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Controlling the Growth of Escherichia coli by Layer-By-Layer Encapsulation

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Abstract.

Escherichia coli is one of the most common commensal aerobic bacteria in the gut microbiota of humans (and other mammals). Nevertheless, if left free to proliferate, it can induce a large range of diseases from diarrhoea to extra-intestinal diseases. In recent years, this bacterium had become increasingly resistant to antibiotics. It is therefore essential to implement new approaches able to maintain both bacterial viability and to control their proliferation. In this context, we developed a process to encapsulate *Escherichia coli* in polymer shells. We took advantage of the fact that this bacterium has a negatively charged surface and modified it via a layer-by-layer process, i.e. with oppositely charged polyelectrolyte pairs (namely chitosan as the polycation and alginate or dextran sulfate as polyanion). We successfully demonstrate the controlled coating of the bacterial surface via zeta potential measurement, the viability of the encapsulated bacteria and a delay in growth due to the multilayer coating. This delay was dependent on the number of polyelectrolyte layers.

Keywords: coated bacteria, layer-by-layer process, commensal gut bacteria, *Escherichia coli*, polysaccharide, polyelectrolytes

1. Introduction

The human microbiota plays a crucial role in the regulation of human health.[1] The microbiota encompasses all microorganisms colonizing specific locations and is not restricted to bacteria, but also refers to other microbes such as viruses, archaea, fungi, and protozoans.[2] Interestingly, via the secretion of antimicrobial agents, the microbiota is able to efficiently protect its host by regulating the pathogenic microorganisms population.[3] Furthermore, the microbiota actively participates in host biological functions (e.g. digestion and metabolism). Alterations in the microbiota community can lead to the development of autoimmune pathologies, inflammatory disorders and allergies.[3] These alterations could be due to a simple variation in diet and hygiene, or even the indiscriminate ingestion of antibiotics. Probiotics are often taken to restore healthy populations of dysbiotic microbiota [4]

There is a strong correlation between human health and the gut microbiota. The human gut microbiota is a huge microbial ecosystem and is considered partially responsible for maintaining human health. Indeed, it is well established that a healthy gut flora influences the overall health of the host. The gut microbiota produces specific host nutrient xenobiotics, and is involved in metabolism and drug metabolism, immunomodulation, conservation of the structural integrity of the gut mucosal barrier, and protection against pathogenic organisms. It stands to reason that particular changes in the ecosystem might contribute to the development of certain diseases. Several parameters impact the normal gut microbiota, e.g. the diet during infancy and adulthood or use of antibiotics deriving from the environment or the gut commensal community. Among the bacteria constituting the gut microbiota of humans and other mammals, *Escherichia coli* (*E. coli*) is the most common commensal pathogenic aerobic bacterium. However, *E. coli* is responsible for a wide range of diseases, from various diarrhoeal diseases to extra-intestinal diseases, and over the last 20 years, has shown the emergence and diffusion of factors inducing resistance to antibiotics. Due to its broad host spectrum and the potential severity of diseases caused by *E. coli*, a strategy is urgently needed to maintain the gut microbiota while regulating its proliferation.

Interestingly, some bacteria are able to enter a “viable but nonculturable” state from which they can be resuscitated upon proper stimulation. [5] Nevertheless, it is difficult to characterize and detect bacteria in this state,[6] and their inclusion in therapeutics or in cosmetics is challenging in terms of regulation. Consequently, more universal processes for answering these issues are required. One possible approach consists of encapsulating bacteria in a porous shell through which nutrients and other vital products are able to diffuse, maintaining bacterial viability but

at the same time delaying bacterial growth. One simple way to achieve this involves the layer-by-layer (LbL) process.

The LbL process can be used to modify macroscopically flat substrates to cover differently shaped surfaces, including particles (metallic,[7] polymeric[8]…), crystals [9] and even cells [10]. This approach presents several advantages, such as no requirement for specific equipment and the fact that numerous chemical and biological materials can be self-assembled in this way. This is why multilayer polyelectrolytes could be highly useful for biological applications. Polyelectrolyte multilayers are based on electrostatic interactions that are able to form ultrathin films with well-controlled thickness and chemical composition. The driving force is the electrostatic attraction between polymer layers that are oppositely charged (polyanion and polycation), which makes the deposition procedure and modification of the charged substrate relatively simple and straightforward.[11] As electrostatic attraction is the driving force, one prerequisite for surface modification is the presence of charged groups. In the present work, we took advantage of the negatively charged surface of the bacteria to modify this biologically charged material via the layer-by-layer process in order to confer new interesting properties.

Cell encapsulation methods have been the subject of previous studies, [12-16] such as for the chemical functionalization of yeast or bacteria cells, e.g. *Alcaligenes faecalis*,[17] *Allochromatium vinosum*,[18] *Bacillus* [19] cyanobacteria,[20] *Escherichia coli*,[21] *Lactobacillus acidophilus*,[22] and *Micrococcus luteus*.[23] Shells of different chemical structures/compositions have been investigated, ranging from soft organic layers based on multilayer assemblies,[4] to hard substrates (calcium carbonate,[24] calcium phosphate,[25] gold,[26] and silica[27]).

In the present work, we focus on the encapsulation of *E. coli* in shells of increasing thickness using a simple method, i.e. LbL assembly via the saturation method. This method was used to coat the bacteria whilst avoiding aggregation. This is the first time that this process has been used to coat bacteria. Furthermore, the direct LbL saturation method means that no washing or purification steps were needed. We used a pair of oppositely charged polysaccharides (natural polymers), i.e. chitosan as the polycation and dextran sulfate or alginate as the polyanions. The impact of the number of polyelectrolyte layers on bacterial viability and growth was investigated.

2. Experimental section

2.1. Materials

These following chemicals were purchased: chitosan (chi^+, low molecular weight Sigma-aldrich), dextran sulfate (dex^-, 40 000 g/moL, Sigma-aldrich), alginate (alg^-, Cargill Baupte, France), acetic acid (Alfa Aesar, + 99%), sodium acetate (Alfa Aesar, 99%) and sodium chloride (NaCl, Sigma-aldrich). Water was purified with a Milli-Q reagent system (Millipore).

2.1. Surface-Modification of Polyelectrolyte Multilayer-Coated Bacteria

Surface modification of the negatively charged bacteria, namely *Escherichia Coli*, was done via the layer-by-layer process starting from a bacteria suspension with an initial optical density at 620 nm.

To remove the culture medium and begin the bacteria coating, the bacteria were washed with a physiological solution (0.15 M NaCl, pH 6) by three successive steps of centrifugation/redispersion in physiological solution. The bacteria were then coated via two different methods: (i) coating via a saturation method or (ii) coating/centrifugation process (Figure S1). All polyelectrolytes were dissolved in 0.15 M NaCl at pH 6 (1 mg/mL). All solutions were sterilized in an autoclave before being used.

Coating via a saturation method: Surface modification of the negatively charged bacteria was performed via a direct LbL saturation method, this implies neither washing nor purification steps. [8, 28, 29] This method proposes the possibility of directly adding the required amount of polyelectrolytes to efficiently coat all the surface of the bacteria. The saturation concentration of each polyelectrolyte must be empirically estimated by measurement of zeta potential. 900 µL of bacterium suspension was progressively covered with the chitosan solution. Then, the polyanion solution (alginate or dextran sulfate) was added similarly. Each polyelectrolyte addition was done under mixing, every 15 min and at room temperature. A zeta potential measurement was done between each addition of polyelectrolyte solution. This enables to determine the required volume of each polyelectrolyte to saturate the bacteria surface. The assembly is denoted as: EC-$(chi^+/dex^-)_n$-saturation or EC-$(chi^+/alg^-)_n$-saturation (with n being the number of bilayers). "EC" designs the bacteria, i.e. *Escherichia Coli.*

Coating/centrifugation process: To 900 µL of a bacterium suspension was added 100 µL of the polycation solution. This mixture was stirred during 15 min and then centrifuged at 5000 rpm during 5 min. The supernatant was removed, and the bacteria were dispersed again in 900 µL of 0.15 M NaCl, pH 6; this rinsing step was repeated three times. The last rinsing water was submitted to Total Organic Carbon (TOC, Shimadzu TOC-L CSN instrument) analysis to check that this solution does not contain any organic matter. To this positively charged coated bacteria,

100 µL of a solution of the polyanion (dextran sulfate or alginate) was added similarly. Free polyanion was removed via the same purification process (centrifugation step). Note that the final volume of the suspension was kept at 1000 µL during each step. This whole process was repeated to reach different number of layers. The assembly is then written as: EC-$(chi^{+}/dex^{-})_n$-centrifugation or EC-$(chi^{+}/alg^{-})_n$-centrifugation (with n being the number of bilayers).

