Mestrado em Processos Químicos e Biológicos

Study of the effect of natural compounds and metal nanoparticles on microorganisms growing in suspension and biofilm

Relatório de Estágio apresentado para a obtenção do grau de Mestre em Processos Químicos e Biológicos

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RESUMO

O corrente trabalho, realizado na *Vysoká Škola Chemicko-Technologická v Praze*, teve como objetivo a investigação, estudo e desenvolvimento de uma sequência de métodos, de forma a avaliar a eficácia antimicrobiana, tanto em suspensão como em biofilme, de nano partículas de prata e ouro, em duas estirpes de *Pseudomonas aeruginosa* (*P. aeruginosa* DBM 3777 - PA 3777 e *P. aeruginosa* DBM 3081 - PA 3081). A *P. aeruginosa* é uma bactéria Gram-negativa, baciliforme e aeróbia, conhecida por ser um patogénico oportunista, resistente a um grande número de antibióticos e antissépticos. O estudo do seu crescimento em biofilme, definido como uma matriz biologicamente ativa de células microbianas contendo substâncias poliméricas extracelulares, tem especial relevância quando estas bactérias se podem proteger no interior do mesmo. Uma vez que estas estruturas podem aderir a diversas superfícies, protegendo as bactérias de ameaças externas, são de especial preocupação para hospitais, indústrias e famílias, para os quais implicam um risco elevado para a saúde.

As nanopartículas são partículas entre 1 e 100 nanómetros de diâmetro, com uma camada circundante interfacial e possuindo uma elevada área de contacto, o que as torna mais reativas a outras moléculas. Embora estejam associadas à indústria moderna, são usadas já há vários séculos e, presentemente, encontram-se presentes numa panóplia diversa de produtos e indústrias. O nível de toxicidade das nanopartículas depende das suas propriedades físico-químicas, composição e das espécies bacterianas. De facto, sabe-se que as nanopartículas afetam bactérias diversas, de diferentes maneiras e por mecanismos ainda desconhecidos, pelo que a pesquisa dos seus efeitos é ainda bastante atual.

No decurso deste estudo foi realizada, em primeiro lugar, uma análise ao crescimento em suspensão das duas espécies em estudo, para investigar a eficiência das nanopartículas. Para este efeito foi utilizada a densidade ótica ($OD_{420\text{-}580nm}$) de uma suspensão que permite aferir da concentração de microrganismos no meio de crescimento. Os resultados obtidos foram de acordo com o esperado. As nano partículas de prata e ouro demonstraram a sua eficácia, reduzindo ou eliminando completamente a população bacteriana presente no meio de crescimento em função da sua concentração. Ainda dentro desta experiência, foi possível observar que a *P. aeruginosa* 3081 revelou ser mais resistente ao efeito tóxico de ambas as nanopartículas, do que a *P. aeruginosa* 3777.

Com estes primeiros resultados, comprovando as capacidades antimicrobianas das nanopartículas, foi possível avançar na investigação ao nível da formação do biofilme. Com este intuito foram realizados mais estudos complementares: determinação da concentração da biomassa no meio de crescimento após 24 horas (pela densidade ótica - OD a 600 nm) para efeitos de normalização de resultados, quantidade de indutores produzidos (pelo método de HSL), quantificação da formação de biofilme pelos métodos de confluência de células (CC) e cristal violeta (CV), e atividade metabólica (pelo método de MTT). Os dois primeiros estudos reportaram-se ao estudo no sobrenadante, e os últimos três no biofilme.

A concentração das nanopartículas usadas foi diferente para cada caso, uma vez que a prata mostrou ser mais eficiente do que o ouro, mesmo a concentrações mais baixas. Deste modo, as concentrações utilizadas para as nanopartículas de prata foram 0, 10, 20, 30, 40 e 50 mg / L e para as nanopartículas de ouro foram 0, 40, 50, 80, 120 e 140 mg / L. Foi ainda necessário efetuar uma correção, recorrendo ao fator de diluição, devido ao facto de a concentração inicial de *P. aeruginosa* ser diferente para cada ensaio.

O método de HSL permite estimar a quantidade de indutores produzidos pelas células face à toxidade induzida pelas nanopartículas, através da regulação da expressão de determinados genes, usando como biossensor a bactéria *A. tumefaciens*. Como resultado, foi possível observar que a estirpe PA 3777 produziu uma maior quantidade de indutores do que a PA 3081, quer em termos absolutos quer por biomassa suspensa. Verificou-se ainda uma maior produção de indutores em resultado da presença das nanopartículas de prata (para concentrações similares às de ouro). Contudo, a capacidade para aderir ao fundo dos poços, verificada pela biomassa aderida, foi similar para ambas as bactérias, sugerindo que o uso dos autoindutores testados não serve apenas para promover a adesão das bactérias.

A formação do biofilme foi estudada pelo método da confluência de células (CC), que determina a percentagem de área do poço ocupado por células aderidas e pelo método do cristal violeta (CV), que permite estimar o volume (ou massa) de biofilme aderido. Os resultados destes dois métodos apresentaram resultados divergentes no tocante à formação de biofilme pelas duas estirpes em estudo. Assim, enquanto que o método CC indiciou uma maior formação de área de biofilme por parte da estirpe PA 3081, o método CV indiciou o oposto, com a colorimetria a indicar uma forte tendência para a produção de uma quantidade maior de biofilme por parte da PA 3777. Saliente-se, contudo que as diferenças metodológicas entre estes dois métodos proporcionam este tipo de discordância entre os dois métodos. Por outro lado, e para ambas as estirpes, verificou-se um efeito maior na prevenção da formação de biofilme em resultado da presença das nanopartículas de prata (para concentrações similares às de ouro).

O método do MTT é um procedimento colorimétrico usado para aferir a atividade metabólica das células, baseando-se na conversão de MTT, em cristais de fomazano pelo sistema de desidrogenases das células vivas, permitindo avaliar, até um certo grau, viabilidade celular. Analisando os resultados obtidos, foi possível observar que a atividade metabólica, principalmente em termos absolutos foi superior para a estirpe PA 3777 do que para a estirpe PA 3081. Por outro lado, a ação das nanopartículas de prata (para concentrações similares às de ouro) revelou ser mais eficaz em termos da diminuição da atividade metabólica do biofilme em valores absolutos, mas menor quando normalizada pela biomassa aderida (em termos de CC).

Com os ensaios efetuados, foi possível, até certo ponto, comprovar a eficiência das nanopartículas sobre estas duas estirpes de *P. aeruginosa*. Os resultados neles obtidos, conjuntamente com a literatura apresentada, permitiram um estudo detalhado do efeito das nanopartículas de prata e de ouro, tanto em suspensão como em biofilme, corroborando a importância das nanopartículas para o mundo moderno e a razão pela qual se deve continuar a investir nesta área. Este trabalho pode ser aproveitado e desenvolvido de forma que a análise seja ainda mais completa, alargando o âmbito das experiências realizadas, incluindo a análise a outros microrganismos e diferentes nanopartículas.

Palavras-chave: Nano partículas, Biofilme, *Pseudomonas aeruginosa*, Inibição de crescimento.

ABSTRACT

This report, carried out in Vysoká Škola Chemicko-Technologická v Praze, aimed at the investigation, study and development of a sequence of methods, in order to evaluate the antimicrobial efficacy, both in suspension and biofilm, of silver and gold nanoparticles, for two *Pseudomonas aeruginosa* strains (*P. aeruginosa* DBM 3777 - PA 3777, and *P. aeruginosa* DBM 3081 - PA 3081). *P. aeruginosa* is a Gram-negative, bacilliform and aerobic bacterium known to be an opportunistic pathogen resistant to a large number of antibiotics and antiseptics. Biofilm (defined as a biologically active matrix of microbial cells embedded in extracellular polymeric substances) growth study has particular relevance due to the fact that bacteria can be protected inside them. Given that these structures can adhere to various surfaces, protecting bacteria from external threats, being of special concern for hospitals, industry and households, which imply a greater health risk

Nanoparticles are particles between 1 and 100 nanometers in diameter, with a surrounding interfacial layer and a high contact area, which makes them more reactive to other molecules. And, although they are associated with modern industry, they have been used for several centuries and, today, nanoparticles are present in a panoply of products and industries. The toxicity level of nanoparticles depends on their physicochemical properties, composition and bacterial species. In fact, it is known that nanoparticles affect various bacteria, in different ways and using still unknown mechanisms, thus leading to be under investigation until the present day.

In the present study, a first bacterial suspension growth analysis was performed to investigate the efficiency of the nanoparticles. To that effect, the optical density ($OD_{420\text{-}580nm}$) of the suspension was used to determine the microbial concentration in the bulk medium. The results were in agreement with the expected: Silver and gold nanoparticles demonstrated their effectiveness by reducing or eliminating the bulk bacterial population in result of their concentrations. Still within this experiment, it was possible to observe that *P. aeruginosa* 3081 was more resistant to the toxic effect of both nanoparticles, than *P. aeruginosa* 3777.

With these first results, proving the antimicrobial capabilities of nanoparticles, it was possible to advance in the investigation. Therefore, the following methods were performed: bulk biomass determination after 24 hours (by measuring the optical density - OD at 600 nm) for biomass normalization; produced inducers (by the HSL method); biofilm formation by the cell confluence (CC) and crystal violet (CV) methods; and metabolic activity by the MTT method. The first two methods address to the supernatant whereas the last three address the biofilm.

The concentration of the employed nanoparticles was different for each case, since silver proved to be more efficient than gold, for the bacterial suspension growth analysis experiment, even at lower concentrations. Thus, the concentrations used for the silver nanoparticles were 0, 10, 20, 30, 40 and 50 mg/L and for the gold nanoparticles were 0, 40, 50, 80, 120 and 140 mg/L. Given that the initial *P. aeruginosa* concentration was different in each case, it was necessary to make a correction using the dilution factor.

The HSL method allows to estimate the amount of autoinducers the cells produced to face the toxicity induced by the studied nanoparticles, through gene expression regulation of particular genes, using the bacterium *A. tumefaciens* as biosensor. As a result, it was possible to observe that the PA 3777 strain produced a larger amount of inductors than the PA 3081 strain, both in terms of absolute values and normalized by the suspended biomass. Furthermore, a larger production of inducers was observable as a result of the presence of the silver nanoparticles (for similar gold NPs concentrations). However, the ability to adhere to the bottom of the wells, verified by the adhered biomass, was similar for both bacteria, suggesting that the use of the tested autoinducers does not only serve to promote the adhesion of the bacteria.

The biofilm formation was studied by the cell confluence (CC) method, which determines the percentage of area of the well occupied by adhered cells and by the crystal violet (CV) method, estimating the volume (or mass) of adhered biofilm. The results of these two methods presented divergent results regarding the biofilm formation by the two strains under study. Thus, whereas the CC method indicated a higher biofilm surface formation by PA 3081 strain, the CV method indicated the opposite, with the colorimetric method pointing towards a strong tendency for the production of a larger biofilm by PA 3777. It should be noted, however, that the methodological differences between these two methods may account for such disagreements between the two methods. On the other hand, for both strains, there was a greater effect on the prevention of biofilm formation as a result of the presence of silver nanoparticles (for concentrations similar to gold NPs).

The MTT method is a colorimetric procedure used to measure the metabolic activity of cells, based on the conversion of MTT to fomazan crystals by the living cells dehydrogenase system, allowing to evaluate, to a certain extent, cell viability. Analyzing the obtained results, it was possible to observe that the metabolic activity, mainly in absolute terms, was higher for the PA 3777 strain than for the PA 3081 strain. On the other hand, the action of the silver nanoparticles (for concentrations similar to gold NPs) were found to be more effective in terms of decreasing the biofilm metabolic activity in absolute values, but lower when normalized by the adhered biomass (in terms of CC).

With the tests carried out in this report, it was possible to prove, to some extent the efficiency of the nanoparticles on these two *P. aeruginosa* strains. The results obtained, combined with the presented literature, allowed to make a detailed study of the effect of nanoparticles, both in suspension and in biofilm, corroborating the importance of nanoparticles to the modern world and why one should continue to invest in this area. This work can be harnessed and developed, in order to further complete the presented experiments, including the analysis to other microorganisms and different nanoparticles.

Keywords: Nanoparticles, Biofilm, *Pseudomonas aeruginosa,* Growth inhibition

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GLOSSARY

AB	Agrobacterium
Ag	Silver
AHL	Acyl-homoserine lactones
AI-1	Auto-inducer 1
AI-2	Auto-inducer 2
AIP	Auto-inducer peptides
ATRP	Atom transfer radical polymerization
Au	Gold
CFU	Colony-forming units
CLSM	Confocal laser scanning microscopy
CRA	Congo Red agar
CV	Crystal violet
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPD	4,5-dihydroxy-2,3-pentanedione
EPS	Extracellular polymeric substances
HSL	Homoserine lactone ring
LB	Luria Bertani
LDH	*Lactate dehydrogenase*
LPS	Lipopolysaccharides
MIC	Minimum inhibitory concentration
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NADPH	Nicotinamide adenine dinucleotide phosphate
nm	Nanometers
NPs	Nanoparticles
OD	Optical density
PA	*Pseudomonas aeruginosa*
PAH	Poly-allylamine hydrochloride
PBS	Phosphate-buffered saline
PEG	Poly ethylene glycol

pOEGMA	Poly(oligo(ethylene glycol) methyl ether methacrylate)
pSBMA	Zwitterionic poly-sulfobetaine methacrylate
QS	Quorum sensing
ROS	Reactive oxygen species
TCP	Tissue Culture Plate
TCSTS	Two-component signal transduction system
WWTP	Wastewater treatment plants
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

1. INTRODUCTION

In the scope of the Masters in Chemical and Biological Processes, the student has the opportunity to undertake an internship or to develop a final course project/dissertation. Having the preference for an internship, and preferably abroad, I applied to an internship at *Vysoká Škola Chemicko-Technologická v Praze* (*VŠCHT Praha*), or University of Chemistry and Technology, Prague, more specifically at the Biotechnology Department, having been accepted by the University. This resulted in a partnership between the Instituto Superior de Engenharia de Coimbra (ISEC) and the *VŠCHT Praha*, with the aim of placing the student under a laboratory environment, not only to do his project, but also with the responsibility to help others in their projects. The internship lasted for 10 months, starting in October 2015 and ending in June 2016.

The internship had as main objectives to investigate, study and develop a sequence of methods to evaluate the antimicrobial efficacy, both for suspension and biofilm growth, in LB growth media, of silver and gold nanoparticles for two strains of *Pseudomonas aeruginosa* (*P. aeruginosa* DBM 3777 (PA 3777) and *P. aeruginosa* DBM 3081 (PA 3081)), a Gram-negative, bacilliform and aerobic bacterium, known to be an opportunistic pathogen resistant to a large number of antibiotics and antiseptics.

The microbial adhesion and growth is a nowadays problem in industry, households and hospitals, playing also an important role in wastewater treatment plants (WWTP), since it is simple for different types of bacteria to develop in a broad range of conditions. For these reasons, it is essential to know how to prevent microbial growth, by studying the different types of microorganisms present in biofilms and the way to inhibit their growth.

Although, bacteria can aggregate in flocs and granules without linking on to a surface, they generally exist in one of two types of organization: planktonic, freely existing in bulk solution, and adherent or sessile, as a community attached to a surface or within the confines of a biofilm. For these two types of organization, there are different factors that influence the way they grow or develop, being the essential requirements for microbial growth the microbes themselves and a substrate (Garret *et al.*, 2008). The chemical and physical nature of their surroundings greatly affect the growth of microorganisms and factors such as nutrients and water activity, temperature, pH, pressure and oxygen levels play an important role (Willey, 2008).

