that hydrophilicity of the surface changed during the time and that surfaces became less and less uniform. So to obtain useable data, SAMs had to be used very soon after preparing.

Therefore all experiments done with different aged SAMs could not be run. This could explain why on graph 2, bacterial adhesion was not different on hydrophilic or hydrophobic surfaces. Also, on these experiments, the number of adhered bacteria decreased during the time what was rather rare for a biofilm formation and this peculiarity should be explained by age of SAMs.

Also, the graph 1 could not be interpreted because different aged SAMs were used. Moreover this graph showed that Matlab program was not very appropriate to know how many bacteria were present. Indeed, all jumps on the curves corresponded to adjustements to be on focus. So it was luminous intensity which increased but not the number of adhered bacteria. Moreover, this program could not study movements of bacteria. So Image Pro was the most appropriate program for this study.

The study of bacterial adhesion during the first hour after adding cells showed that there were more adhesion on hydrophobic surfaces than on hydrophilic surfaces according to graph 3 and graph 6. And this difference of adhesion between the 2 kinds of surfaces was significant. (graph 4) To understand what was happened, it was important to know that numerous factors came into play and affected the forces that determined whether adhesion would occur (Wiencek, 1995) (fig. 1).

Also, when a bacterium moved closer to the surface, another potential barrier to adhesion was water adsorbed to the bacterial or substratum surfaces. Displacement of adsorbed water to allow bacterium to be close enough to surface was energetically unfavorable. But a hydrophobic surface had a tendency to exclure water, that permited bacteria to attach preferentially to hydrophobic surface because of facilitating close approch (Fletcher, 1996). So adsorbed water that must be displaced for adhesion to occur on hydrophilic surface could explain why bacteria had a greater affinity for hydrophobic surfaces.

By using *Pseudomonas putida GFP 9*, the difference of the number of adhered bacteria was significant (graph 4) but it was clearly impossible to generalize this result on all bacteria because for example, for *Pseudomonas sp. NCIMB 2021*, the difference in number of bacteria attached on hydrophobic and hydrophilic SAM was small (Wiencek, 1995).

So bacterial structural components could modify attachment to surface. Indeed, the presence of flagella used during transport, pili and pilus-associated adhesins had been shown to be important for the adherence and colonization of surfaces. None studies could claim their presence or absence on *Pseudomonas putida GFP 9*. Membrane proteins might also influence bacterial attachment processes. But different factors like time of incubation, temperatures… caused little changes in morphology of bacteria. Also, studies had shown that conditions at surface could induce expression of additionnal genes, morphology changes and synthesis of macromolecules (Fletcher, 1996). Indeed, *Pseudomonas putida* undergoes a variety of structural and metabolic changes following initial adhesion to a surface (Sauer and Camper, 2001). That was why on the same kind of surface, there was not the same number of adhered bacteria. But for dodecanethiol E and F, it was exactly the same bacteria used (same concentration, same condition of growth) and it was not the same results. This difference could come from the SAM used. To eliminate unbounding thiol, SAM was rinced with

ethanol but some traces of ethanol could remain (because SAM was used just after being prepared) and ethanol has a bactericidal effect. But the high number of adhered bacteria of mercaptoundecanol E was not explicable.

Graph 6 showed that number of bacterial attachment every 2 minutes decreased during the time on the 2 kinds of SAM. According to Wiencek and Fletcher (1994), this was a result of several possible effects: "the existence of a limited number of attachment sites, which were saturated during the early stages of exposure; repulsion of bacteria that were already attached and the conditioning of the substratum by substances released from the bacteria and adsorbed onto the substrata, which could begin to alter the surface properties of the SAMs 30 minutes after adding cells". This last point could be an explication of the decrease of adhered bacteria on mercaptoundecanol H.

Moreover, graph 6 illustrated between 2 and 4 minutes after adding cells the number of attachment was the same on the 2 kinds of SAMs. This suggested that the interfacial forces responsible for the initial binding of *Pseudomonas putida GFP 9* were greater on hydrophilic SAMs (Wiencek and Fletcher, 1994). That was due to the hydrophilicity of the bacterial surface according to BATH test results. This result was confirmed by contact angle measurements carried out on *Pseudomonas putida* since 40° contact angle was found. (Rijnaarts and *al*.,1995) However, after initial bacterial adhesion, the number of adhered cells was reduced on charged hydrophilic surfaces because of the thermodynamic approach (Campbell, 2000).

As studies done by Fletcher and Wiencek (1994), we found that detachment was greater on the hydrophilic SAM because of strenght of binding. However, they said that desorption from the hydrophobic SAM was almost negligible. But it was not the case on our experiments. Detachment of bacteria on hydrophobic surface was lower than hydrophilic surface but 22 minutes after adding cells, this difference was not very pronounced. These different result could be due to flow of bacteria used in their experiments.

Studies had indicated that binding might be stronger on hydrophobic surfaces than hydrophilic surfaces (Fletcher, 1996). In contrast, a bacterium that encountered an hydrophilic surface was first weakly held at the surface at a separation distance (Fig. 2). This weak adhesion was reversible what was explained the more numerous detachment on hydrophilic surfaces.

However, this theory did not quite correspond to our results for bacterial movements. Indeed, the number of movements should be less numerous on SAM made with dodecanethiol. Between 8 and 10 minutes after adding cells, figure 2 representation could explain why bacteria moved more on hydrophilic surface than on hydrophobic surface. But after this time, bacteria moved more on hydrophobic surfaces.

The only explication that I have it is because we haven't used flow and not the same growth way.