For both approaches, the zeta potential measurement was performed on a Zetasizer Nano-ZS (Malvern Instrument) to monitor the adsorption of each polyelectrolyte onto the bacteria surface. The size of the bacteria was determined via DLS measurement (Malvern Instrument).

2.2. Assay for antibacterial activity:

Microbial cultures. To study the impact of the bacteria coating, *Escherichia coli* (coated and non-coated) were grown under shaking in lysogeny broth (LB) medium at 37°C. For the assay, 96-well microplates were used in each 200 µL bacterial suspension was added (eight replicates) and then were incubated at 37°C. The following samples were studied bare bacteria and coated bacteria i.e. EC-$(chi^{+}/dex^{-})_n$ or EC-$(chi^{+}/alg^{-})_n$. The growth of uncoated and coated bacteria was followed periodically by reading the optical density of bacterial suspensions at 620 nm using a UV-Visible spectrophotometer.

The time-dependent microbial growth was simulated using an exponential growth model (equation 1):

$$X = X_0 e^{\mu t} \qquad \text{equation 1}$$

where X and X_0 are final and initial biomass concentrations, respectively, as reflected by the absorbance intensity. Specific microbial growth rate, µ, was determined via least-squares error analysis.

2.3. Bacterial visualization and viability assay

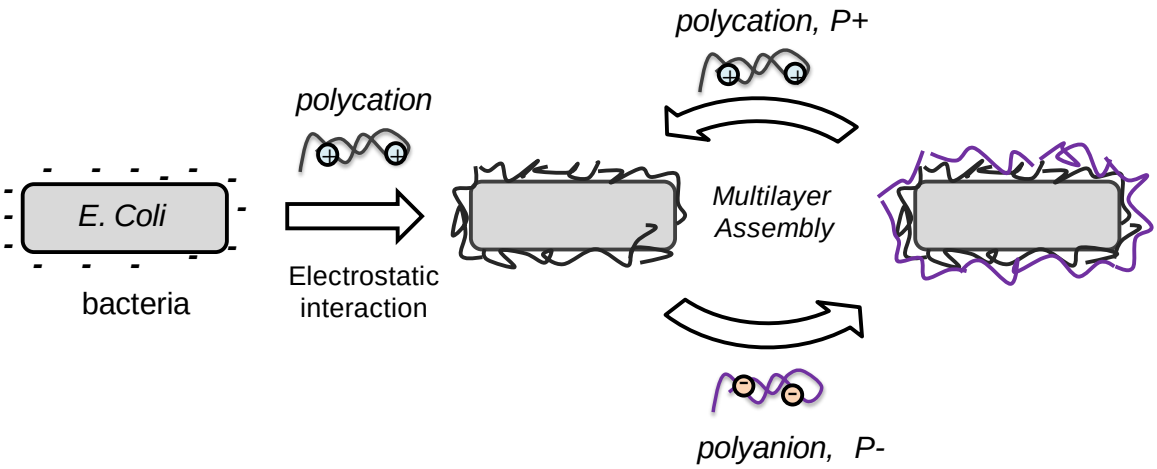
Bacterial visualization and viability were assessed using an acridine orange staining followed by fluorescence microscopy observation. 10 µL of acridine orange (0.1 % wt) was added to 100 µL of bacterial suspension (uncoated or coated bacteria). A 10 μL drop was then introduced on a cover slip to observe the bacteria.[30] Images were obtained with an optical fluorescence ZEISS microscope equipped with a CCD camera and a fluorescence illuminator and various mirror units (ZEN software).

3. Results and discussion

As presented in Figure 1, bacterial coating via LbL assembly was used to control bacterial growth (Figure 1). *E. coli* which is a Gram-negative bacterium and an important component of the normal intestinal microflora of humans, was studied in the present work. This bacterium was coated via multilayer assembly of charged polysaccharides that were oppositely charged (Figure 1A). We used chitosan/alginate and chitosan/dextran sulfate as polyelectrolyte pairs (Figure 1B).

Below we briefly investigate the growth of uncoated bacteria in LB media. Then we describe the surface modification of the bacteria, i.e. bacteria coating via two LbL processes: (i) saturation method or (ii) coating/centrifugation method (Figure S1). Then, we will study the impact of the coating on the growth and viability of the bacteria.

A)



B)

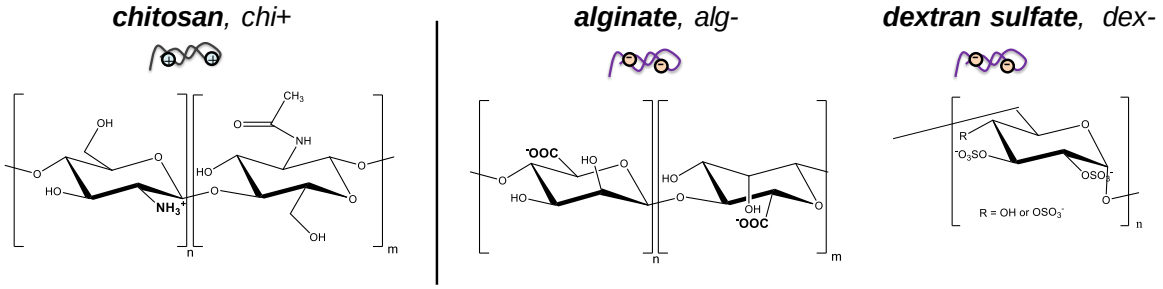


Figure 1. A) Schematic illustration of the coating with polyelectrolytes onto *Escherichia Coli* bacteria. B) Charged polysaccharides used for the coating of bacteria, i.e. the chitosan, the alginate and the dextran sulfate.

A suspension of uncoated *E. coli* bacteria was grown in LB medium in batch culture for 27 h starting from different optical densities (OD), i.e. 0.025, 0.05 and 0.75. This medium was used because it enables rapid and good growth of *E. coli.*[31] We first correlated the initial OD value with the number of bacteria by counting them in a Malassez cells. The OD values of 0.025, 0.05 and 0.75 corresponded to 9 $x10^{-4}$ bacteria/mL, 1.3 $x10^{-5}$ bacteria/mL and 1.8 $x.10^{-5}$ bacteria/mL indicating linear variation of the OD and the number of bacteria suspended in the culture media. Then, the OD value of each bacterial suspension was read periodically to monitor growth (Figure 2A). The growth curves are shown in Figure 2 B. The growth rate was 0.08 h^{-1}, 0.07 h^{-1} and 0.06 h^{-1} for suspensions with an OD of 025, 0.05 and 0.075 respectively. The four classical phases of growth were observed, i.e. lag phase (less than 30 min), exponential phase (up to 30 min to 12 h), stationary phase (12 to 22 h) and death phase (22 h to 27 h).[32] These stepwise

changes in growth rate could be correlated with the changes seen in expression profiles of genes coding for enzymes involved in nutrient assimilation or biosynthesis. [31] The growth phases distinguished in this microbial growth curve can also be defined in terms of the metabolic processes and physiological states occurring during growth, which have been directly correlated to the nutritional condition of the growth media.[33] Note that there was no impact of the initial OD i.e. no influence of the initial number of bacteria on growth in the studied conditions.