In Nature microorganisms nourish mostly on substrates mixtures, and growth may not be controlled by a single nutrient but, rather, by two or more nutrients simultaneously. Consequently, the kinetic properties of cells may change due to adaptation (Kovárová-Kovar & Egli, 1998). For experimental proposes, the kinetics of microbial growth in suspension must be known before being grown in biofilms (Lewandowski & Beyenal, 2014).

2. BIOFILMS

Antonie van Leeuwenhoek (1684) was the first to observe biofilms, found in scrape from his own teeth plaque (Garret *et al.,* 2008). He described them as “animalcules” in a report to the Royal Society of London: "The numbers of these animalcules in the scurf of a man’s teeth are so many that I believe they exceed the number of men in a kingdom." (Paraje, 2011). Characklis, in 1973, studied microbial slimes in industrial water systems and discovered that they were highly resistant to disinfectants (Characklis, 1973). In 1978, Costerton coined the term biofilm. He also played an important role in alerting the world about the importance of biofilms, and how we can benefit in studding them, by introducing a theory explaining how biofilm microorganisms adhere to living and nonliving surfaces (Costerton *et al.*, 1978). In the last two decades, tools such as scanning electron microscopy (SEM) represented the standard techniques for biofilm characterization. More recently, the utilization of the confocal laser scanning microscopy (CLSM), and the investigation of the genes involved in cell adhesion and biofilm formation, have played an important role in understanding the world of biofilms (Donlan, 2002).

Biofilms consist of many different types of microorganisms, such as bacteria, algae, fungi, and sessile (or even crawling) protozoa (Cohn *et al.*, 2010) and are now defined as a biologically active matrix of microbial cells enclosed in an extracellular polymeric substances (EPS) matrix in association with a surface (Garret *et al.,* 2008; Donlan, 2002). EPS consist of various organic substances such as polysaccharides, proteins, nucleic acids and lipids, most of which created, and excreted, by the microorganisms during growth (Tsuneda *et al.*, 2003; Singha, 2012)

Biofilms are difficult to remove and can be found in a panoply of surfaces, such as living tissues, indwelling medical devices, industrial or potable water piping systems, or natural aquatic systems (Donlan, 2002). The production of an exopolysaccharide matrix, or glycocalyx, by the microorganisms, has been suggested to prevent the access of antibiotics to the bacterial cells embedded in such community, which makes them more resistant to disinfection processes (Mah & O’Toole, 2001).

Depending on the species involved, the micro-colonies may be composed of 10–25% cells and 75–90% of an EPS matrix (Garret *et al.,* 2008). The composition, and quantity of EPS, will depend on the type of microorganisms, the biofilms age and the different environmental conditions, such as oxygen and nitrogen levels, extent of desiccation, temperature, pH, and availability of nutrients (Vu *et al.*, 2009). Non-cellular materials, such as mineral crystals, corrosion particles, clay or silt particles, can also be observed inside the biofilm, depending on the environment in which the biofilm has developed (Donlan, 2002).

The initial stage in biofilm formation is the bacterial adhesion onto solid surfaces, promoted by the EPS, due to the fact that the EPS surrounding the bacteria alters its physicochemical characteristics such as the charge, hydrophobicity, and a number of other surface properties (Tsuneda *et al.*, 2003). Although the EPS composition and structure are still not fully comprehended, due to their particular heterogeneous nature, Tsuneda *et al*. (2003), they have a special importance for the scientific community, since they are able to produce natural polymers, or biopolymers, for various industrial and biotechnological applications (Singha, 2012). More *et al*. (2014) describe EPS as an ecological, low cost, highly effective and sustainable alternative to substitute the present chemical flocculants used in water, wastewater and sludge treatment processes. Already, Govender (2011), successfully demonstrated, on a laboratory scale, the potential use of EPS as a possible flotation agent during the bioflotation of sulfide minerals.

2.1. What drives bacteria to produce a biofilm?

Several bacteria predominantly exist as adherent multicellular biofilms, coexisting in diverse environmental niches, and allowing them to survive under unfavorable conditions (Fuente-Nuñez *et al.*, 2013). The transition from the planktonic state to biofilm growth occurs as a consequence of environmental changes. The development of biofilms, in response to stress signals, must be a prompt and highly efficient process, to avoid the death of the bacteria population (Fuente-Nuñez *et al.*, 2012).

Microorganisms within biofilms can withstand nutrient deprivation and pH changes, as well as the presence of oxygen radicals, disinfectants and antibiotics (Jefferson, 2004). The EPS matrix encloses the biofilm and works as a protective shield against degradation, predators, antimicrobial agents and toxins. The existence of biofilms in a widely variety of environments suggests that those microorganisms are capable to react to their environment and change their EPS and adhesion abilities, depending on the properties of the surfaces onto which they attach (Vu *et al.*, 2009). Figure 1 represents the different phases of biofilm development.

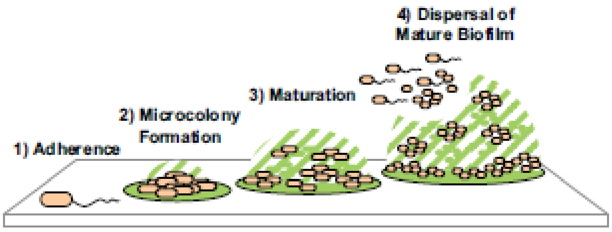

Figure 1 - Phases of biofilm development. (1) Initial attachment of a cell to a surface with subsequent production of an extracellular matrix, making the attachment even stronger. (2) Within 24 h, micro colony formation occurs, over several rounds of cell division, and adherence of cells to the surface becomes stronger. Association with other cells initiates the protection from the environment. (3) Over 24–72 h, continued growth leads to the formation of a mature biofilm structure and nutrients exist between the exterior and interior layers of a biofilm micro colony. (4) After 48 h, external cues and physical disruption lead to some cells from the outer surface of the biofilm micro colony becoming motile and dispersing. Adapted from (Taylor *et al*, 2014).

3. FACTORS INFLUENCING THE GROWTH OF BIOFILMS

Factors influencing the development of biofilms are highly dependent on the microorganism in question. Different occurrences motivate bacteria to transition to one of a variety of biofilms (Jefferson, 2004). This section will focus on a general report of the main parameters affecting the growth of microorganisms.

3.1. Substratum effect

In response to the chemical modification of surface properties, promoted by the substrate, cells secrete nucleic acids, proteins, lipids and lipopolysaccharides that accumulate and form the EPS, being this the first step towards biofilm formation (Renner & Weibel, 2011). Even quite low nutrient concentrations may be sufficient for biofilm growth, given that the biofilm matrix is often negatively charged, and for this reason, nutrients are attracted to the biofilm surface. Biofilm bacteria gather nutrients by concentrating organic compounds in their extracellular polymers. Therefore, bacteria may also use the “waste” products from their neighbors, and secondary colonizers around them, to acquire their biochemical resources by using different enzymes to break down the complex food sources (Prakash *et al.*, 2003).

Ramli *et al.* (2012) studied the effect of glucose in biofilm formation of *Burkholderia pseudomallei*, evaluated in Luria Bertani (LB) broth supplemented with different concentrations of glucose, and verified that biofilm formation increased significantly with the glucose concentration increase in the medium. Vivas *et al.* (2008) stated that the addition of glucose to the LB broth affected biofilm formation in 75% (6/8) of the tested strains at 37 °C and 25 °C, but did not affect biofilm formation at 16 °C.

Cohn *et al.* (2010) indicate, in their report, that the presence of sodium chloride (10 g per liter of wastewater) in the substrate affected the development and growth of biofilms, presumably by the alteration of cell surface hydrophobicity. This has also been showed by Poosaran *et al.* (2005) reporting that, by increasing the cellular age, and in the presence of 0.5% w/v sodium chloride, the cell surface hydrophobicity is reduced. On the other hand, it is also important that microorganisms can survive changes in the osmotic concentration of the surrounding environment. It is known that high osmotic concentrations may cause plasmolysis and low osmotic concentrations may cause cellular lysis (turgidity) (Willey, 2008).

3.2. Hydrodynamics

A boundary layer is described as the fluid thickness representing the practically negligible flow velocity immediately adjacent to the substratum/liquid interface. This thickness depends on the fluid linear velocity and, in this way, at lower velocities the biofilm will be thicker. As the velocity increases, the boundary layer decreases, and cells will be subjected to increasingly greater turbulence and mixing (Donlan, 2002). In this manner, growth and detachment increase as a function of flow velocity: Increased mass transfer leads to a higher biofilm growth rate, whereas increased shear stress causes a higher detachment of the biofilm (Stoodley *et al.*, 1999).

In faster flows, more cells come into contact with the biofilm surface due to better mixing, making it easier to attach, however the sticking efficiency, due to the higher stress shear acting upon the microorganisms, may be reduced.

As such, biofilms growing in laminar flow conditions colonize a glass surface at a greater rate than in turbulent flow, but reach steady state earlier (Stoodley *et al.*, 1999). On the other hand, it is possible that, in laminar flow, the cell accumulation rate will be higher because the detachment rate is reasonably low in relation to the growth rate. However, due to nutrient transfer limitations, steady state is reached sooner (Stoodley *et al.*, 1999). Furthermore, biofilm density increases with both increasing turbulence and substrate load.

3.3. Cell surface properties

Cell surface hydrophobicity, incubation time and EPS production, all influence the rate and extent of the microbial cells attachment (Donlan, 2002; Choi *et al.*, 2015). Another important factor is cell size and cell mobility. It is known that fimbriae, flagella, curly, and pili improve bacteria adhesion in the early stage (Donlan, 2002; Renner & Weibel, 2011). Furthermore, in response to low nutrients concentration and toxicity effects, bacteria can modify their cell surfaces hydrophobicity to allow direct hydrophobic-hydrophobic interactions with the substrates. The hydrophobicity of the cell surface is important in adhesion because hydrophobic interactions tend to increase with increasing nonpolar nature of the microbial cell surface and substratum (Donlan, 2002).

Bacterial cells can be divided into two major groups: Gram-negative and Gram-positive. Their adhesion properties are different, being the Gram-negative more attracted to positively charged surfaces, during the adhesion process (Al Abbas *et al.*, 2012). In fact, increasing the cell negative charge will increase the repulsion against a negatively charged surface (Sheng *et al.*, 2001). Gottenbos *et al.* (2001) suggested that positively charged biomaterial surfaces exert an antimicrobial effect over Gram-negative strains, but not over Gram-positive. It is also thought that the antimicrobial potential is also influenced by the thickness and composition of the microorganisms' cell wall. The distinction between these two bacterial groups is mainly influenced by the organization of a key component, the peptidoglycan layer which is significantly thicker in Gram-positive than in Gram-negative bacteria (Rai *et al.*, 2012). This fact, combined with the Gram-positive peptidoglycan negative charge, and according to Feng, *et al.* (2000), may increase the number of silver (Ag) ions stuck onto the cell wall. On the other hand, Franci *et al.* (2015), defend that the peptidoglycan cross-linking rigid structure, composed of linear polysaccharidic chains, not only reduces the bacterial cell wall anchoring sites for AgNPs, but also difficults its penetration to the cell interior. In contrast, Gram-negative present a thinner cell wall, with less peptidoglycan in its composition, covered by lipopolysaccharides (LPS) and phospholipids, effectively promoting the adhesion of AgNPs (Rai *et al.*, 2012).

Resistance to antimicrobial agents can also depend on the thickness of the biofilm. Thicker biofilms averaging cell densities of 7.6 log colony-forming units (CFU) cm^{-2} were more effective against the penetration of hydrogen peroxide than thinner biofilms (average cell density of 3.5 log CFU cm^{-2}) (Mah & O'Toole 2001).

Despite microorganisms being able to switch between hydrophobic and hydrophilic phenotypes, in response to changes in environmental conditions and growth phases, usually cells with higher hydrophobicity adhere strongly to hydrophobic surfaces. On the other hand, hydrophilic cells are more likely to adhere to hydrophilic surfaces (Krasowska & Sigler, 2014). Gram-negative cell wall contains proteins, lipids, lipoproteins and peptidoglycan with glycolipids and lipopolysaccharides on the external surface. This last constituent of the cell wall can have a variety of polysaccharide chains. This way, bacteria also vary according to the hydrophobicity degree of the cell surface and rate of attachment. Gram-positive bacteria cell walls are simpler, mostly consisting of peptidoglycan with small portions of teichoic and teichuronic acids, proteins and polysaccharides (Lappin-Scott & Costerton, 1995). Cell-surface hydrophobicity is also associated to the presence of certain proteins. For instance, the major hydrophobic sites, at the cell-wall surface of Gram-positive bacteria, are proteins sensitive to pepsin (Mamo, 1989). Furthermore, Gram-negative bacteria, when sensing stress, release outer membrane vesicles that significantly increase cell surface hydrophobicity, facilitating the attachment to surfaces, and to each other, thus leading to a higher tendency to form biofilms Baumgarten *et al.*, 2012.

3.4. Gene regulation

When studying bacterial biofilm formation, some genes are considered of special interest, given their importance for biofilm development (Johnson, 2008). For example, changes in gene expression may lead to increasing aggregation abilities, down-regulated expression of polar flagella, or up-regulated expression of *Type IV pili*, leading to the attachment of the cell onto a surface (Taylor *et al.*, 2014). It is also known that bacteria activity within a biofilm is regulated by quorum sensing (QS). QS makes possible for bacteria to communicate throw small diffusible signaling molecules called auto-inducers. This way is possible for them to switch from individual cells to a group behavior, with a globally regulated gene expression (Cady *et al.*, 2012).

Furthermore, genes involved in antibiotic or antimicrobial agents' resistance are also crucial in biofilm establishment. In recent studies, hydrogen peroxide was able to penetrate a thick biofilm formed by a mutant strain of *P. aeruginosa* lacking one of the major catalase (hydrogen peroxide degradative enzymes) genes – *katA* (Mah & O'Toole, 2001). In another study, Anderl *et al.* (2000) induced the growth of *Klebsiella pneumoniae* biofilms on agar plates both with and without the ampicillin antibiotic. In the end, their study showed that ampicillin was unable to penetrate the biofilm of normal strains, due to the production of a *β-lactamase* ampicillin-degrading enzyme. On the other hand, in *β-lactamase* lacking mutants the ampicillin was able to penetrate the biofilm (Anderl *et al.*, 2000).

3.5. pH and temperature effects

The solution pH is an important property that influences both bacterial growth and cell adhesion. First of all, each species has its own pH growth range and optimal growth pH. On the other hand, it is also known that changes in the solution pH alters the cells surface charge and, therefore, increasing the cell negative charge will increase the repulsion against a negatively charged surface (AlAbbas *et al.*, 2012). Sheng *et al.* (2008) investigated the adhesion ability of a *Pseudomonas sp.*, *Desilfovibrio desulfuricans* and *Desilfovibrio singaporenus* in a pH range between 3 and 9, and reported that, at pH 3, all three species showed maximum adhesion ability (when the pH solution was close to the microorganisms' surface isoelectric points, respectively of pH 2.1, 3.5 and 3.7). Furthermore, they also reported that the adhesion abilities at pH 9 were higher than at pH 7 due to the electrostatic interactions on the metal surface of ferrous ions with the negative carboxylate cell surface functional groups.

A number of other parameters, including temperature, must also be taken into account regarding bacterial growth and attachment. For instance, microorganisms are not able to regulate their internal temperature, and because of this, they present a temperature growth range, as well as an optimal growth temperature. An important factor influencing the effect of temperature on growth is the temperature sensitivity of enzyme-catalyzed reactions. In fact, when metabolism, as a whole, becomes more active, by increasing the temperature within the allowed temperature range, the microorganism grows faster (Willey, 2008). However, when further increasing the temperature the enzymes will denature, growth will cease and cell death occurs.