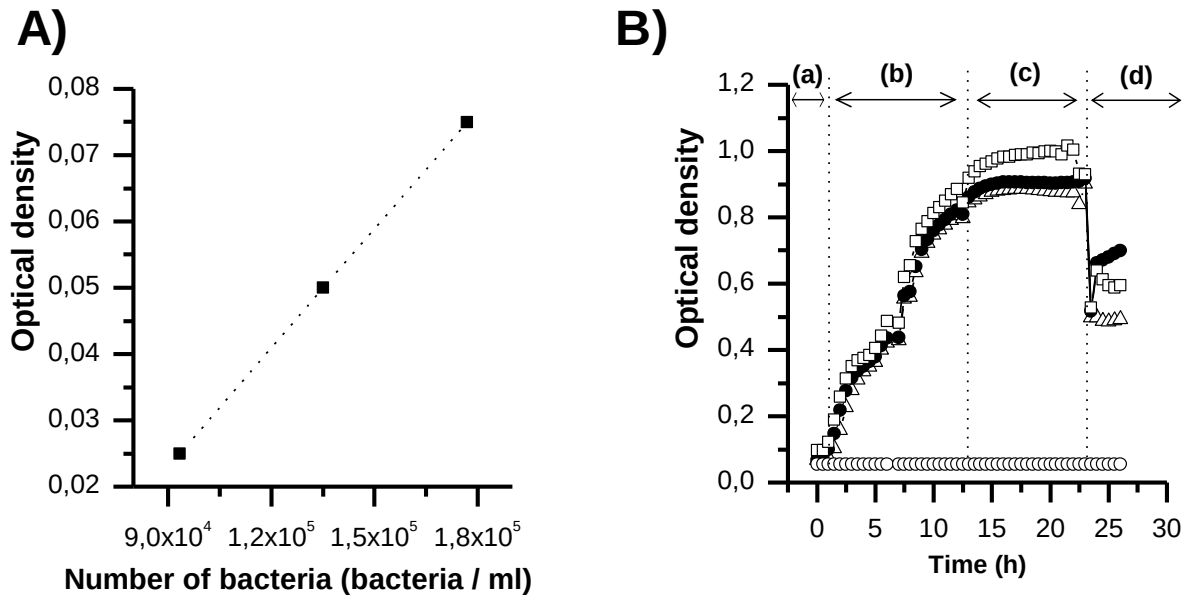


Figure 2. A) Correspondence between the optical densities of the suspension of the bacteria and the number of the bacteria determined by a Malassez cells, B) Growth of E. Coli in a LB medium at 37°C, determined by following the optical density of a suspension of bacteria according to the time and starting from a suspension of bacteria presenting an optical density at Δ 0.025, ● 0.05 and □ 0.075 (○ LB medium without bacteria)

After studying the growth of uncoated bacteria, a coating was applied and the impact of this coating on growth and viability was evaluated. Two coating processes were used. The first was a saturation method that consists of determining the required amount of each charged polysaccharide. The second one used a standard LbL process (coating/centrifugation process), in which the bacteria were immersed and mixed in a stock solution of the polycation or polyanion, with three intermediate washing steps before each polyelectrolyte adsorption.

In the studied conditions, polyelectrolyte multilayer growth was based on electrostatic interactions between carboxylate (alginate) or sulfate (dextran derivative) and ammonium (chitosan) groups.

The measurement of zeta potential is a standard technique used to demonstrate the adsorption of each polyelectrolyte layer that is oppositely via charge reversal on the surface of the substrate. The zeta potential of a suspension of bacteria was first measured. The value for *E. coli* was −17 mV indicating that the bacteria have a negatively charged surface.

We will begin by describing the coating of the surface of the bacteria via the saturation method and then by the second method, using centrifugation. We will then discuss the merits of each of these approaches.

The saturation method is an attractive option as it does not require post-processing procedures such as centrifugation and washing steps and therefore is less time-consuming. The amount of polycation or polyanion solution required to form each layer of the shell was experimentally determined by zeta-potential measurements. [8, 34] This was called the "saturation concentration". The addition of charged polysaccharides was stopped when the zeta potential of the surface of the bacteria reached a constant positive value (saturation concentration, C_{sat}) At C_{sat}, it was assumed that the amount of free polyelectrolyte in the suspension was very low/insignificant and that the surface of the bacteria was saturated by a polyelectrolyte layer. Above the C_{sat}, it was assumed that there was an excess of polyelectrolyte that was free in the bacterial suspension. It was thus crucial to estimate the saturation concentration for the polycation and the polyanion. As shown in Figure 3A, the bacterial surface was initially negatively charged (-17 mV), but the addition of the chitosan solution led to a progressive increase in the potential, which then reached a plateau. Under our conditions, the C_{sat} of chitosan was 0.12 g/L. The optimal concentration for forming the second layer was 0.005 g/L, which was correlated with a zeta potential above + 25 mV and was the optimal concentration for forming a stable first layer on the surface of the bacteria. The deposition of the negatively charged layer was done similarly. The polyanion was added to the positively charged bacteria to form a stable second layer. As shown in Figure 3B, the zeta potential progressively decreased from + 25 mV to - 17 mV to reach a negative zeta potential. The C_{sat} of alginate was 0.05 g/L. The same approach was followed using dextran sulfate. The optimal concentration for forming a second layer on the positively coated bacterial surface was 0.08 g/L (Figure S2).

Figure 4A shows the variation in the zeta potential after the adsorption of successive layers of the polycation/polyanion shell on the bacteria. The negatively charged surface was coated with the first layer of chitosan then a layer of negatively charged alginate and then a final layer of chitosan until the required number of layers had been adsorbed. The concentration used to form each polyelectrolyte layer corresponded to the saturation concentration previously determined. Regular layer-to-layer variation in the zeta potential was demonstrated, ranging from +25 mV to −17 mV for chitosan and alginate polyelectrolytes pairs. Coating with chitosan and dextran sulfate polyelectrolyte pairs was successful, as illustrated by the charge reversal after each charged polysaccharide had been adsorbed (Figure S3).

In the second method used for coating, the bacteria were immersed alternatively in solutions of the polycation and then of the polyanion, with three intermediate washing steps (centrifugation/redispersion in the buffer). The zeta potential (Figure 4B, Figure S3) started at -17 mV for a suspension of bare bacteria and then increased to around -2 mV after exposing the bacteria to a solution of chitosan. These results show that the first chitosan layer was unable to overcompensate for the negative starting charge of the bare (uncoated) bacterial surface. This incomplete charge overcompensation after adsorption of the charged polysaccharide resulted in aggregation of the bacteria coated using the centrifugation method. The polyanion layer was then added and alternating zeta-potential values were observed during the LbL process. This was a good indicator of the multilayer film growth on the surface of the bacteria, but the "saturation method" seems to be more suitable as there was no problem with charge overcompensation.

The bacteria were stained with acridine orange (AO) to visualize them. [35] Acridine orange is a cell-permeant nucleic acid binding stain that can be used both to visualize the bacteria and to determine if they are dead or alive because it emits red fluorescence in dead bacteria and green fluorescence in living bacteria. Green fluorescence was seen after staining bare bacteria, and LbL-coated bacteria (bacteria coated with polyanion-ended shells made of chitosan/polyanion), following both methods (Figure 5, Figure S4). Thus the bacteria were alive even after being coated/encapsulated with the charged polysaccharide shells.

We observed that bacteria coated with polycation-ended shells have a stronger impact on the bacteria viability, i.e. led in the most of the case to the death of the bacteria. Polyamines are well-known to be cytotoxic because they induce destructuring interaction with the cell membrane and/or interact with other polyanion component of the cells. Adsorption of a supplementary polyanionic layer on the shell of the positively charged bacteria leads to a

reversal of the situation, with the addition of an excess of innocuous negatively charged carboxylic acid or sulfate groups. In the work that follows, we will refer to bacteria coated with polyanion-ended shells made of chitosan/polyanion.