3.6. Adhesive properties of biofilms

Despite the lack of a detailed knowledge of the adhesion mechanism, the surfaces properties that influence microorganisms' adhesion are roughness, polarization, oxides coverage and chemical composition. In fact, the interactions between the bacterial cell wall and a given surface are mainly influenced by interfacial electrostatic, Van der Waals, hydration and repulsive forces, alongside hydrophobic interactions. From these, the hydrophobic (regarding nonpolar surfaces) and electrostatic interactions regulate biofilms occurrence, since they highly influence the attachment of bacterial cells to surfaces (Renner & Weibel, 2011; Donlan, 2002). Also, divalent cationic ions (e.g., Mg^{2+}, Ca^{2+}) may enhance the attachment of the bacteria to a surface by reducing the electrostatic repulsion and stabilizing interactions between the negatively charged bacteria surface and an anionic substratum (Renner & Weibel, 2011).

On the other hand, the study of Characklis *et al.* (1990) indicates that the extent of microbial colonization appears to increase as the surface roughness increases, because rougher surfaces have a larger surface area for bacteria to adhere. Furthermore, surface roughness reduces the shear stress on bacterial cells and communities positioned in flowing streams (Renner & Weibel, 2011). In another study, Ivanova *et al.* (2009) focused on the effect of roughness in commercial purity grade 2 titanium (normal and modified), and how it influenced bacterial adhesion. They concluded that both the titanium micro and nanomorphology are the factors with most impact, regarding the attachment of bacteria to this material type.

4. BIOFILM GROWTH INHIBITION

Controlling cell attachment via modifying the material surface chemistry, hydrophobicity, roughness or topography, and controlling the bacterial cell wall chemistry, are the most common forms of preventing biofilm establishment. Furthermore, a number of chemical compounds can also be used for biofilm prevention.

As an example of this last case, Fuente-Núñes *et al.* (2012) have discovered in their studies a small synthetic cationic peptide with high antibiofilm activity. They stated that it can reduce cell mobility, inhibit bacterial swarming and stimulate twitching motility. The 9-amino-acid peptide, 1037, showed great effect against biofilm formation of both Gram-negative (*Pseudomonas aeruginosa, Burkholderia cenocepacia*) and Gram-positive (*Listeria monocytogenes*) bacteria. In another study, Perumal & Mahmud (2013) used a methanol extract of *Euphorbia hirta L.*, with concentrations ranging from 0.031 to 1 mg/mL, and found that it could be used to eradicate *P. aeruginosa* biofilms, making it useful for nosocomial infection therapies. Furthermore, it can prevent contamination in medical devices. They also proved that this compound is useful for other pathogens biofilm control, such as *Salmonella typhi*, *Bacillus subtilis*, *Bacillus cereus* and others. Furthermore, Augustine *et al.* (2012) recently studied the antibiofilm activity of Artic actinomycetes against *V. cholera*. They tested thirty-one actinomycetes species from four sediment samples, but only three (A731, A733, A745) of them showed a significant reduction in biofilm formation. The isolates A733 and A745 were identified as *Streptomyces sp*., and the A731 as *Nacardiopsis sp.*, with the A745 showing the maximum inhibition.

Also, the presence of reactive oxygen species (ROS) plays an important role on biofilm formation. Oxidative stress occurs when cells cannot perform an effective antioxidant response, whether due to an increase in ROS levels or a decrease in the cellular antioxidant ability (Ray *et al.*, 2012). This causes the oxidative damage of different macromolecules, first leading to loss of function, increased rate of mutagenesis, and eventually cell death (Kashmiri & Mankar, 2014). Moreover a build up of ROS is thought to be one of the primary mechanisms of nanoparticles toxicity (Markowska *et al.*, 2013).

5. BACTERIAL QUORUM SENSING

As previously stated, bacteria activity within a biofilm is regulated by quorum sensing (QS), making it possible for them to switch from individual cells to a group behavior, by auto-inducers signaling (Cady *et al.*, 2012). By inhibiting this type of communication, it is possible to reduce bacterial biofilm formation. There are two ways to inhibit QS: i) natural (prokaryotic, animal, plant, fungus, marine organisms and antibody based) and ii) synthetic (signal synthesis, modifications in the AHL side chain, modifications in the AHL ring moiety, antagonists of receptor ligand interactions, alkyl DPD analogues, QseC signals, AIP-II signal modification and metallo-complex) (Kalia, 2013).

Both Gram-negative and Gram-positive bacteria use QS for communication, but they produce different auto-inducers. Gram-negative bacteria mainly depend on N-acyl homoserine lactones (AHL) molecules (auto-inducer-1, AI-1) while Gram-positive bacteria use modified oligopeptides (auto-inducer peptides, AIP) (Taga & Bassler, 2003; Li & Tian, 2012).

QS systems are usually divided into three classes. Gram-negative bacteria use a LuxI/LuxR–type QS, which use acyl-homoserine lactones (AHLs) as signal molecules. AHLs are produced by more than 70 species of Gram-negative bacteria, diffusing across the cell membrane and binding to regulatory proteins within the cell (Kalia, 2013). The AHLs are small neutral lipid molecules, synthesized by enzymes known as AHL synthases, and composed of a homoserine lactone ring (HSL) with an acyl chain, that bacteria use to sense and signal their cell density (Churchill & Chen, 2011). The AHL QS systems can be identified via the use of bacterial biosensors, such as *A. tumefaciens*, whom do not produce AHLs and contain a functional *LuxR*-family gene (regulated by an AHL QS system), which regulates the gene's expression codifying the *β-galactosidase* intracellular enzyme (Steindler & Venturi, 2007). Gram-positive bacteria use oligopeptide-two-component-type QS with small peptides as signal molecules, operating through histidine kinases membrane bound receptors (Kalia, 2013). The third class, common in both Gram-negative and Gram-positive bacteria, is the LuxS-encoded auto-inducer 2 (AI-2) (Taga & Bassler, 2003). To understand the importance of these studies, it is relevant to summarize the different synthetic inhibition mechanisms mentioned above.

Gram-negative bacteria rely on an N-acylhomoserine lactone (AHL) based QS (see Figure 2), where a synthase-regulator complex is responsible for the expression of the corresponding genes. This mechanism is usually mediated by two proteins, LuxI and LuxR, interacting directly, at QS concentration. The AHL binds to the protein encoded by the *LuxR* gene, forming an activated LuxR-AHL complex, which decreases the repression of H-NS proteins on the operon containing the *LuxR* and *LuxI* genes. Then, the LuxI protein catalyzes the AHL synthesis (in this way the AHL acts as a self-inducer of its own synthesis). The produced AHL is specific for each species, and as result, the unique AHL can only be recognized by the members of the same species, in order to trigger a response reaction (Taga & Bassler, 2003; Engebrecht & Silverman, 1987). Furthermore, Szenthe & Page (2003) state that when the cells reach high densities, the auto-inducer HSL produced per cell is constant. Such should also be the case in a confined space, for high cell densities, with high and constant HSL concentrations produced per cell, and a tendency to reach higher HSL concentrations inside the cells. In fact, autoinducers tend to increase with population density and bind to a regulatory LuxR protein (LasR and RhIR), subsequently activating it as a transcription factor (Smith *et al.*, 2003). When this phenomenon occurs, an auto-inducing loop of communication is activated (producing even more HSL), through the transcription of genes encoding the regulatory LuxR protein as well as an auto-inducer LuxI-synthase (Lasl and RhII) (Smith *et al.*, 2003; Szenthe & Page, 2003).

Different bacteria produce molecules with different acyl-chain moiety length. Both length and atom substitution have impact in interspecies communication with AHL (Churchill & Chen, 2011; Steindler & Venturi, 2007; Schauder & Bassler, 2001). Figure 2 Gowda *et al.* (2013) identified AHLs belonging to C4-HSL, C6-HSL, C8-HSL, C10-HSL and C12-HSL of *P. aeruginosa* isolates from nosocomial infections. In a study by Shaw *et al.* (1997), these authors used chromatography to identify N-acyl-homoserine lactone signal molecules, using *A. tumefaciens* harboring *lacZ* fused to a gene regulated by autoinduction. Their essay detected four species, N-(3-oxohexanoyl)-, N-(3-oxooctanoyl)-, N-(3-oxodecanoyl)-, and N-(3-oxododecanoyl)-l-HSL in extracts prepared from culture supernatants of *P. aeruginosa*.

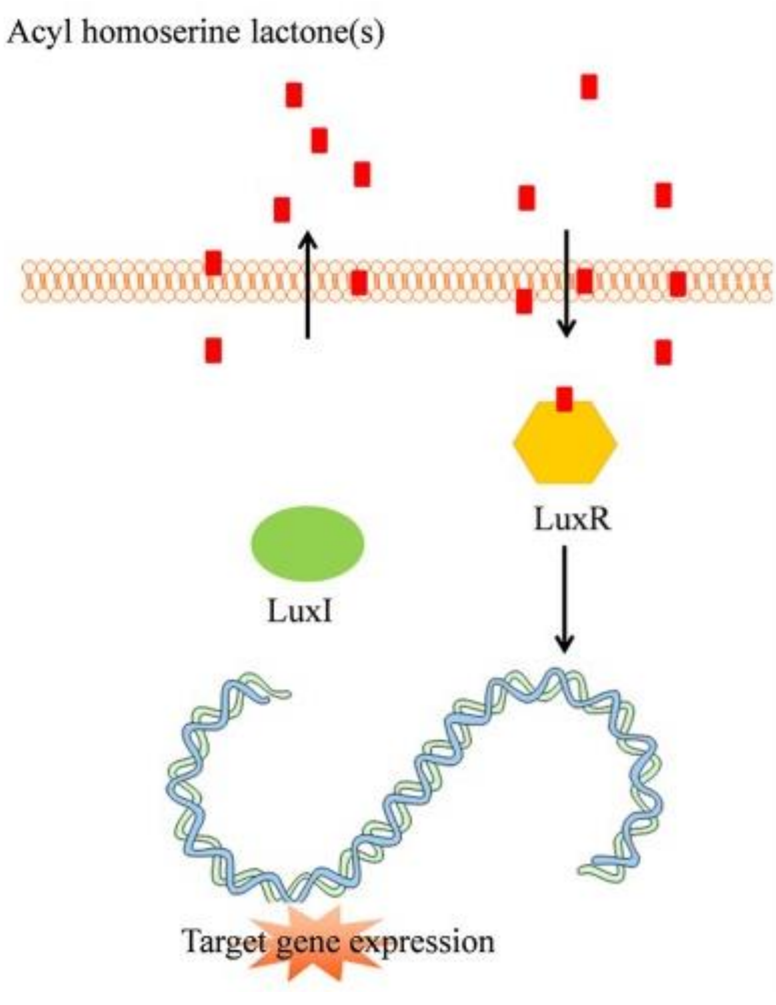

Figure 2- Gram-negative QS mechanism. AHL molecules are synthesized by LuxI. They can passively pass through the cell membrane and, in high concentrations, bind to the intracellular LuxR, activating the target gene expression. Source: (Verbeke *et al.*, 2017)

Gram-positive bacteria have two identified QS systems. The first consists of a two-component signal transduction system (TCSTS) that specifically detects and responds to an AIP (auto-inducer peptide). Contrary to Gram-negative bacteria, the Gram-positive cell membrane is not permeable to AIP, depending on an oligopeptide transporter to diffuse AIP to the extracellular matrix (see Figure 3) (Li & Tian, 2012). AIP are used by these bacteria to bind and trigger the activation of the histidine kinase receptors (Sturme *et al.*, 2002). These receptors sense the AIP, and a cytoplasmic response regulator protein, via gene expression regulation, leads the cell to respond to the peptide (Sturme *et al.*, 2002). The biosynthesis of AIP can ultimately increase secretion of virulence factors (Sturme *et al.*, 2002). The second QS system is called ComRS, a communication system, which has been identified in many Gram-positive streptococci. In this system a signal peptide pheromone (auto-inducer), called XIP, interacts with a transcriptional regulator (ComR), inside the cell, activating genes for genetic transformation (Mashburn-Warren *et al.*, 2010).

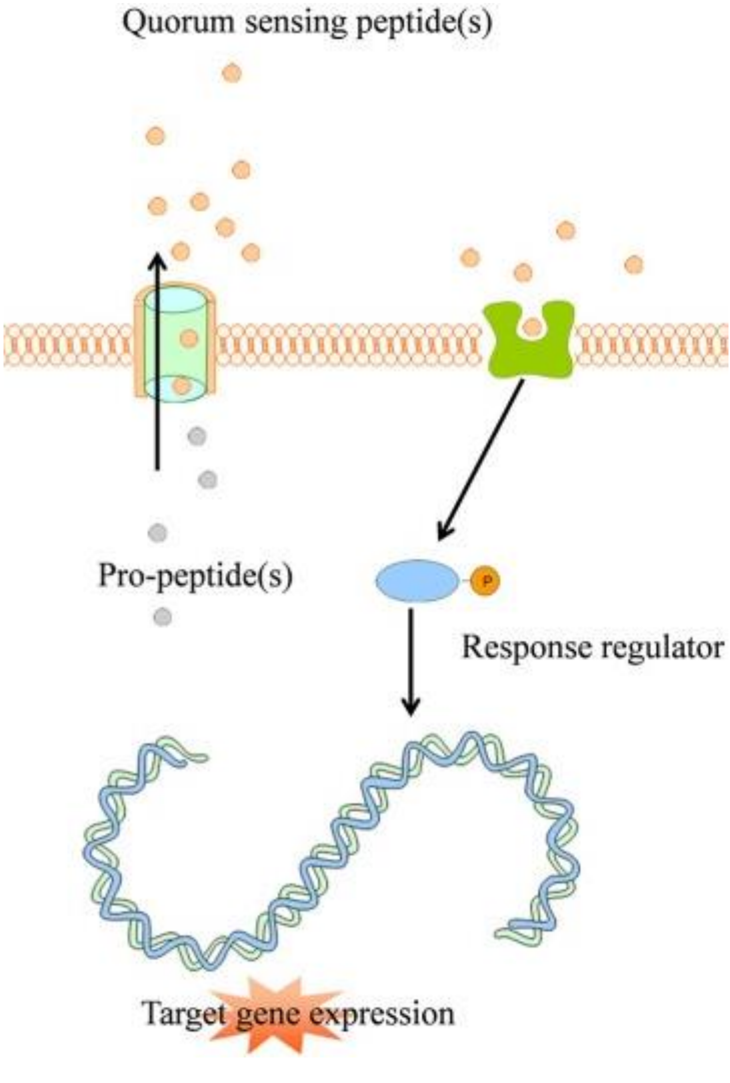

Figure 3 – QS peptides are synthesized by the bacterial ribosomes as pro-peptidic proteins and undergo posttranslational modifications during excretion by active transport. The QS peptides bind to membrane-associated receptors, are further autophosphorylated, and activate intracellular response regulators via phosphor-transfer. These phosphorylated response regulators induce increased target gene expression. Source: (Verbeke *et al.*, 2017).

As referred above, both Gram-negative and Gram-positive organisms have also a common QS system, used for inter-species communication. This system uses AI-2 (LuxS-encoded auto-inducer 2), a QS auto-inducer used by several bacteria, which has the potential to be modified and further modify bacteria behavior. A LuxS encoded auto-inducer converts ribosyl-homocysteine into homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), a precursor of AI-2 (Chen *et al.*, 2002). A study by Guo *et al.* (2012), using ester derivatives of DPD analogs (methyl, propyl, butyl and pentyl), reveal a selectivity of QS modulation amongst closely related bacteria (*Escherichia coli* and *S. typhimurium*).

Regarding the QseC receptor (a bacterial adrenergic receptor), it is a membrane-bound histidine (α-amino acid used in protein biosynthesis) sensor kinase, used by pathogens to diffuse virulence factors, through an inter-kingdom signaling, in response to stress hormones (epinephrine and norepinephrine) and the bacterial signal AI-3 (Curtisa *et al.*, 2014; Clarke *et al.*, 2006).

Finally, some surface material types may also have antibiofilm proliferation abilities. Chenga *et al.* (2007) performed a study on long-chain zwitterionic poly-sulfobetaine methacrylate (pSBMA) surfaces, grafted via atom transfer radical polymerization (ATRP), to test their resistance to bacterial adhesion and biofilm formation, using poly(oligo(ethylene glycol) methyl ether methacrylate) (pOEGMA) as comparison. They concluded that both zwitterionic materials, pSBMA and hydrophilic/neutral pOEGMA brushes grafted via ATRP, highly reduced bacteria accumulation and biofilm formation. They reported that this was partially due to the material longer chains and higher densities, but mostly due to their intrinsically strong hydration via electrostatic interactions for pSBMA, and hydrogen bonding interactions for pOEGMA. Moreover, Cohn *et al.* (2010) indicate, in their study, that a magnetic field influences negatively on the formation of biofilms in wastewater and observed that metal ions compete against organic compounds for active sites on the biofilm, impeding organic degradation.

6. NANOPARTICLES

The prefix “nano-“ means a measure of 10^{-9} units, the nature of this unit being determined by the word that follows (meter, second and others). Therefore, nanoparticles (NP) can be seen as particles within the nanometer scale. However, to consider the term “nanometer“ as referring solely to 10^{-9} m is far from being practical (Williams, 2008), and, taking that into account, the scientific community accepted that nanoparticles are particles in the size range of 1-100 nm (El-Nour *et al.*, 2010). Moreover, nanoparticles can be used to improve drug delivery in the human system and applications in areas such as optics, optoelectronics, catalysis, photography, nanostructure fabrication, chemical/biochemical sensing, photonics and surface enhanced Raman scattering, among others (Bindhu & Umadevi, 2014).

It is known that a number of NP affect various bacteria, although the mechanisms are not completely understood. In fact, nanoparticles can affect the cell in different ways (see Figure 4 and Figure 5), by damaging the DNA and mitochondria, inducing proteins adducts, enzyme dysfunction, interrupt transmembrane electron transport and disruption of cell membrane. The toxicity level depends of the NPs physicochemical properties and composition and the bacterial species (Hajipour *et al.*, 2012). For instance, Bao *et al.* (2015) demonstrated that AgNPs can induce apoptosis in *E. coli*, directly related to the AgNP concentration. In their studies, they also proved that DNA synthesis can be inhibited by AgNPs, once again directly related to AgNPs concentration.

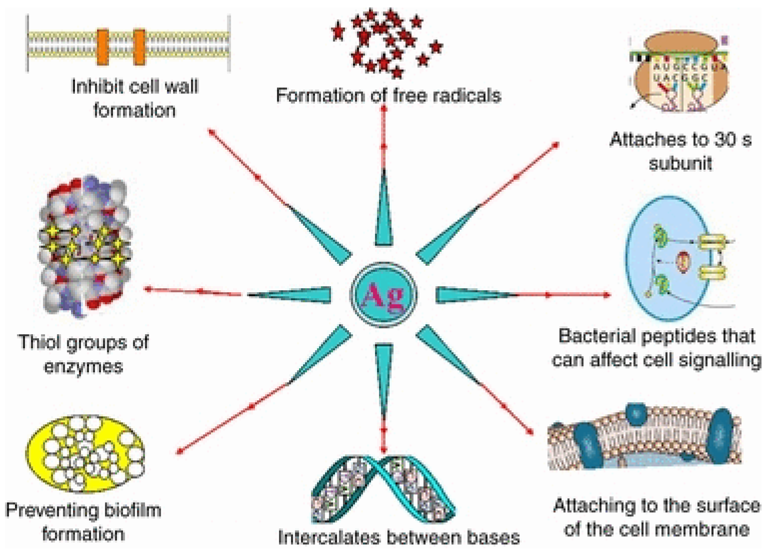

Figure 4 - Different mechanisms of nanoparticles action on bacteria. Source: (Rai *et al.*, 2012).

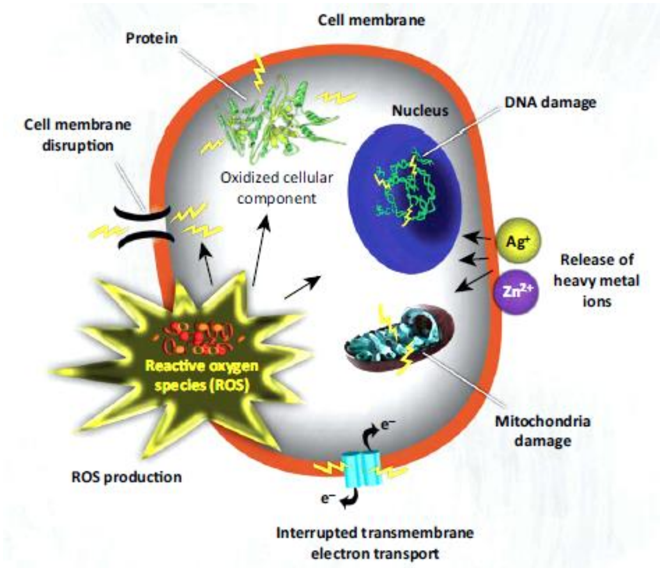

Figure 5 - Mechanisms of AgNP's toxic action. Source: (Hajipour *et al.*, 2012).

Nanoparticles also induce oxidative stress, either through the oxidative properties of the NP themselves, or by oxidative substances or radicals´ generation upon interaction with the microorganisms´ intracellular structures (see Figure 6). This may also involve immune cell activation, mitochondrial respiration, and NADPH oxidase system (Manke *et al.*, 2013). The toxicity is exerted when free oxygen radicals (ROS) are formed, by oxidative stress induction, after the administration of NPs (Hajipour *et al.*, 2012). There are two sub-species of ROS: radical ROS (nitric oxide and hydroxide radicals) or non-radical ROS (hydrogen peroxide) (Soenena *et al.*, 2011). Most of the cells can tolerate small ROS concentrations with self-defense mechanisms like the glutathione redox system. However, the presence of higher ROS levels over a longer period of time is more likely to affect the cell (Soenena *et al.*, 2011).

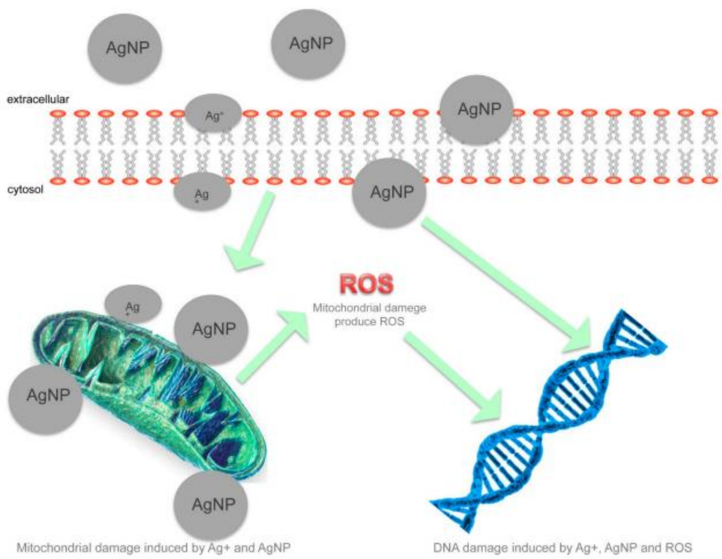

Figure 6 - Schematic representation of ROS response AgNP-induced toxicity. Source: (Franci *et al.*, 2015).

One field of application of nanoparticles falls directly in preventing biofilm occurrence. Indeed, many examples of silver (AgNPs), gold (AuNPs), and other, nanoparticles with anti-biofilm activity have been described over the last years. For instance, coating medical surfaces with silver nanoparticles (AgNPs) has demonstrated to be successful at limiting the formation of surface biofilms (Rizzello & Pompa, 2014). Indeed, AgNPs have been already successfully used in medical and pharmaceutical nano-engineering for the delivery of therapeutic agents, in chronic disease diagnostics, and as part of sensors (Markowska *et al.*, 2013). The mechanisms of the AgNPs bactericidal action are mostly due to the silver ions released from their surface, although less effective than free silver ions at the same concentration (Rizzello & Pompa, 2014). AgNPs in the range of 1–10 nm attach to the surface of the cell membrane with higher affinity, as compared to larger nanoparticles, drastically perturbing the membrane functions (Rizzello & Pompa, 2014). In addition, it has been proven that triangular shaped particles of silver display more bacterial killing activity than rods or spherical particles (Markowska *et al.*, 2013). Also, Zhou *et al.* (2012) already demonstrated the antibacterial effect of gold and silver nanoparticles against the Gram-negative *E. coli* bacteria and the Gram-positive *Calmette-Guérin* bacillus, being especially effective with low nanoparticles levels of aggregation. Moreover, changing the surface agents of AuNPs caused different inhibitory effects, as PAH (poly-allylamine hydrochloride) capped AuNPs caused lysis, unlike citrate capped AuNPs. In another study, Lima *et al.* (2013) supported AuNPs onto clinoptilolite, mordenite and faujasite zeolites, making the size, dispersion and roughness of the nanoparticles dependent of the zeolite support. Then they determined the efficiency of the zeolite support AuNPs on *E. coli* and *S. typhi*, reporting that this zeolite dispersed nanoparticles were excellent biocides even at short contact periods. The more active materials were pointed out to be Au-faujasite, eradicating 90-95% of the tested bacteria at periods as short as 90 minutes.

In other studies, Gurunathan *et al.* (2014) have already used AgNPs treated with leaf extract of *Allophylus cobbe* (with concentrations from 0.1 to 1.0 μg/mL) to inhibit the activity of biofilm formation of Gram-negative *P. aeruginosa* and *Shigella flexneri* and Gram-positive *Staphylococcus aureus* and *Streptococcus pneumoniae* bacteria under *in vitro* conditions. They showed that these NPs were effective against all the tested bacterial strains. After incubation, for 24 h with 0,5 μg/mL of AgNPs, the biofilm activity of *P. aeruginosa* and *S. flexneri*, decreased by more than 90%. The same happened for *S. aureus* and *S. pneumoniae* for a concentration of 0.7 μg/mL. Additionally, they tested the effect of AgNPs inhibitory action alone, and combined with antibiotics, on Gram-negative and Gram-positive bacteria. Their results indicated a higher inhibitory effect when the silver nanoparticles were combined with antibiotics. AgNPs alone inhibited biofilm activity by approximately 20%. However, when combined with ampicillin, they inhibited biofilm activity by 70% and 55%, for Gram-negative and Gram-positive bacteria, respectively. Furthermore, AgNPs combined with vancomycin inhibited biofilm activity in Gram-negative and Gram-positive bacteria by 55% and 75%, respectively. Also, Kalishwaralal *et al.* (2010) synthesized AgNPs from *Bacillus licheniformis* and determined the biofilm formation ability by the Congo Red agar (CRA) method and the Tissue Culture Plate method (TCP) on *P. aeruginosa* and *Staphylococcus epidermidis*. TCP showed that, for a concentration of 50 nM of silver nanoparticles, the biofilm stopped its formation, without affecting the viability of both microorganisms. However, at the 100 nM concentration the microorganisms' growth was impeded and the formed biofilm retroceded. Furthermore, the CRA method indicated that AgNPs have the ability to block exopolysaccharide synthesis (at a concentration of 50 nM), essential for biofilm development.

Regarding the effect of gold nanoparticles, Sathyanarayanan *et al.* (2013) tested the effect of these particles, as well as iron-oxide nanoparticles, in the *in vitro* growth of *S. aureus* and *P. aeruginosa*. In their experiments, they verified that a concentration of 0.01 mg/mL of AuNPs reduced by 13% the growth of *S. aureus* biofilm, while iron-oxide nanoparticles exhibited increased biofilm growth compared to a control assay. On the other hand, 0.01 mg/mL of AuNPs, increased the growth of *P. aeruginosa* biofilm, when compared to a control assay. However, a noteworthy reduction in the biofilm growth was verified when *P. aeruginosa* was exposed to higher concentrations (0.05 mg/mL, 0.10 mg/mL, and 0.15 mg/mL) of AuNPs.

With time, bacteria have developed resistance mechanisms to antibiotics by modifying the antibiotic target site, removing the antibiotics from cell through efflux pumps, antibiotic inactivation through enzyme activity and alteration of metabolic pathways (Shah *et al.*, 2014). Keeping this in mind, it is important to develop a way of improving the efficacy of antibiotic against drug resistant bacteria. For instance, Singh *et al.* (2015) combined AgNPs with 14 antibiotics of seven classes against seven pathogenic bacteria, by the disc-diffusion method. The viability of the strategy was found successful, with different levels of activity obtained, depending of the antibiotic class. Furthermore, they tested bacteria strains resistant to the *β-lactam* class, combining again AgNPs with antibiotics. They showed that the antibiotic minimum inhibitory concentration (MIC) was reduced, making them susceptible to antibiotic treatment. In fact, Singha *et al.* (2012) has already referred that the use of AgNPs, in combination with antibiotics, can reduce by up to 1000-fold the required dose to achieve the same effect. Furthermore, the antibiotic half-life longevity has been shown to be enhanced by AuNPs, presenting a synergistic effect (Shah, et al., 2014).

Up until now, nanoparticles have been commonly prepared in one of two ways: i) physical or ii) chemical approach (El-Nour *et al.*, 2010). Chemical methods are expensive and often involve environmental risk due to the use of toxic and hazardous chemicals (Bindhu & Umadevi, 2014). Furthermore, in recent years it has been observed an increase in the use of plants and microorganisms as biological sources to synthesize metal nanoparticles, which can be considered a third way to prepare nanoparticles. Some of the main processes are presented in sections 7.1 to 7.3.

6.1. Physical processes

In the physical processes, metal nanoparticles are generally synthesized by evaporation-condensation, which could be carried out using a tube furnace at atmospheric pressure, or prepared by laser ablation of metallic bulk materials in solution (Mafuné *et al.*, 2001). The absence of solvent contamination in the prepared thin films and the uniformity of NPs distribution are the advantages of physical synthesis methods in comparison with chemical processes (Iravani *et al.*, 2014). However, the use of tube furnaces has some disadvantages, such as high energetic consumption, time to achieve thermal stability and temperature increase around the material (El-Nour *et al.*, 2010).

For these reasons, the laser ablation method (process of removing material from a solid surface by irradiating with a laser beam) seems more suitable (Prathn *et al.*, 2010). In fact, this method allows for pure and uncontaminated metal colloids production without the disadvantages presented before. Yet the efficiency and the characteristics of the produced nanoparticles depend on the laser wavelength impacting the metal target, laser impact duration, laser fluence (energy delivered per unit area), ablation time and effectiveness of the liquid medium, with or without surfactants (Iravani *et al.*, 2014).

6.2. Chemical processes

Chemical reduction is the most common approach for NPs synthesis and is simply the reduction of an ionic salt in an appropriate medium, in the presence of a surfactant, using organic or inorganic reducing agents (Prathn *et al.*, 2010; Iravani *et al.*, 2014).

Some chemical agents such as sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol, Tollens reagent, N,N-dimethylformamide (DMF), and poly ethylene glycol (PEG)-block copolymers can reduce silver ions (Ag^+), in organic or aqueous solutions, leading to the formation of metallic silver (Ag), followed by agglomeration into oligomeric clusters (Iravani *et al.*, 2014). Selecting the appropriate reducing agent, and a nontoxic stabilizer for nanoparticles stability, can be tricky, because shape and size distribution strongly depend on the properties of these agents.