Staining also showed that as expected the bare bacteria were well suspended, but coating the bacteria using the centrifugation process led to aggregated bacteria, unlike coating via the saturation method. In the latter case, the coated bacteria were non aggregated. Although not often acknowledged, aggregation is a common issue for the LbL coating of living cells via the centrifugation process.[36, 37]

Our results were consistent with those of Jonas et al.[37] They demonstrated that coating *Staphylococcus epidermidis* bacteria with the following polyelectrolyte pairs (poly(styrene sulfonate) / poly-(allylamine hydrochloride))$_n$ or (chitosan/alginate)$_n$ via the LbL process using centrifugation led to aggregated bacteria. In comparison with Jonas et al.'s work, we coated bacteria with a different shape and bacterial cell envelope. Jonas et al. coated *Staphylococcus epidermidis*, which is a spherical gram-positive bacterium. Herein, we coated *E. coli*, which is a rod-shaped gram-negative bacterium and proposed another method to coat the bacteria to avoid aggregation phenomenon. They explained that the problem of incomplete charge overcompensation after the adsorption of the first polycation onto the surface of the bacteria. Subsequently, the driving force for the adsorption of the second layer (the polyanionic polysaccharide) is mainly hydrogen bonding. To overcome this problem of aggregation, Jonas et al.[37] developed another method to coat bacteria, still using the LbL process but involving the agarose gel route in which aggregation is avoided and supernatant removal is made easier. This process was modified from a method initially proposed by Richardson et al.,[38] in which colloids to be coated are embedded in a block of agarose gel. In our study, we then suggest another route, i.e. "the saturation method" which also avoids aggregation. This is the first time that this process has been used for the coating of bacteria, and it is of particular interest as the direct LbL saturation method does not require washing or purification steps.

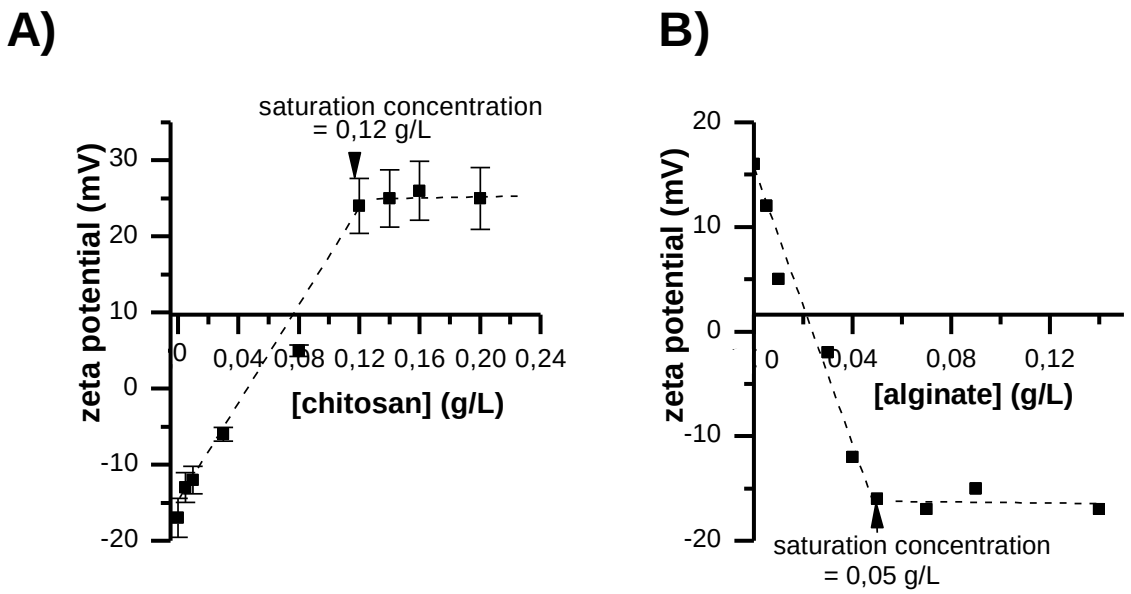


Figure 3. Determination of the saturation concentration over zeta potential measurements to coat the surface of E. coli with A) chitosan and B) alginate.

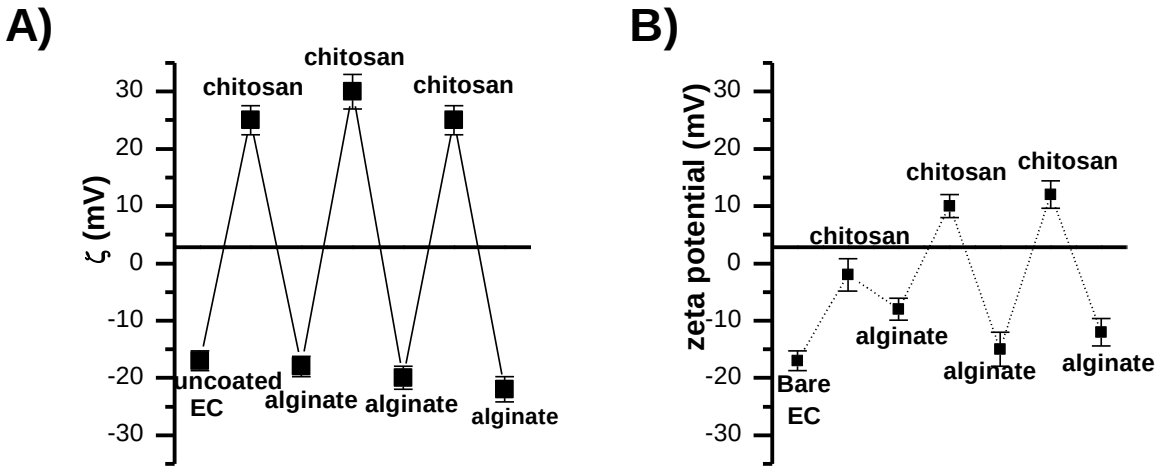


Figure 4. Zeta potential of the suspension of E coli bacteria after the adsorption of the charged polysaccharide (chitosan/ alginate) for: A) the saturation process and B) the coating/centrifugation process.

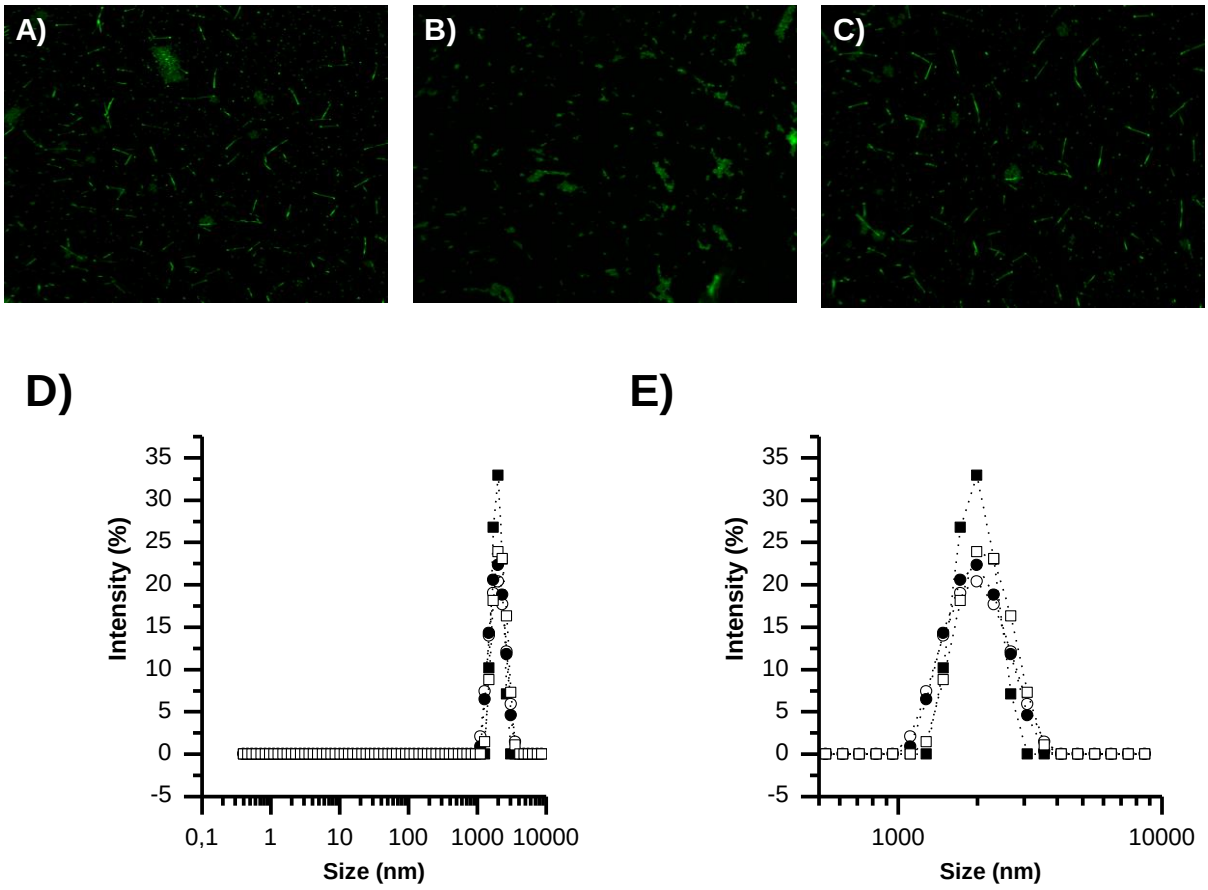


Figure 5. *E. coli* stained with acridine orange, A) bare bacteria, layer-by-layer coated bacteria (EC-(chi+/dex$^-$)n) B) using the centrifugation process, C) using the saturation method (magnification: x200), E) DLS result obtained from a suspension of bare E. Coli (curve (■)) and LbL coated bacteria (bacteria coated with polyanion-ended shells made of (chi+/dex$^-$), curve (□): one (chi+/dex$^-$) bilayer ; curve (●): two (chi+/dex$^-$) bilayers; curve (○): two (chi+/dex$^-$) bilayers) via the saturation process and E) zoom-in of graph E.