6.3. Biosynthesis by microorganisms and plants

Microorganisms are able to synthesize gold, silver and cadmium sulphide nanoparticles. This is a relatively new approach of synthesizing nanoparticles, although it is known for a long time that bacteria can, either intra or extracellularly, synthesize inorganic materials (Prathn *et al.*, 2010). Furthermore, the mixture of simple bacteria with complex eukaryotes makes possible to produce nanoparticles with the desired size and shape (Mohanpuria *et al.*, 2008).

The biosynthesis process is carried out by reduction/oxidation reactions. The microbial enzymes or the plant phytochemicals with biological activity (antioxidant capacity) reduce metal compounds into their respective nanoparticles (Prathn *et al.*, 2010). For instance, *P. aeruginosa* can be used as an intermediate to produce silver and gold nanoparticles, as proven by Oza *et al.* (2012). Silver nanoparticles were tested at 30 °C and 100 °C for a pH of 2, 6, 8, 9 and 10. It was found that 100 °C and pH 10 were the optimum conditions to biosynthesize silver nanoparticles from a 100 ppm aqueous solution of $AgNO_3$. In a study conducted by Husseinya *et al.* (2007), two clinical samples of *P. aeruginosa* were isolated from skin burns. Gold nanoparticles were produced by a *P. aeruginosa* strain, synthesizing the pyoverdine soluble fluorescent pigment whereas another *P. aeruginosa* strain produced the pyocyanin blue pigment, when cultured on cetrimide agar media. *P. aeruginosa* ATCC 90271 was used as a control. The later *P. aeruginosa* strain produced the largest particle size and the first produced the largest particle distribution.

6.4. Silver Nanoparticles

Silver-based compounds have been in use as antimicrobial agents to inhibit bacterial growth, for a long time. Data reports back to the 18th century, during which silver nitrate ($AgNO_3$) was used in the treatment of ulcers (Klasen, 2000). With time, silver become recognized and useful in treatment for wounds and bacterial infections, being now in market in a variety of products (Chopra, 2007).

The most critical physicochemical parameters that affect the antimicrobial potential of AgNPs include size, shape, surface charge, concentration and colloidal state. AgNPs positive charge electrostatically attracts the negatively charged cell membrane, facilitating AgNPs attachment. There are four prominent mechanisms of action for AgNPs: i) adhesion to microbial cell surface, resulting in membrane damage and dysfunctional transport activity; ii) penetration inside the microbial cells and interaction with cellular organelles and biomolecules, affecting the cellular machinery; iii) increment in ROS inside the microbial cells, leading to cell damage; and iv) modulation of cellular signal system, ultimately causing cell death (Dakal *et al.*, 2016).

Different sized AgNP have different modes of action. For instance, in *P. aeruginosa*, nanoparticles with 3 to 7 nm interact with cell membrane and with S- and P-containing compounds (Morones *et al.*, 2005). 10 nm AgNPs have reportedly penetrated the cell wall, synergistically boosting antimicrobial effectiveness of the antibiotic aztreonam (Habash *et al.*, 2014). Larger nanoparticles, around 28 nm, attenuate QS (Singh *et al.*, 2015).

When AgNPs penetrate the microorganism cell wall, they can interact with cellular structures and biomolecules and cause damage to the cells (Habash *et al.*, 2014; Rai *et al.*, 2012). In particular, AgNPs may interact with proteins, resulting in their inactivation or functional defects (Klueh *et al.*, 2000). Other example, described by Feng *et al.* (2000), shows that AgNPs may interact with DNA, causing it to change from a relaxed to a condensed form, resulting in loss of the replication ability.

Kim *et al.* (2011) recognized, in their studies, the potential antibacterial, antifungal and antiviral ability of AgNPs, such as their effect on the *lactate dehydrogenase* (LDH) activity in *S. aureus* and *E. coli*. They also found that the AgNPs produced ROS and radical species, such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), hydroxyl radicals (OH•), hypochlorous acid (HOCl) and singlet oxygen, capable of increasing oxidative stress in cells. Indeed, the mechanism of ROS-mediated antibacterial activity was sufficient to cause cell death. It was also found that heavy metals ions, such as Ag^+, increase cellular oxidative stress in the microorganisms in a manner directly dependent on their concentration, and can alter the protein structure and function by modifying critical amino acid residues, inducing protein dimerization (Thannickal & Fanburg, 2000). ROS can also be produced in a process called mitochondrial oxidative phosphorylation, catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, with molecular oxygen generating oxygen radicals (O_2•). Dismutation and metal-catalyzed Fenton reaction cause a further reduction of molecular oxygen, forming H_2O_2 and OH•, respectively (Thannickal & Fanburg, 2000).

In Figure 7 is a photography of a used AgNPs colloidal solution, and TEM images of AgNPs agglomerates.

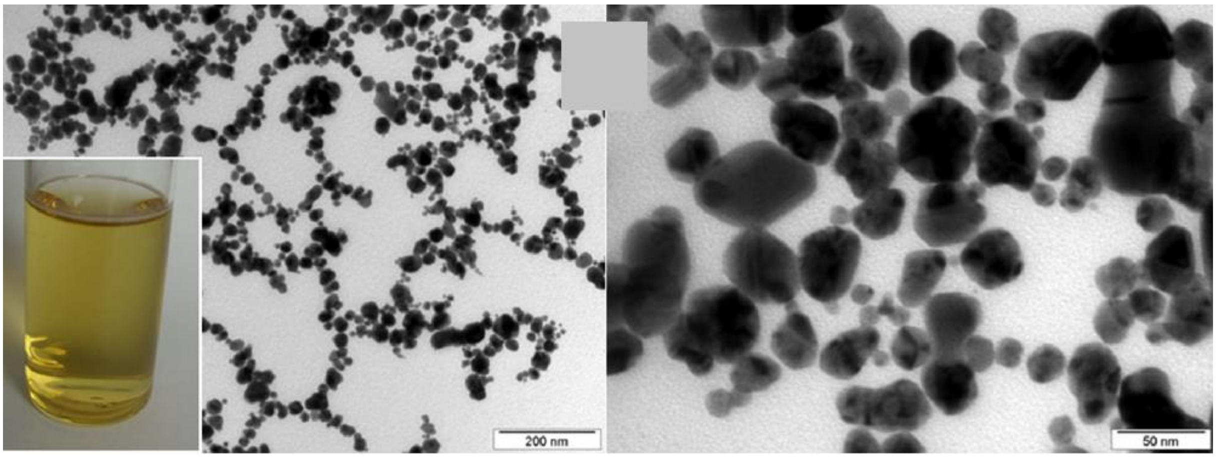

Figure 7 - Silver nanoparticles colloidal solution, and TEM images of agglomerates bigger than 50 nm (on the right) and 200 nm (on the left). Adapted from: (Slepička *et al.*, 2015).

6.5. Gold Nanoparticles

It is believed that gold compounds have been used for medical proposes since 2500 BC. Although, Michael Faraday, in 1857, was the pioneer in the discover of the AuNPs, when he was able to synthetize a colloidal gold solution by the reduction of an aqueous solution, in a two-phase system of chloroaurate ($AuCl_4^-$), using phosphorus in CS_2 (Daniel & Astruc, 2004). In the early 20th century, the enthusiasm for the use of gold nanoparticles grew. Due to the ease of synthesis and the minimal biologic repercussions, AuNPs were widely used to treat rheumatoid arthritis diseases (psoriatic arthritis, juvenile arthritis, and discoid lupus erythematosus). Today AuNPs are used in many fields, with special focus on medicine for drug delivery, therapy and biosensors (Thakor *et al.*, 2011; Thompson, 2007).

As reported before, nanoparticles can interact with the cell wall of Gram-negative and Gram-positive bacteria, and AuNPs are no exception. Formation of distinct aggregation patterns and lysis of bacterial cell was observed for 2 and 6 nm AuNPs, demonstrating a size dependency (Hayden *et al.*, 2012). In another study, Li *et al.* (2014), effectively tested AuNPs against 11 clinical multi drug-resistant isolates, referring that surface chemistry played an important role in AuNP antimicrobial properties. Also, Zawrah & El-Moez (2011) used spherical gold nanoparticles, and tested it against major foodborne pathogens, determining that, in the MIC test, *P. aeruginosa* treated with AuNPs presented larger inhibition regions than the reference drugs.

In Figure 8 is a photography of a used AuNPs colloidal solution, and TEM images of AuNPs agglomerates.

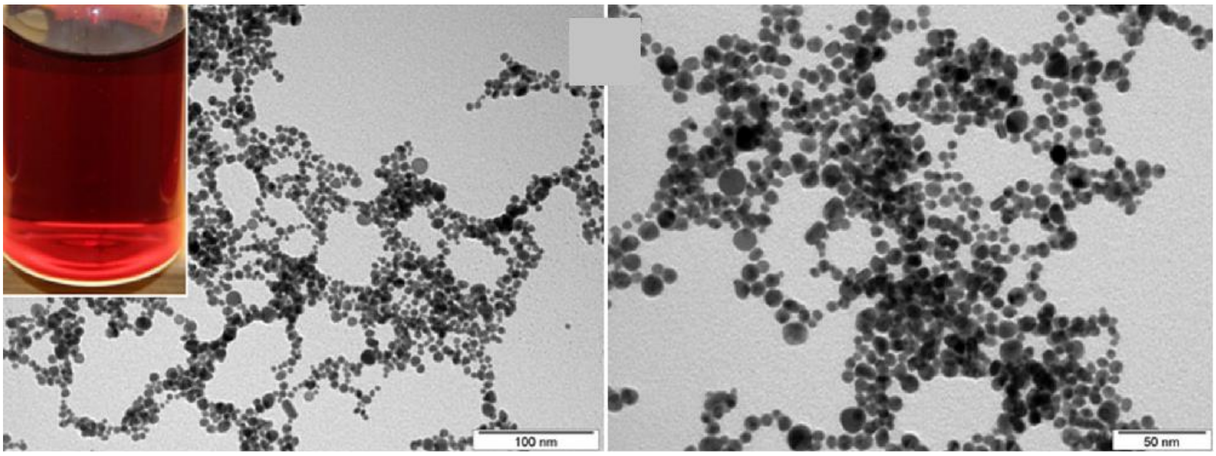

Figure 8 - Gold nanoparticles colloidal solution, and TEM images of agglomerates bigger than 50 nm (on the right) and 100 nm (on the left). Adapted from: (Slepička *et al.*, 2015).

7. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (Figure 9) is a Gram-negative, aerobic, coccobacillus bacterium with unipolar motility and highly resistant to antibiotics. It is found in soil, water, skin, flora, and most man-made environments (Döring, 2015). Incredibly, *P. aeruginosa* bacteria seems to adapt to the microgravity. Kim *et al.* (2013) reported that the biofilms formed during spaceflight exhibited a column-and-canopy structure that has not previously been reported. Effectively, *P. aeruginosa* can grow using uniquely extracellular DNA from the culture medium, as source of phosphate, nitrogen and carbon (Mulcahy *et al.*, 2010). In this sense, it is no surprise that *P. aeruginosa* is highly resistant to a variety of antibiotics, especially when you take in to account the exceptionally low outer-membrane permeability and the presence of multidrug efflux pumps (Nikaido, 1996).

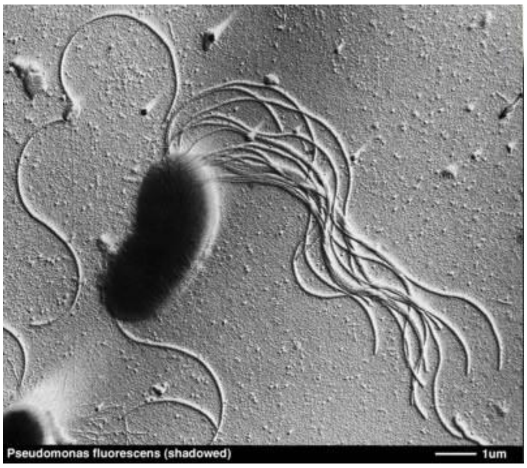

Figure 9 - *P. aeruginosa*, seen with a Low-Angle Rotary Shadowed TEM. Source: (Electron Microscopy Sciences, 2017).

Depending on the *P. aeruginosa* strain and nutritional conditions, different biofilm phenotypes can be developed. For instance, in glucose minimal media, the biofilm cycle of *P. aeruginosa* PAO1 can be subdivided into five major phenotype steps. First, bacteria reversibly adhere onto a suitable surface. Then stage I of the biofilm formation begins, with irreversible attachment to the surface, where the microorganisms form micro-colonies inside an EPS matrix. Progressively the micro-colonies expand and less colonization space is available (stage II). Eventually the bacteria fill all the space (Stage III), growing into observable three-dimensional communities (Stage IV). Finally, bacteria are released from the attached structure, returning to the planktonic state to spread out and colonize other surfaces (Stage V) (Rasamiravaka *et al.*, 2015).

Such as other microorganisms, *P. aeruginosa* biofilms are formed from individual planktonic cells in a complex and highly regulated developmental process. These bacteria can produce several exopolysaccharides, contributing to biofilm formation, including alginate, Psl, LPS and Pel (Ghafoor *et al.*, 2011). Each has different function. Alginate (a linear unbranched polymer composed of D-mannuronic acid and L-guluronic acid) retains water and nutrients and protects the biofilm (Sutherland, 2001). Pel is a glucose-rich matrix, although its composition is not entirely known. Psl contains a repeating pentasaccharide consisting of D-mannose, L-rhamnose and D-glucose. Both serve as a primary skeleton structure, being involved in the development of the biofilm at its early stages (Ryder *et al.*, 2007; Franklin *et al.*,2011). *P. aeruginosa* growing on a surface has increased expression of *algC*, a gene required for the synthesis of extracellular polysaccharides (Davies & Geesey, 1995). As shown by Ma *et al.* (2012), *algC* encoded enzymes are fundamental, and required, for the control of sugar precursors, alginate, lipopolysaccharides (LPS), Psl and Pel critical biofilm matrix exopolysaccharides.

The expression of certain target genes, in *P. aeruginosa*, depends of two AHL QS systems acting sequentially. At high cell density, the concentration of both the referred AHLs systems, LasIR and RhlIR, is high. The genes controlled by the LasIR system are expressed first. The AHL-LasIR complex activates *rhlR*, a gene that encodes a second AHL receptor, RhIR. This receptor binds to its equivalent AHL autoinducer, producing its own target genes (Brint & Ohman, 1995).

P. aeruginosa is known to express different virulence factors at different stages of the biofilm life cycle. The potential for infection and virulence factors are showed by the different types of AHL detected. The two QS systems used by these bacteria, Las system and the RhlI and RhlR proteins, produce different AHL signals (Brint & Ohman,1995; Holm *et al.*, 2015). As reported by Smith *et al.* (2003), LasI is essential for the production of the AHL signal molecule N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL). Kiratisin *et al.* (2002) further demonstrated that LasR forms multimers only when 3O-C12-HSL is present. The second mechanism depends on the production of the AHL N-butyryl-L-homoserine lactone (C4-HSL), by the RhlI synthase, with the *rhlR* as the transcriptional regulator, being responsible for the expression of other genes (Smith & Iglewski, 2003). Pearson *et al.* (1999) observed that these are freely diffusible compounds, although the diffusion of 3O-C12-HSL is significantly slower than that of C4-HSL.

Although it is not studied in this work, it makes sense to refer one extra cellular component that influences biofilm formation, Extracellular appendages such as flagella, type IV pili and Cup (chaperone-usher pathway) fimbriae are associated with irreversible attachment, and micro-colonies formation in biofilms (Anyana *et al.*, 2014). O'Toole (2011) clarifies this in their work with *P. aeruginosa* mutants (with flagellar-mediated mobility and defective in type IV pili), which did not develop a biofilm, when compared to the wild type strains.

8. MATERIALS AND METHODS

For the quantification of the nanoparticles inhibition properties, four different methods were used: biomass optical density (OD) at 600 nm, crystal violet (CV) method, assessment of cell metabolic activity (MTT assay) and measurement of homoserine lactones (HSL). These assays will be explained further ahead. Cell confluence was also performed in *Cellavista* as a method to determine the percentage of biofilm occupation in the wells.

Both gold and silver nanoparticles were obtained by preparing a colloid solution of metal nanoparticles in polyethylene glycol (PEG)/H_2O, a simple technique, where the metal is directly sputtered (using high energy ions to eject metal particles) into PEG, produced at the Department of Solid State Engineering of the University of Chemistry and Technology in Prague (Slepička *et al.*, 2015). The silver and gold nanoparticles standard solutions had a concentration of 102 mg/L and 360 mg/L respectively. Both Au and Ag nanoparticles had an average diameter of 20 nm.

The microorganisms used in the experiments were *P. aeruginosa* DBM 3777 (PA 3777), *P. aeruginosa* DBM 3081 (PA 3081) and *Agrobacterium tumefaciens* NTL4(pZLR4) ATCC BA-2240. A list of the equipment used in the laboratory experiments is presented in Table 1.

Table 1 - Equipment used in the experiments and their respective manufacturers.

Equipment	***Manufacturer***
Cellavista	Innovatis
Universal 32R centrifuge	Hettich
Orion 290A pH meter	Orion
DU 730 Life Science UV/Vis spectrophotometer	Beckman Coulter
Kern 572 scale	Kern & Sohn GmbH
Kern 770 scale	Kern & Sohn GmbH
Bioscreen C microbiology reader	Oy Growth Curves Ab Ltd
Infinite M200PRO reader	Tecan

Two different media were used: Luria Bertani (LB) media and minimal *Agrobacterium* (AB) media, alongside a phosphate-buffered saline (PBS) solution and a lysis buffer. The composition of each of these is presented in Table 2.

Table 2 - Media used in the experiments, with their respective composition and manufacturers.

Media	***Composition***	***Manufacturer***
LB media:		
Tryptone	10 g/L	Oxoid
NaCl	10 g/L	Fermtech
Yeast extract	5 g/L	Penta
AB media (pH=7.4):		
Yeast extract	1 g/L	Merck
KCl	0.15 g/L	Dorapis
K_2HPO_4	3 g/L	Penta
NaH_2PO_4	1 g/L	Lachema
NH_4Cl	1 g/L	Lach-Ner
$MgSO_4*2H_2O$	0.3 g/L	Chemapol
$CaCl*2H_2O$	0.01 g/L	Lachema
$FeSO_4*7H_2O$	0.0025 g/L	
Phosphate-buffered saline (pH=7,4):		
NaCl	8.01 g/L	Penta
KCl	0.2 g/L	Chemapol
$NaHPO_4*12H_2O$	3.578 g/L	Lachema
KH_2PO_4	0.27 g/L	Penta
Lysis buffer in 40 mL of PBS		
$MgCl_2$	0.174 g	Penta
Cetyltrimethylammonium bromide (CETAB)	0.008 g	Lachema
N_3Na	0.032 g	Aldrich

8.1. Bacterial growth analysis with nanoparticles

Bacterial growth analysis is the first step to be performed when analyzing the efficiency of an anti-microbial agent in the laboratory, as it monitors the microorganisms' growth in a suspension by measuring the OD. This test was prepared by adding an inoculum of PA 3777 and PA 3081 strains to different microculture plates with a wide range of gold or silver nanoparticles concentration (Table 3). *Bioscreen C* was employed to monitor the optical density measurements between 420-580 nm, every 30 minutes during 24-hour periods. Temperature in *Bioscreen C* was set to 30 °C for PA 3777 and to 37 °C for PA 3081.

Table 3 - Concentration used for bacterial growth analysis with nanoparticles for PA 3777 and PA 3081 with gold and silver NPs.

Microorganism + Nanoparticle	***Concentration (mg/L)***
PA 3777 + AgNP	0; 10; 13; 16; 19; 22; 25; 28
PA 3777 + AuNP	0; 5; 8; 10; 12; 15; 20; 22; 24; 26; 28; 30; 32
PA 3081 + AgNP	0; 20; 22; 24; 26; 28; 30;32; 34; 36; 38; 40;42; 44; 46; 48
PA 3081 + AuNP	0; 80, 83; 86; 89, 92, 95; 98; 101; 104

8.2. Determination of antibiofilm activities of silver and gold nanoparticles.

The microtiter dish assay is an important tool for the study of the early stages of biofilm formation. Microtiter dishes allow for the formation of a biofilm on their walls and/or bottom, and are applied primarily for the study of bacterial biofilms. The optimal conditions for biofilm formation must be predetermined empirically, or by literature survey, for each tested microorganism (O'Toole, 2011).

In the present study, bacteria were first cultured, in *Erlenmeyer* flasks containing LB medium, at 30 °C (PA 3777) or 37 °C (PA 3081), at 150 rpm for 24 h. The suspension was then centrifuged and resuspended in LB medium until an OD of 0.6 (at 600 nm) was obtained. Biofilm growth was then performed in a clear polystyrene microtiter plate (TPP 96F). Such plates allowed the formation of a biofilm on the bottom of the microtiter dishes. As showed by Cappello (2008), the adhesion to this type of surface largely depends on the bacteria strain and media composition. The suspension volume and silver nanoparticles concentration added into each microtiter plate well is presented in Table 4.

Table 4 - Volume of medium + *P. aeruginosa* and AgNPs (and respective concentration) added into each well.

Well	*AgNP concentration (mg/L)*	*LB Medium + P. aeruginosa (µL)*	*AgNP solution (µL)*
1-8 and 49-56	**0**	**200.0**	**0.0**
9-16 and 57-64	**10**	**180.4**	**19.6**
17-24 and 65-72	**20**	**160.8**	**39.2**
25-32 and 73-80	**30**	**141.2**	**58.8**
33-40 and 81-88	**40**	**121.6**	**78.4**
41-48 and 89-96	**50**	**102.0**	**98.0**

The suspension volume and gold nanoparticles concentration added into each microtiter plate well is presented in Table 5.

Table 5 - Volume of medium + *P. aeruginosa* and AuNPs (and respective concentration) added into each well.

Well	*AuNP concentration (mg/L)*	*LB Medium + P. aeruginosa (µL)*	*AuNP solution (µL)*
1-8 and 49-56	**0**	**200.0**	**0.0**
9-16 and 57-64	**40**	**177.8**	**22.2**
17-24 and 65-72	**50**	**172.2**	**27.8**
25-32 and 73-80	**80**	**155.6**	**44.4**
33-40 and 81-88	**120**	**133.3**	**66.7**
41-48 and 89-96	**140**	**122.2**	**77.8**

The microtiter plates were incubated for 24 h at 30 °C (PA 3777) or 37 °C (PA 3081), after which 100 µL of each well suspension was placed in a plate to measure the OD at 600 nm. The remaining 100 µL of supernatant were centrifuged and the supernatant was collected into an *Eppendorf* for posterior HSL measurement. Wells 1 to 48 were used for the crystal violet test and wells 49 to 96 for the MTT test.

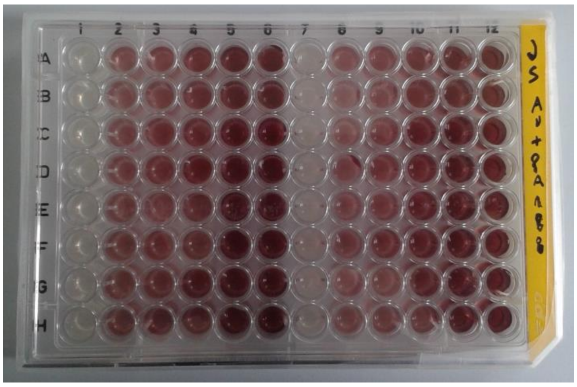

Figure 10 - Example of a PA 3777 with AuNPs assay.

8.3. Crystal violet method

Crystal violet (CV) dying is a basic coloring method, with CV binding to negatively charged surface molecules and polysaccharides. Although this method can be used to determine, at some extent, the biomass (including EPS substances) of a biofilm, it is poorly suited to evaluate the viability of the biofilm cells, as it stains both living and dead cells. However, as dead bacteria lose their adherence and are, subsequently, detached from the biofilm, it reduces the number of cells stained by CV. This fact makes this method useful to determine if more, or less, bacteria are contributing for the biofilm proliferation.

This experiment was performed using wells 1 to 48, from the microtiter plate used for the bacterial growth analysis with nanoparticles assay. First, the wells containing the adhered bacteria are washed 3 times with 200 μL of a 9% saline solution, to remove the remaining supernatant. Before removing the last saline wash solution, a cell confluence assay in *Cellavista* is performed. This method, better explained in section 8.6, measures the area occupied by the adhered cells in the wells. Afterwards, 200 μL of a 0.1% CV solution is added and left in the wells for 20 min, staining and fixing the cells to the bottom of the plate. The CV solution is then removed and the wells are washed, again, 3 times with 200 μL of the saline solution. Finally, ethanol (96%) is added to the wells, to suspend the CV after staining the biomass, and left to act for 10 min, before transferring 100 μL to another plate to measure the absorbance of bound CV (at 590 nm). Figure 11 represents an example of a performed CV assay.

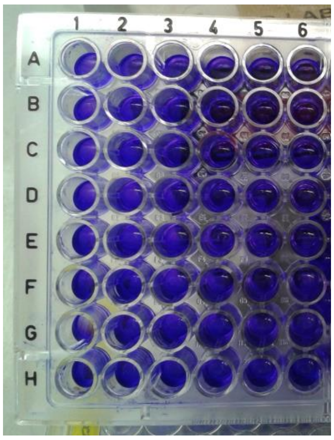

Figure 11 - Example of a CV assay.

8.4. Assessment of cell metabolic activity (MTT method)

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay is a colorimetric test for measuring cell metabolic activity based on the conversion of MTT (water-soluble yellow dye) into formazan crystals by the dehydrogenase system of living cells (Stockerta *et al.*, 2012; Meerloo *et al.*, 2011). Formazan crystals are insoluble purplish precipitates which may be formed inside the cells, deposited near the cell surface or even in the culture medium (Riss *et al.*, 2013). Riss (2014), in his review paper points to MTT reduction studies stating that formazan crystals are not only associated with mitochondria, but also the cytoplasm and membranes in the endosome/lysosome compartment, and even in the plasma membrane.

Generally, formazan formation can be affected by reducing agents, mitochondrial respiratory chain inhibitors (in eukaryotes), light exposure, pH, D-glucose concentration and the physiological state of the cells (Chiba *et al.*, 1998). Furthermore, ascorbic acid, vitamin A, sulfhydryl-containing compounds (including reduced glutathione), coenzyme A and dithiothreitol are also associated to non-enzymatic reduction of the MTT to formazan (Riss, 2014). As the MTT assay is based on the activity of NAD(P)H-dependent oxidoreductase enzymes to convert MTT into the formazan product, it can be concluded that the NAD(P)H concentrations in the cytoplasm and plasma membrane are determinant for the final result of the MTT assay (Riss, 2014).

Both glucose and MTT solutions are needed for this test. The MTT solution is prepared in phosphate buffer for a final concentration of 1 mg/mL, and then filtered with a 0.2 μm filter. The glucose solution is also prepared in phosphate buffer for a final concentration of 57.4 g/L.

To perform this experiment, wells 49 to 96 from the microtiter plate used for the biofilm cultivation assay, are employed. The wells with the adhered cells are washed 3 times with 200 μL of PBS, to remove the remaining supernatant. A cell confluence analysis in *Cellavista* is then performed, to determine the area covered by the biofilm. Afterwards the PBS is removed and 60 μL of glucose and 50 μL of MTT solutions are added into each well. A volume of 10 μL of menadione (with a concentration of 0.11g/L) is also added to the wells, to increase the crystals formation rate. A dark incubation is then performed, by covering the microtiter plate in aluminum foil for 2 h, at 150 rpm, and 30 °C for PA 3777 and 37 °C for PA 3081. After this period the aluminum foil is removed and 100 μL, of a solution to help diluting the formazan crystals (40% Dimethylformamide + 2% acetic acid + 16% dodecyl sulfate sodium), is added and the plate is incubated for 30 min at 230 rpm. Finally, 100 μL are transferred to a new plate to measure the absorbance at 570 nm by the *Infinite M200PRO* reader. Figure 12 represents an example of a performed MTT assay.

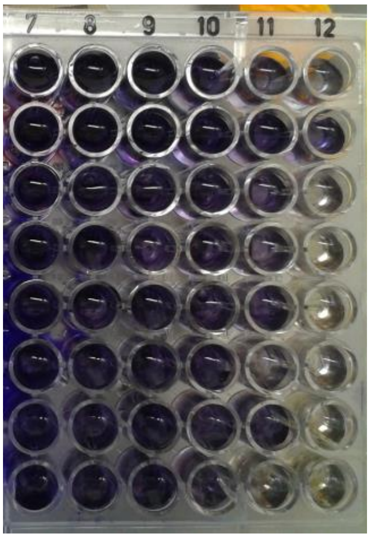

Figure 12 - Example of an MTT assay.

8.5. HSL method

Quorum sensing (QS) is a cell-to-cell communication mechanism employed by a variety of bacterial species to coordinate their behavior at a community level, through the regulation of gene expression (Nievas *et al.*, 2012). In Gram-negative bacteria, such as *P. aeruginosa*, homoserine lactones (HSL) and N-acyl homoserine lactones (AHL) are commonly involved in QS mechanisms. The HSL method uses a genetically modified bacterial strain, *A. tumefaciens* NTL4(pZLR4) with a plasmid for the synthesis of the *β-galactosidase* intracellular enzyme, expressed when homoserine lactones are present (Nievas, *et al.*, 2012). After cell lysis, this enzyme decomposes X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) into a colored product that can be determined by spectrophotometry at 660 nm. Thus, in the presence of the HSL QS molecule, this activator molecule binds to the *A. tumefaciens β-galactosidase* gene promoter, activating its expression, and resulting in *β-galactosidase* production and consequent bluish color response of the medium inside the wells (Verbeke, *et al.*, 2017).

Regarding this experiment procedure, first 1 mg of gentamicin is diluted in 1 mL of AB media. Then 500 μL of the previous solution, and 100 μL of a 20% glucose solution, are added into 200 mL of AB media. Afterwards *A. tumefaciens* is inoculated in this media and cultivated for 24 h, at 30 °C and 150 rpm. After this period, the bacteria are separated by centrifuge and re-suspended in AB media for an OD of 0.5 (400 nm). A microtiter plate is filled with 2 μL of the samples taken from the supernatant (previously stored from the biomass suspension during MTT and CV methods) and 50 μL of the *A. tumefaciens* suspension. After pipetting, the plate is incubated at 30 °C, on a shaker at 150 rpm, for 16 to 18 hours. After this period, 50 μL of the lysis buffer is added and incubated at room temperature for 90 min. Meanwhile a solution of lysis buffer with X-Gal is prepared: 5 mg of X-Gal is diluted in 1 mL of dimethyl sulfoxide (DMSO) and subsequently added to 4 mL of lysis buffer (quantity for one plate). After 90 min, 50 μL of lysis buffer with X-Gal is added to each well. Finally, the plate is covered in aluminum foil and the absorbance is read after 60, 90 and 120 minutes at 660 nm.

For a better interpretation of the results, it is necessary to know the concentration of autoinducers present in the QS assay. Therefore, it was necessary to construct three calibration curves, for the different incubation times (60, 90 and 120 min). For this, 2 µL of calibration solutions (0.1; 0.25; 0.5; 0.75; 1.0; 1.25 mg/L) were added to 50 µL of an *A. tumefaciens* suspension in four wells, and incubated as described before.