Using bacteria coated via the saturation process, we then studied the impact of coating on bacterial growth in the LB medium. According to the DLS results presented in Figure 5D, the size of the bare bacteria was around 2 µm, which is consistent with the size reported in the literature. [39] The DLS confirmed the aggregation phenomena due to the bacteria coating via the coating/centrifugation process (Figure S5). We also found that coating the bacteria with different numbers of charged polysaccharide layers had no effect on their size. This was expected as the bacteria were not aggregated (demonstrated previously via AO staining) and the multilayer shell was only expected to be around a nanometer in thickness.

The kinetics of bacterial growth was monitored in LB medium via the variation in optical density (OD) versus time. The influence of the number of bacteria was studied. Each assay was replicated eight times. Typical growth curves are shown in Figure 6 for the bare bacteria. The classical phases of growth were seen, i.e. lag phase (less than 30 min), exponential phase (up to 30 min to 12 h) and stationary phase (12 to 22 h). However, we observed a longer lag time for the growth of E. coli coated with the charged polysaccharide pair than for uncoated bacteria. After the lag time which increased with the number of bilayers, the bacteria entered the classical growth phases. The lag time was defined on the optical density curve as the time corresponding to the intercept of the baseline with the line of the highest slope in the exponential growth phase. Prior to these experiments, we checked that the lag times were not dependent on the starting OD representing bacterial concentration. As shown in Figure 3, starting from an OD of 0.025 or 0.05 or even 0.075 had no effect on the lag time. Thus, the observed lag time in Figure 6 could only be due to the bacterial coating and more precisely was found to depend on the number of bilayers. Indeed, increasing the number of bilayers from 1 to 3 increased the lag time as follows: 2.3 h, 2.5 h and 9 h respectively. Note that the same result was obtained no matter the polyanion used (alginate or dextran sulfate). We can explain the differences in lag times between bare and coated bacteria based on Jonas *et al.'s* work [37]. They coated *S. epidermidis* via the LbL method combined with agarose. The lag time reflects a purely physical attributes, i.e. the presence of the shell on the bacteria may induce ~~a~~ the delayed diffusion of nutrients through the shell to the bacteria. This may explain why the lag time increased with the number of layers. The LbL process was performed in physiological solutions and under these conditions the viability of cells was preserved, as shown via AO staining. In Jonas et al's work,[37] the lag times was attributed to a bacteriostatic or bactericidal effect of the shell involving paired and unpaired amine/ammonium groups (from the chitosan), which affected the nature of the last adsorbed layer. It is interesting that the lag times seen in the present work due to the coating of living organisms were also observed in germination delays, *e.g.* for yeast (e.g. *B. coagulans*) coated with glycol chitosan and alginate. The lag time for the germination of this yeast when coated with three bilayers of glycol chitosan/alginate was 15 h.[4]

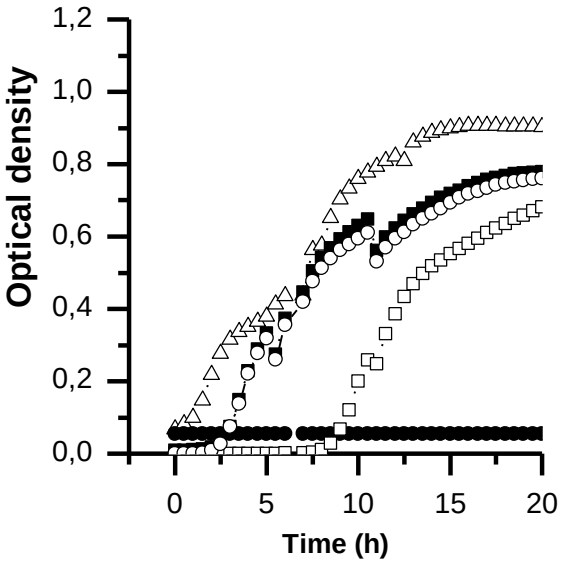


Figure 6. Growth of *E. Coli* in a LB medium at 37°C, determined by following the optical density of a suspension of bacteria according to the time and starting from a suspension of bacteria presenting an optical density at 0.05 and from Δ bare bacteria and coated bacteria with polyanion-ended shells of (■ $(chi^{+}/ alg^{-})_1$, ○$(chi^{+}/ alg^{-})_2$ and □ $(chi^{+}/ alg^{-})_3$). Note the reference is the LB medium without bacteria, symbolised by ●

After showing the impact of the LbL coating on bacterial growth, bacterial viability was also assessed. Acridine orange staining was used to distinguish between dead and live bacteria. At the end of the assay, a suspension of bacteria was stained with AO.[35] Whatever the conditions used (starting from bare or coated bacteria), green fluorescence was seen after staining the bacteria. This shows that the bacterial cells were alive.

4. Conclusion

The LbL process can be used to control the growth of bacteria, based on the bacteriostatic effect of oppositely charged paired polysaccharides. We used two LbL methods to coat *E. coli* bacteria, i.e. i) saturation method and ii) classical coating/centrifugation method. For both approaches, we demonstrated each step of polyelectrolyte adsorption onto the bacteria walls via zeta potential measurements and then showed the resulting charge-charge interaction. The saturation method led to non-aggregated coated bacteria, whereas the bacteria became

aggregated in the centrifugation method. The first method appears to be more appropriate for controlling the bacteriostatic effect.

Interestingly, multilayer coating delayed bacterial growth, which thus could be controlled according to the number of coated bacteria with polyanion-ended shells. Moreover, within the range of layers used for the coating of the bacteria, there was no significant impact of coating on nutrient diffusion, as growth resumed later (coating only slowed it down). Thus, a large number of adsorbed polyelectrolytes does not seem to be required to delay bacterial growth as a few layers are sufficient to achieve this effect. The lag times reported in our study could be sufficient to prevent bacterial proliferation in standard applications.

Considering the low number of polyelectrolyte layers required, the simplicity of the saturation method for coating the bacterial wall via the LbL process, and the wide availability of charged polysaccharides, our strategy to encapsulate bacteria appears to be a promising approach.

Disclosure of potential conflict of interest: none

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Figures Caption

Figure 1. A) Schematic illustration of the coating with polyelectrolytes onto Escherichia Coli bacteria. B) Charged polysaccharides used for the coating of bacteria, i.e. the chitosan, the alginate and the dextran sulfate.

Figure 2. A) Correspondence between the optical densities of the suspension of the bacteria and the number of the bacteria determined by a Malassez cells, B) Growth of E. Coli in a LB medium at 37°C, determined by following the optical density of a suspension of bacteria according to the time and starting from a suspension of bacteria presenting an optical density at Δ 0.025, ● 0.05 and □ 0.075 (○ LB medium without bacteria)

Figure 3. Determination of the saturation concentration over zeta potential measurements to coat the surface of E. coli with A) chitosan and B) alginate.

Figure 4. Zeta potential of the suspension of E coli bacteria after the adsorption of the charged polysaccharide (chitosan/ alginate) for: A) the saturation process and B) the coating/centrifugation process.