8.6. Cell confluence

Cellular confluence refers to the percentage of the culture vessel occupied by attached cells. A 100% cell confluence means that the entire surface is covered by cells, while a 50% confluence means that roughly half of the surface is covered. When, beginning with the same initial biofilm cell concentration, a smaller confluence percentage indicates a slower biofilm growth than a larger confluent percentage (*Smith et al.*, 2003; ATCC, 2012). *Cellavista* cell confluence can be performed without a fluorescent dye, with the results obtained for each well directly converted into cell number (Cellavista, n.d.). The cell confluence is analyzed by the *Cellavista* automatic inverse microscope, which is capable of thoroughly analyze the acquired images, thus monitoring the biofilm formation throughout time. Figure 13 represents an example of a performed CC assay.

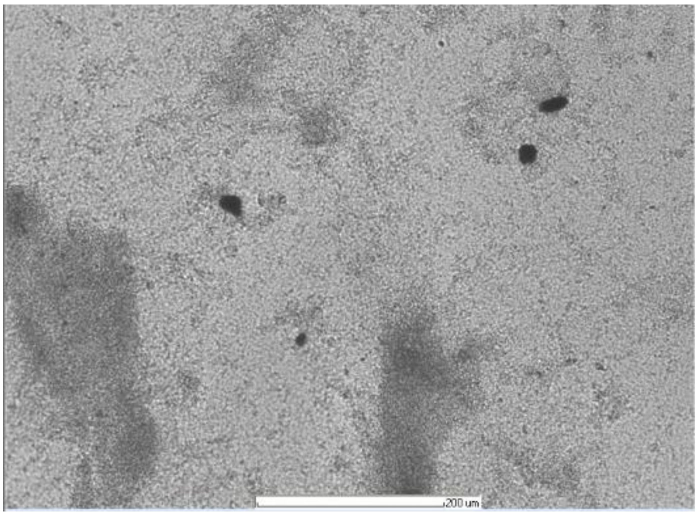

Figure 13 - *P. aeruginosa* biofilm, at 40x, in *Cellavista*.

Figure 14 is a sketch of the OD600, CV, MTT, HSL and Cell Confluence assays.

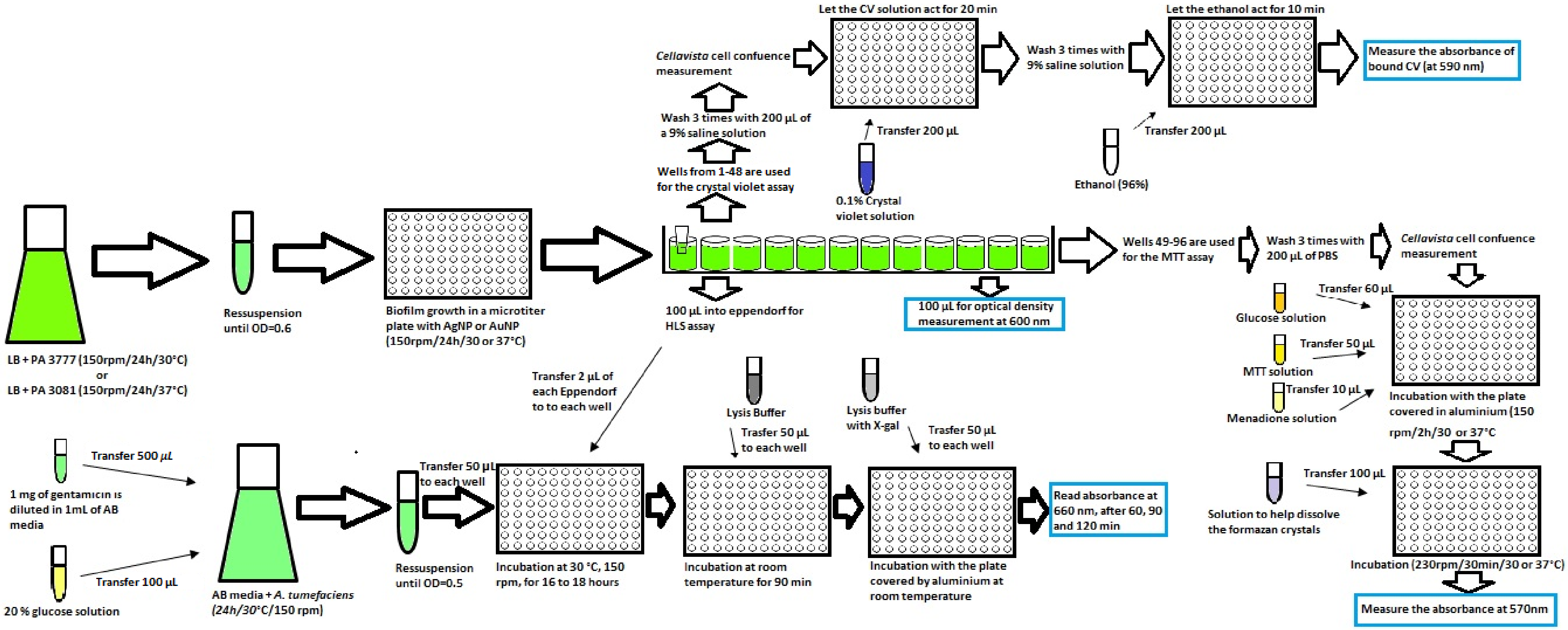

Figure 14 - Representation of the methods present in section 10.2.

8.7. Dixon's Q test

The Dixon's Q test was used to improve the data analysis of the OD, CV, MTT and HSL tests. This test is used for outliers' identification and rejection, assuming a normal distribution of the data. First, the data is organized (sorted) in an ascending order of values, allowing for the determination of the Q parameter using the following equation 1:

$$Q = \frac{x_{i+1} - x_i}{x_n - x_1} \tag{1}$$

The next step is to compare the calculated Q value with the values of Table 6 for a 95% confidence interval ($\alpha = 0.05$). Choosing the correct confidence interval depends of the number of observations (n). Given that, in the present case, the number of observations was 8 (n=8), 16 (n=16), 32 (n=32), 48 (n=48) or 96 (n=96), depending of the experiment, if the Q value is higher than the correspondent interval of confidence to $\alpha = 0.05$, the x_i value is an outlier and should be rejected.

Table 6 - Table used for the interval of confidence, with the used confidence interval highlighted in yellow. Adapted from: (Verma & Quiroz-Ruiz, 2006).

n	*α = 0.05*	*α = 0.01*
8	*0.4673*	*0.5914*
16	*0.3293*	*0.4268*
32	*0.2541*	*0.3357*
48	*0.2241*	*0.2991*
96	*0.1865*	*0.2521*

9. RESULTS AND DISCUSSION

In this section, the results of the conducted experimental work will be presented. The Dixon's Q test was used to improve data analysis, by identifying and rejecting outliers.

9.1. Bacterial growth analysis with nanoparticles

Figure 15, Figure 16, Figure 17 and Figure 18, present the growth curves of the studied bacteria (PA 3777 and PA 3081) in a bulk suspension, when exposed to different concentrations of silver and gold nanoparticles, between 420-580 nm, with measurements every 30 min, for a period of 24 h.

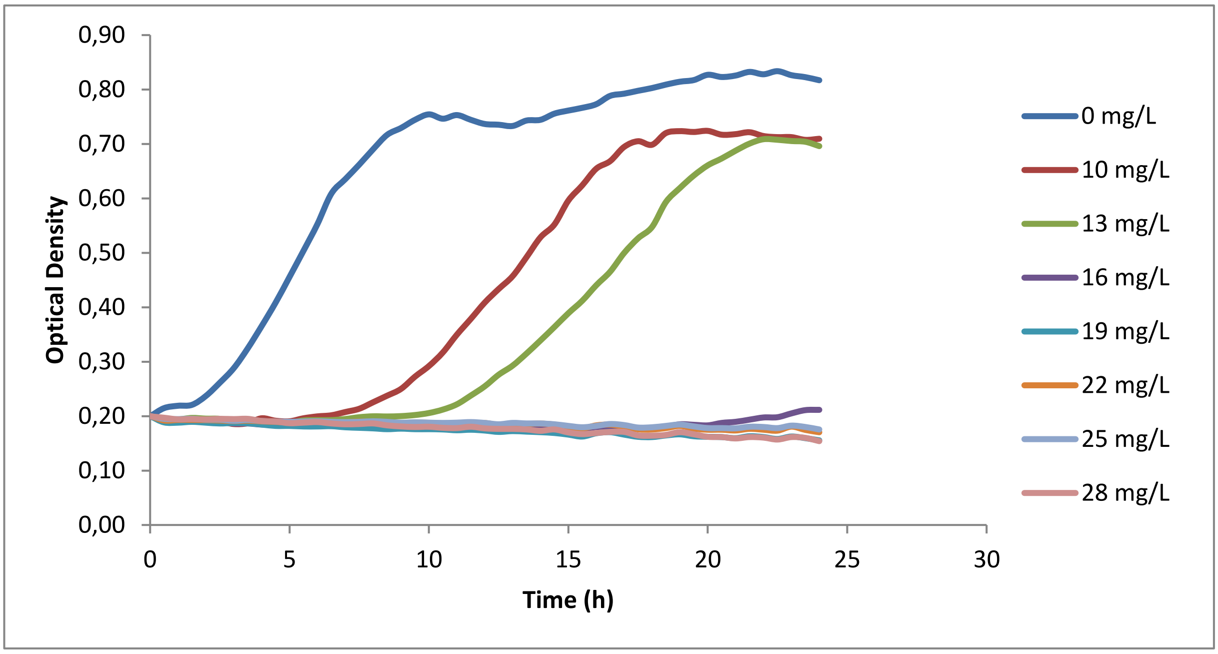

Figure 15- Bacterial growth analysis of PA 3777 in the presence of AgNP over 24 hours.

In Figure 15 is clearly visible the inhibition effect that silver nanoparticles (AgNP), even at low concentrations, presented against *P. aeruginosa* 3777. In fact, analyzing Figure 15, it can be concluded that AgNP concentrations as low as 16 mg/L of AgNP are enough to completely prevent these bacteria from growing.

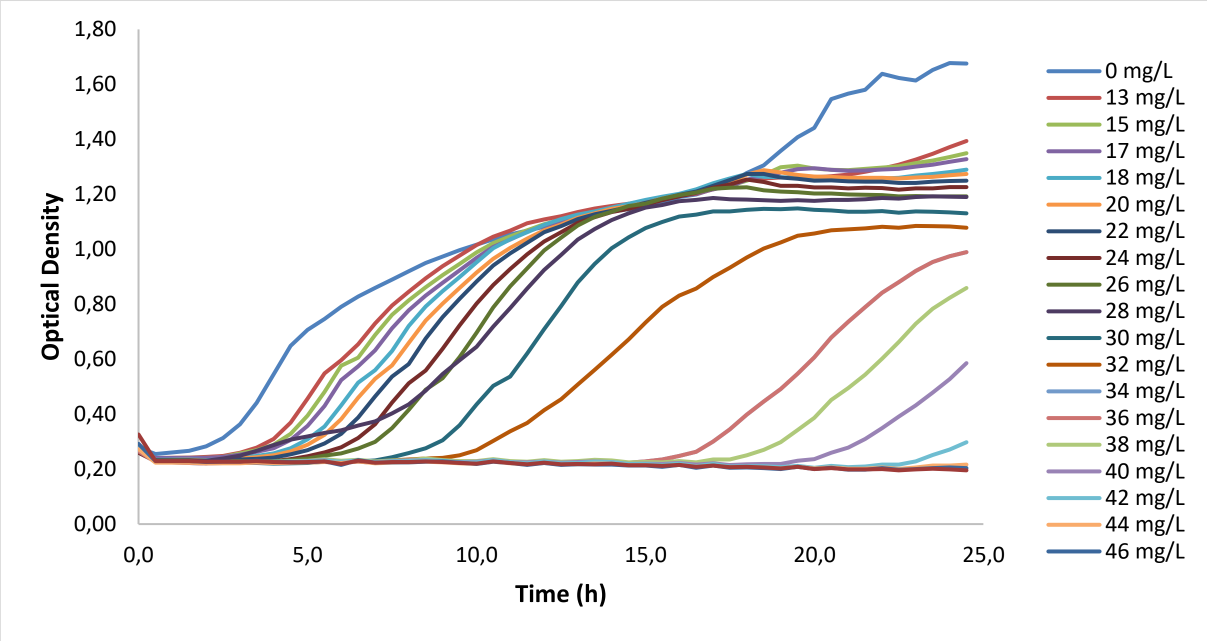

Figure 16 - Bacterial growth analysis of PA 3081 in presence of AgNP over 24 hours.

Comparing Figure 15 with Figure 16, we can determine that *P. aeruginosa* 3081 is more resistant to the effect of silver particles (AgNP) than *P. aeruginosa* 3777. Indeed, analyzing Figure 16, it can be concluded that it is necessary a concentration of at least 44 mg/L of AgNP to completely prevent the growth of PA 3081.

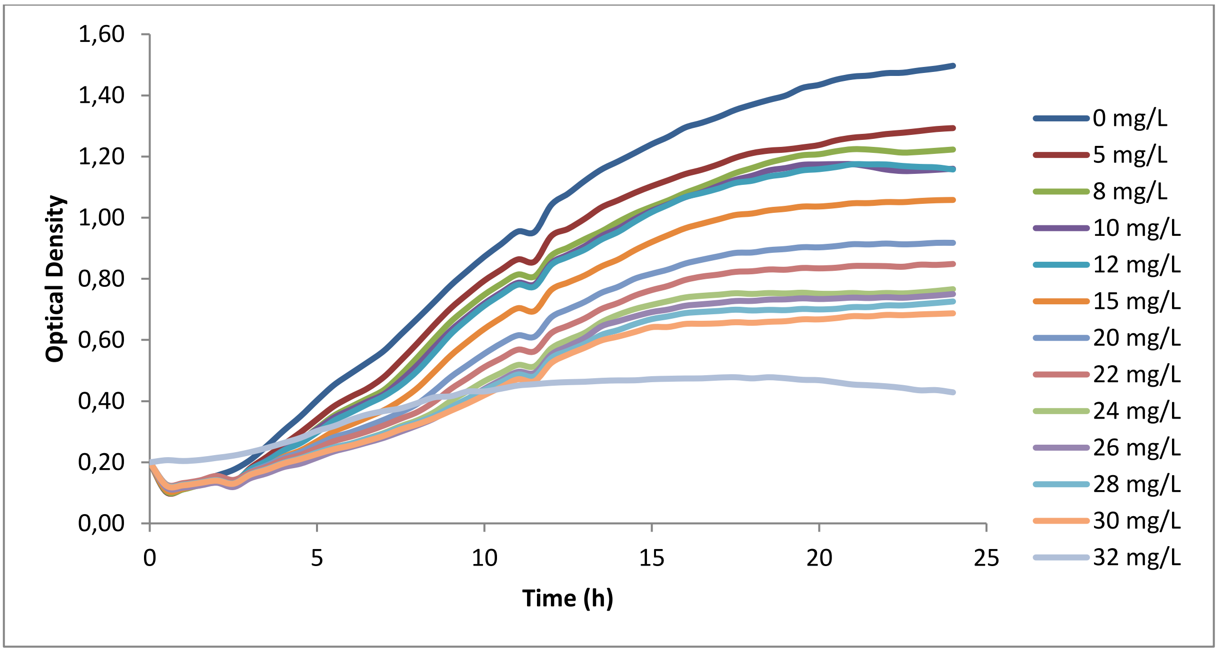

Figure 17 - Bacterial growth analysis of PA 3777 in presence of AuNP over 24 hours.

Analyzing Figure 17, it can be concluded that the gold nanoparticles (AuNP) demonstrated to be less effective than silver (AgNP) for PA 3777 strain. In fact, it seems to be needed a concentration higher than 32 mg/L AuNP to prevent these bacteria to significantly grow (and even at this concentration it could be found an initial growth up until 10 hours of incubation).