Figure 5. *E. coli* stained with acridine orange, A) bare bacteria, layer-by-layer coated bacteria (EC-(chi+/dex$^-$)n) B) using the centrifugation process, C) using the saturation method (magnification: x200), E) DLS result obtained from a suspension of bare E. Coli (curve (■)) and LbL coated bacteria (bacteria coated with polyanion-ended shells made of (chi+/dex$^-$), curve (□): one (chi+/dex$^-$) bilayer ; curve (●): two (chi+/dex$^-$) bilayers; curve (○): two (chi+/dex$^-$) bilayers) via the saturation process and E) zoom-in of graph E.

Figure 6. Growth of *E. Coli* in a LB medium at 37°C, determined by following the optical density of a suspension of bacteria according to the time and starting from a suspension of bacteria presenting an optical density at 0.05 and from Δ bare bacteria and coated bacteria coated with polyanion-ended shells of (■ $(chi^+/ alg^-)_1$, ○$(chi^+/ alg^-)_2$ and □ $(chi^+/ alg^-)_3$). Note the reference is the LB medium without bacteria, symbolised by ●

Figure S1. Schematic illustration of A) the "saturation method" and B) “coating/centrifugation process”

Figure S2. Determination of the saturation concentration of dextran sulfate over zeta potential measurements to coat the surface of E. coli coated with chitosan

Figure S3. Zeta potential of the suspension of E coli bacteria after the adsorption of the charged polysaccharide (chitosan/ dextrane sulfate) for: A) the saturation process and B) the coating/centrifugation process.

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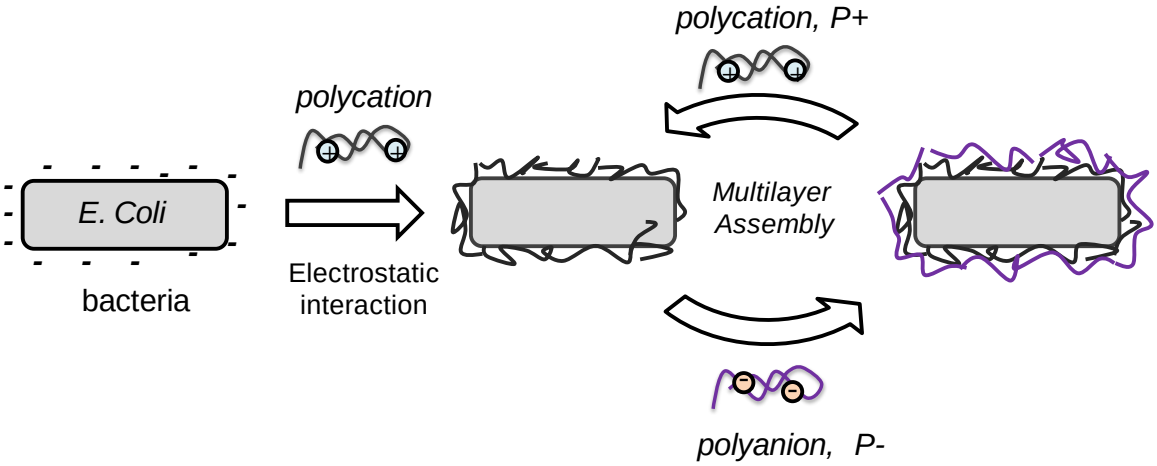
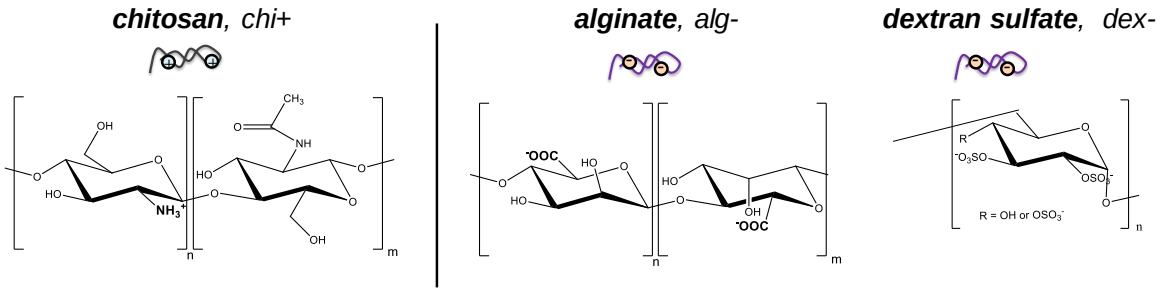
Figure S5. DLS result obtained from a suspension of LbL coated bacteria (chitosan/alginate)$_2$ via the "coating/centrifugation process.

A)



B)



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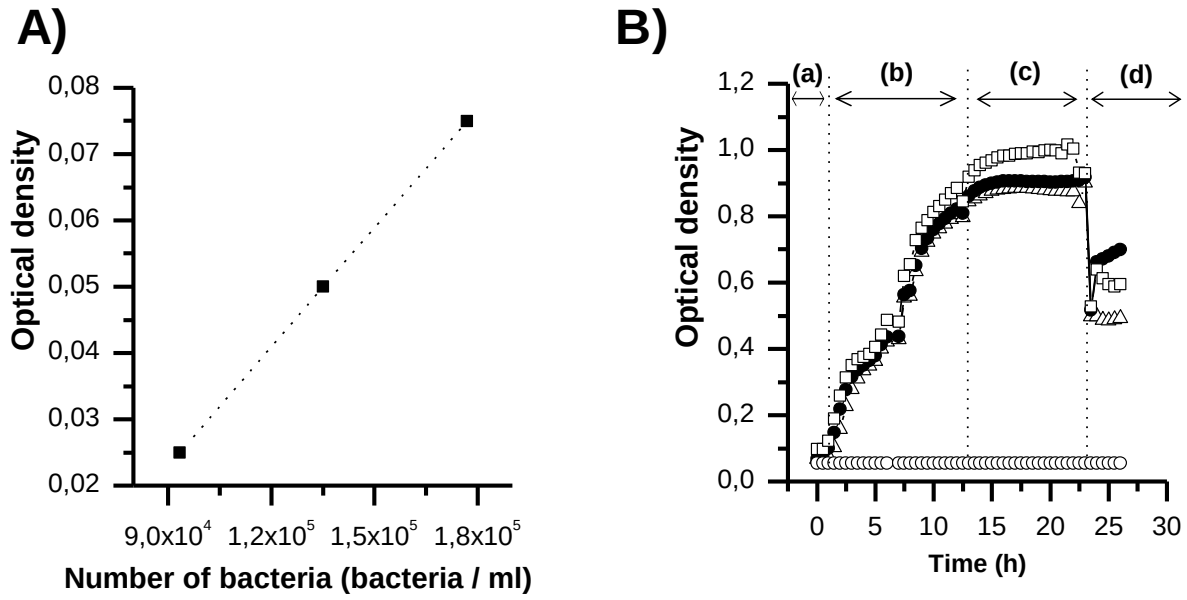

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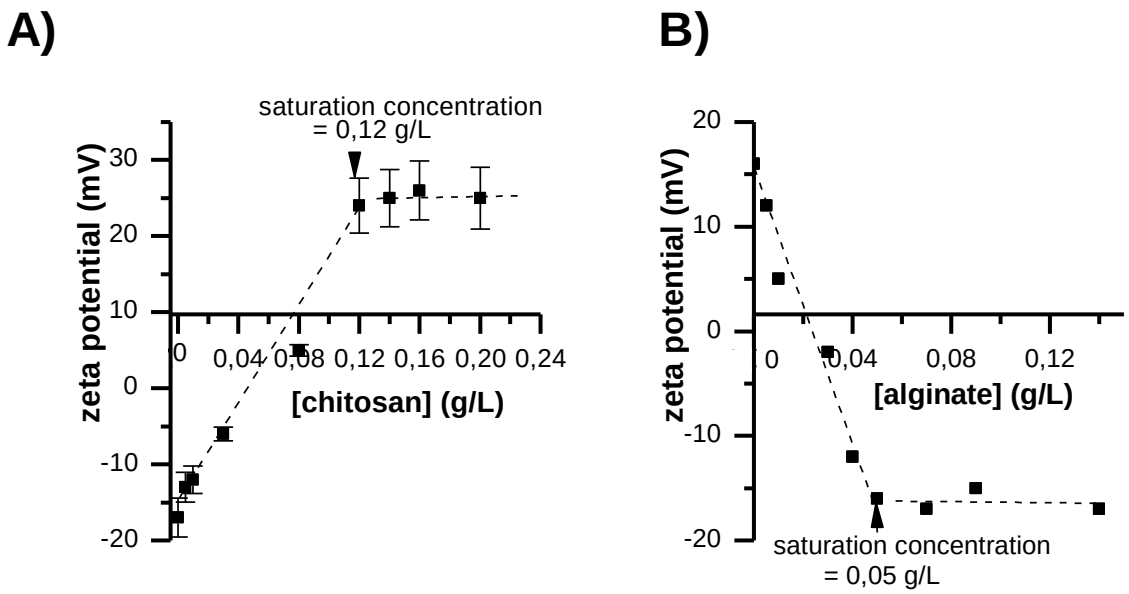

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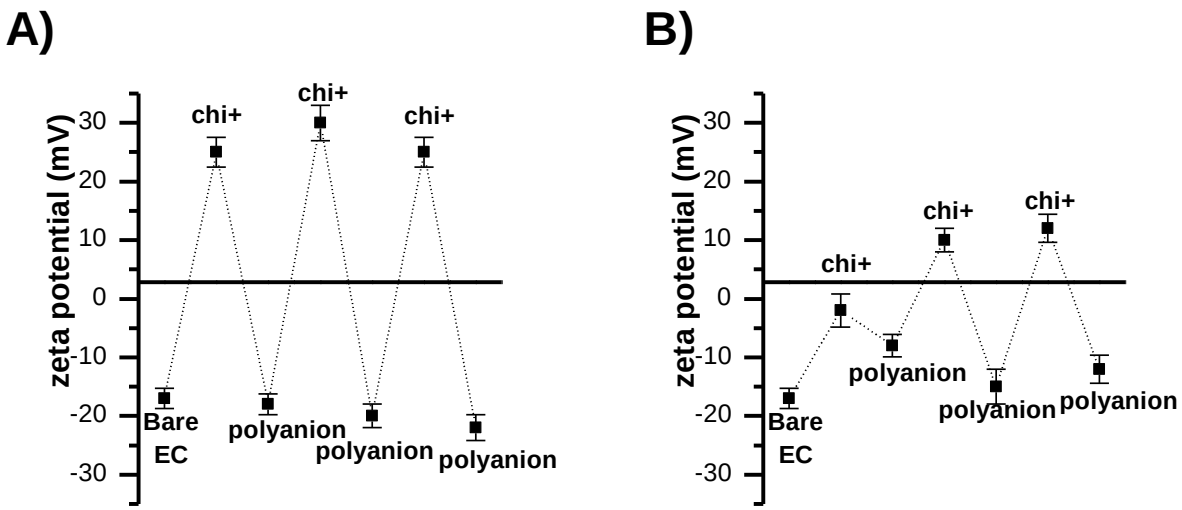

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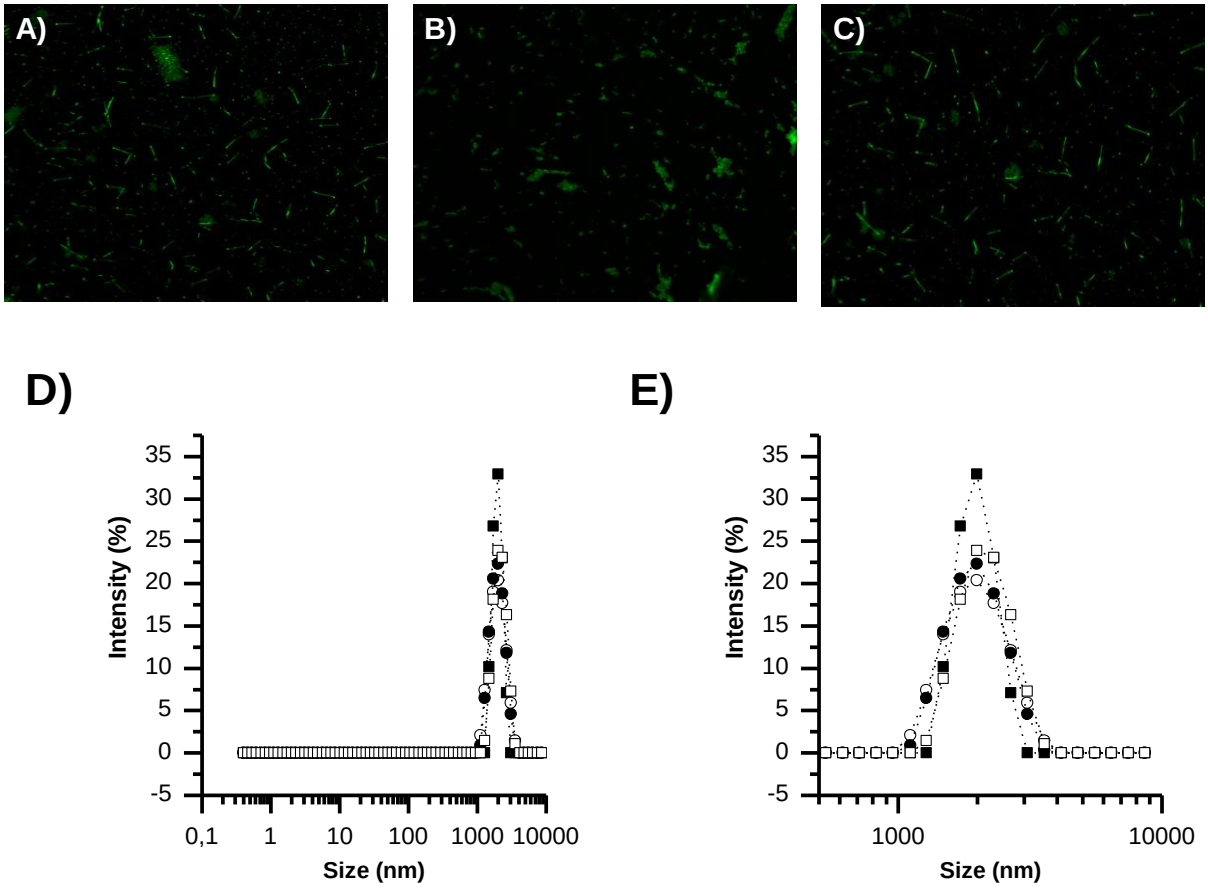

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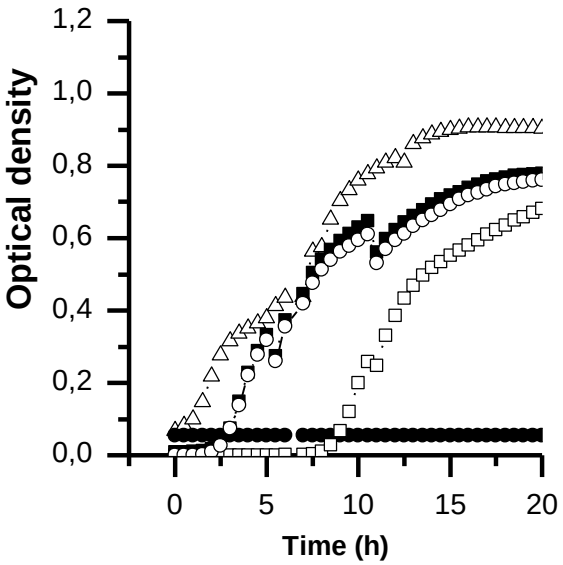

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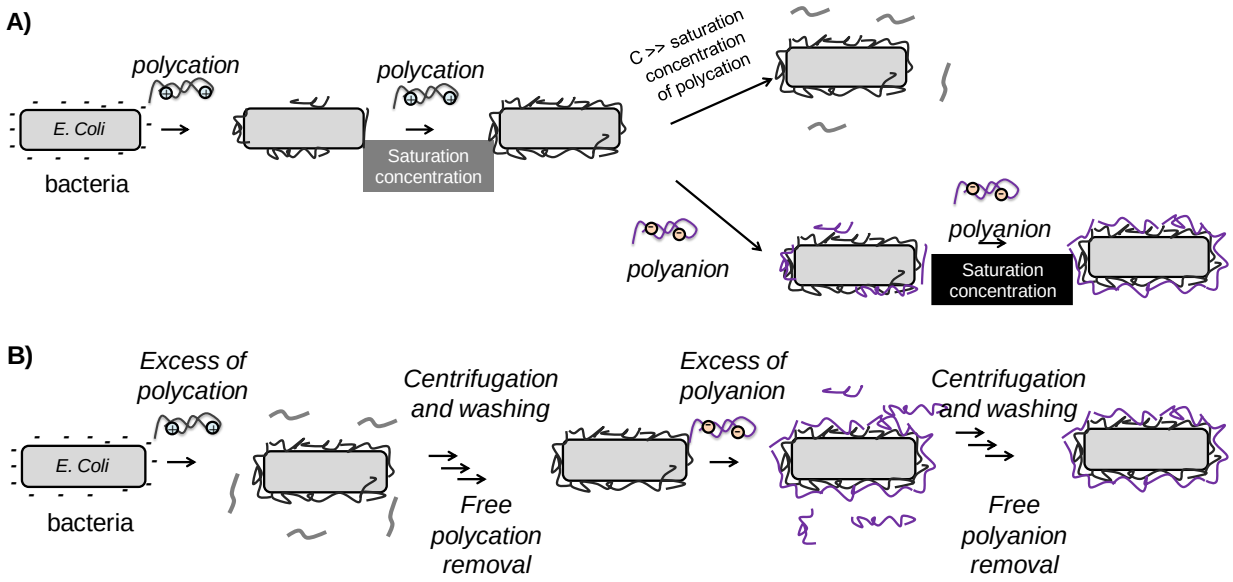
Supporting information



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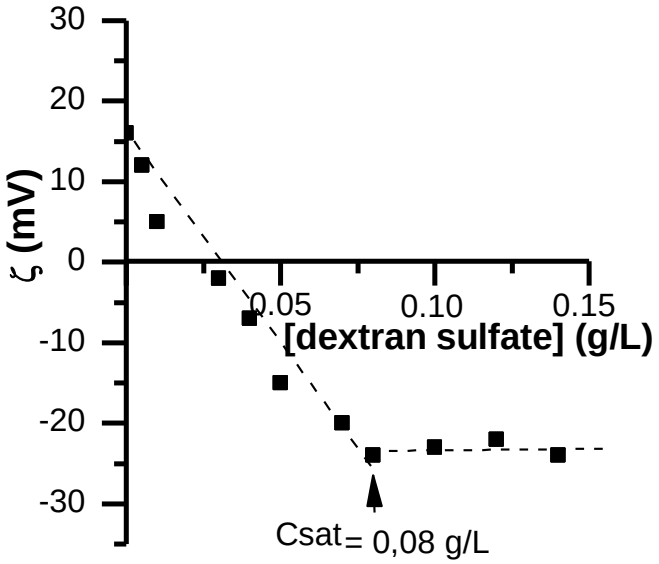

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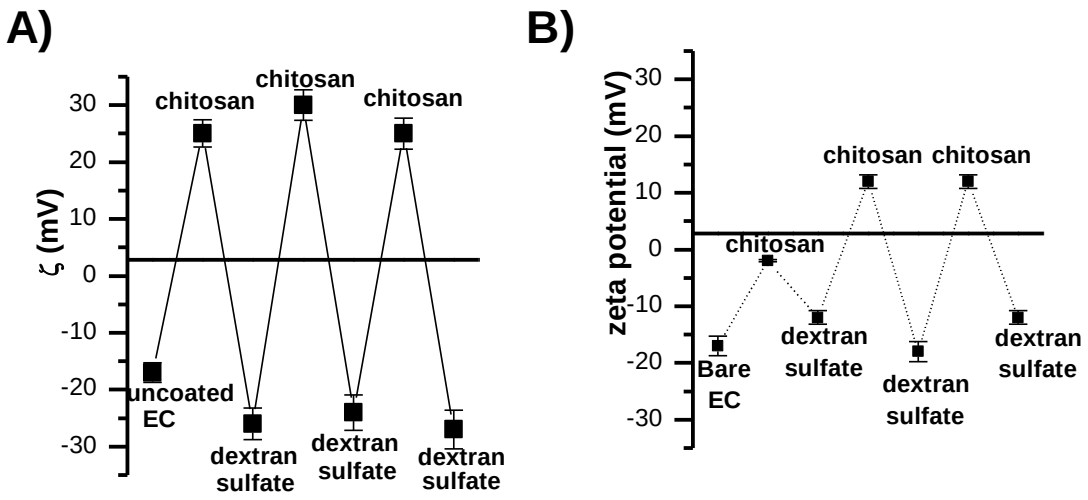

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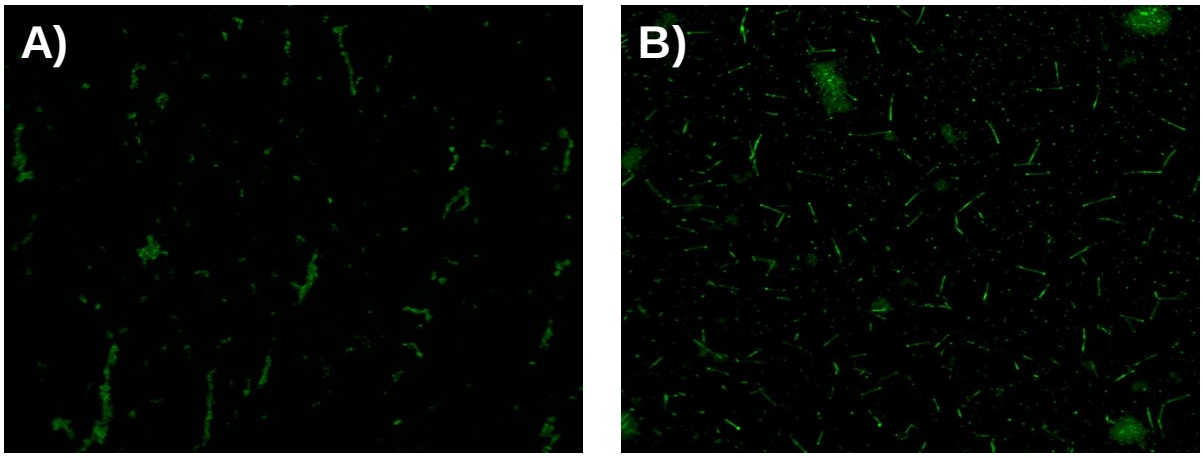

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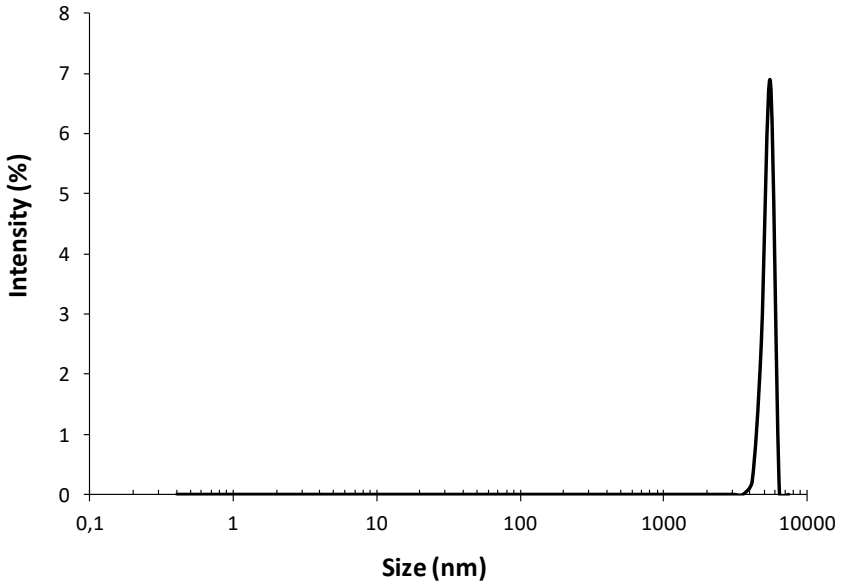

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