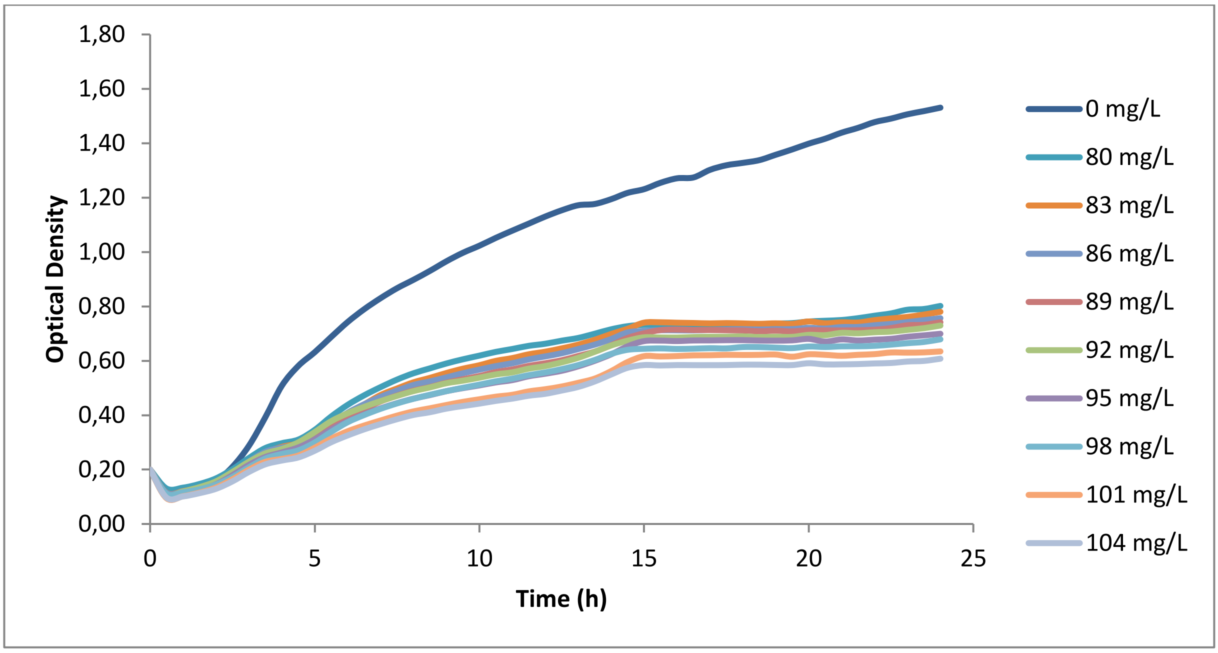

Figure 18 - Bacterial growth analysis of PA 3081 in presence of AuNP over 24 hours.

Figure 18 shows that the AuNP was less effective against PA 3081 than AgNP, as it had already occurred for PA 3777. Furthermore, it can also be confirmed that the growth of the PA 3081 strain was less affected, for both AgNP and AuNP, than the PA 3777 strain. Although there was no total bacterial inhibition in some cases, it can be concluded that the addition of nanoparticles, at the concentrations present in this study, decreased the bacterial growth. As no AuNP concentration, up until 104 mg/L was able to completely prevent PA 3081 to grow, it would be necessary to test concentrations higher than 104 mg/L to define the concentration able to completely stop this bacteria growth.

9.2. Crystal violet, MTT, HSL and Cell Confluence assays

To perform a proper interpretation of the results obtained in the assays, the results analysis was divided in two parts: supernatant analysis and adhered biomass analysis. The supernatant analysis refers to HSL assays, and the adhered biomass analysis to the CC, CV and MTT assays. The initial biomass concentration for these tests was different for each trial. In this regard, it was necessary to correct the raw data with the dilution factor, for the biomass concentration determined by the OD600 results.

9.2.1. SUPERNATANT ANALISYS

In this section is analyzed the HSL assay. The results were obtained using an optical density reader (*Bioscreen C* microbiology reader).

9.2.1.1. HSL ASSAY

P. aeruginosa uses a quorum-sensing (QS) mechanism of gene regulation to interact within the confines of the biofilm. Silver nanoparticles have been studied before as QS inhibitors by Anju & Sarada (2016) in *P. aeruginosa*, with the inhibitory effect being confirmed by the authors. This microorganism may use this mechanism in order to alter gene expression dependent on the density of microorganism population. According to Taga & Bassler (2003), the concentration of an autoinducer in a given environment is dependent on the number of both suspended and adhered bacteria present. The HSL test is usually applied to monitor the effect of external stress (nanoparticles in this case), in the *P. aeruginosa* communication, inside the biofilm.

For a better interpretation of the HSL results, it was used a calibration curve, that correlates the measured OD at 660 nm in the QS test with the HSL concentration, present in the appendix. In this section is only presented the HSL concentration, whereas the HSL concentration correlated to the final biomass concentration is presented in the appendix.

Figure 19**Erro! A origem da referência não foi encontrada.** presents the HSL concentration for PA3777 and PA3081, cultivated in a shaker for 24 h, at 150 rpm, and 30 or 37 ºC, with silver NPs.

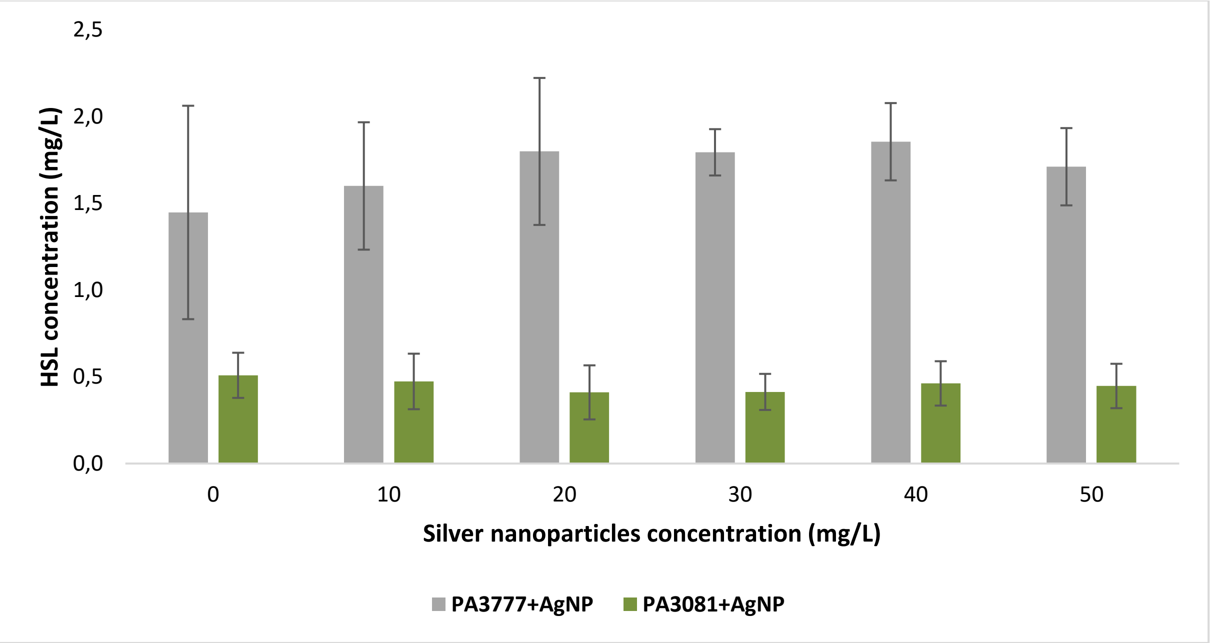

Figure 19 - Graphical representation of the HSL assay, for PA3777 and PA3081, with Ag nanoparticles.

Figure 20 presents the concentration results of the HSL method, for PA 3777 and 3081, cultivated in a shaker for 24 h, at 150 rpm, and 30 or 37 ºC, with gold NPs.

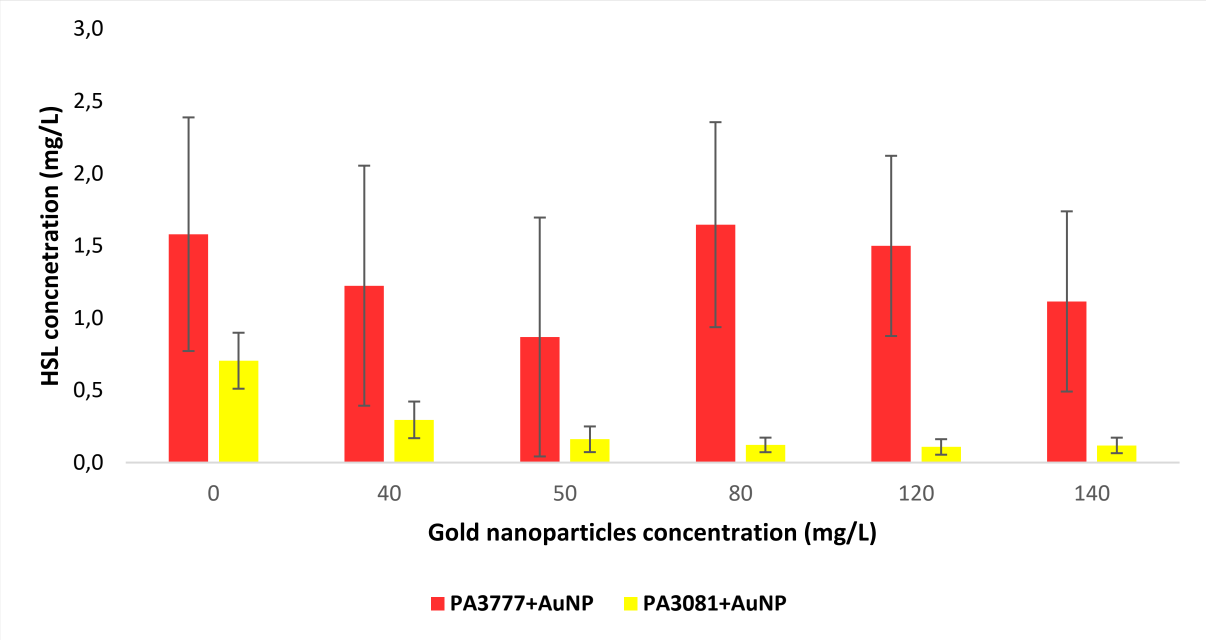

Figure 20 - Graphical representation of the HSL assay, for PA3777 and PA3081, with Au nanoparticles.

Analyzing Figure 19 and Figure 20, it is possible to conclude that the PA 3777 strain produced more total HSL autoinducers than the PA 3081 strain. Furthermore, overall, the PA 3777 also produced more HSL per suspended biomass (0.967 mg HSL/g Biomass) than PA 3081 (0.236 mg HSL/g Biomass). Given the fact that the overall (taking into account both the AgNPs and AuNPs) adhesion ability of both strains was somewhat similar (see CC results), it seems that PA 3777 presented a larger use of HSL communication for other purposes beyond triggering adhesion. And, even if the CV results point towards a larger biofilm formation for the PA 3777 strain, the magnitude of the difference for the PA 3081 strain is not able to justify the differences found for the HSL results. In fact, as previously stated in Chapter 5, autoinducers may be produced and used by QS systems in response to stress conditions. Keeping this in mind, the maintenance of the HSL levels, even when the biomass contents were falling (decreasing suspended biomass with the NPs concentrations increase), may imply the increase of cell communication (higher HSL levels per cell), associated with the attempt of the microorganisms to signal, and react, to the presence of the NPs toxicity effect. In fact, as the PA 3777 bulk growth seemed to be more affected than the PA 3081 strain (see section 10.1) to the presence of the studied NPs, this could justify the presented higher HSL levels.

It should also be noticed that, for similar NPs concentrations (40 and 50 mg/L), the AgNPs seemed to lead to larger HSL concentrations, for both strains, in accordance with the higher toxicity effect of AgNPs found in section 10.1. However, mixed results were obtained for HSL per suspended biomass, for similar NPs concentrations, with respect to the two *P. aeruginosa* strains (higher values for PA 3777 for AgNPs and smaller for PA 3081).

9.2.2. ADHERED BIOMASS ANALISYS

In this section the Cell Confluence, Crystal violet and MTT assays are presented and discussed. The results for CV and MTT were obtained using an optical density reader (*Bioscreen C* microbiology reader). Regarding Cell Confluence, the results were obtained using *Cellavista*.

9.2.2.1. CELL CONFLUENCE

The cell confluence results for both PA 3777 and PA 3081 strains, with gold and silver nanoparticles, are presented in Figure 21 and Figure 22. The cell confluence tests were performed to determine the percentage of area covered by *Pseudomonas* biofilm in the wells (including EPS and non-dislodged dead cells), as an indirect measure of the microorganisms' biofilm growth according to different conditions. This method depends of an image processing software, which may incur in non-negligible errors (as it can be confirmed by the obtained standard errors). Furthermore, before the measurement, it was necessary to remove the supernatant inside the wells, possibly dislodging some of the cells attached to the wells, thus increasing this analysis' errors.

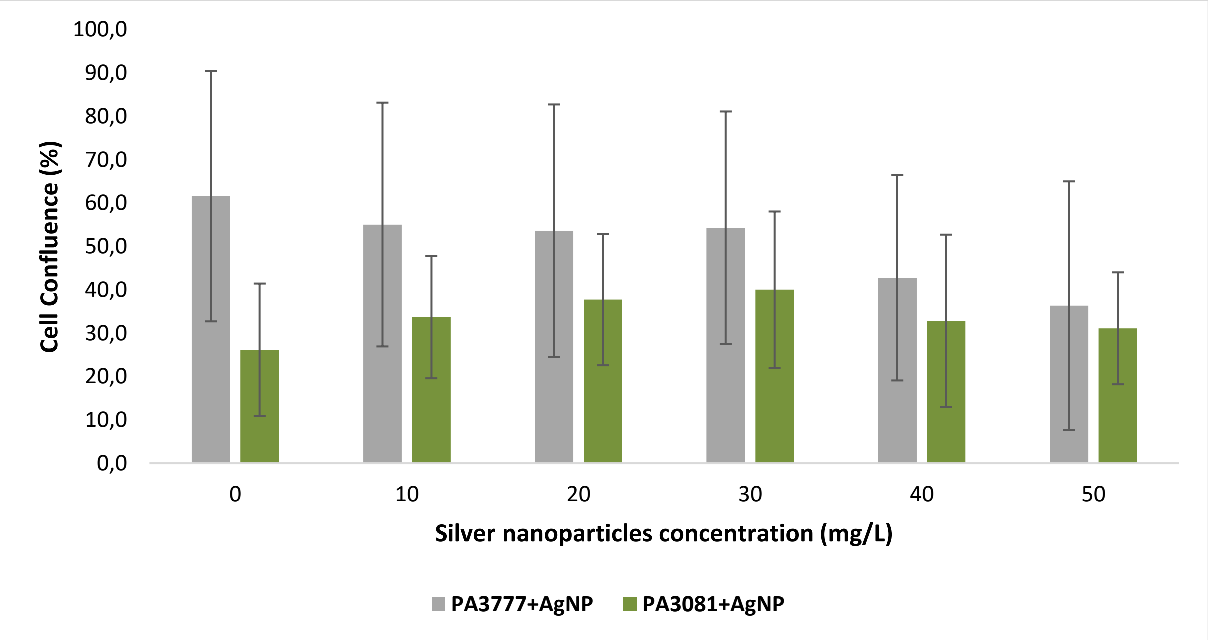

Figure 21 - Cell confluence percentage obtained by analysis in *Cellavista* for AgNP, with PA 3777 and PA 3081.

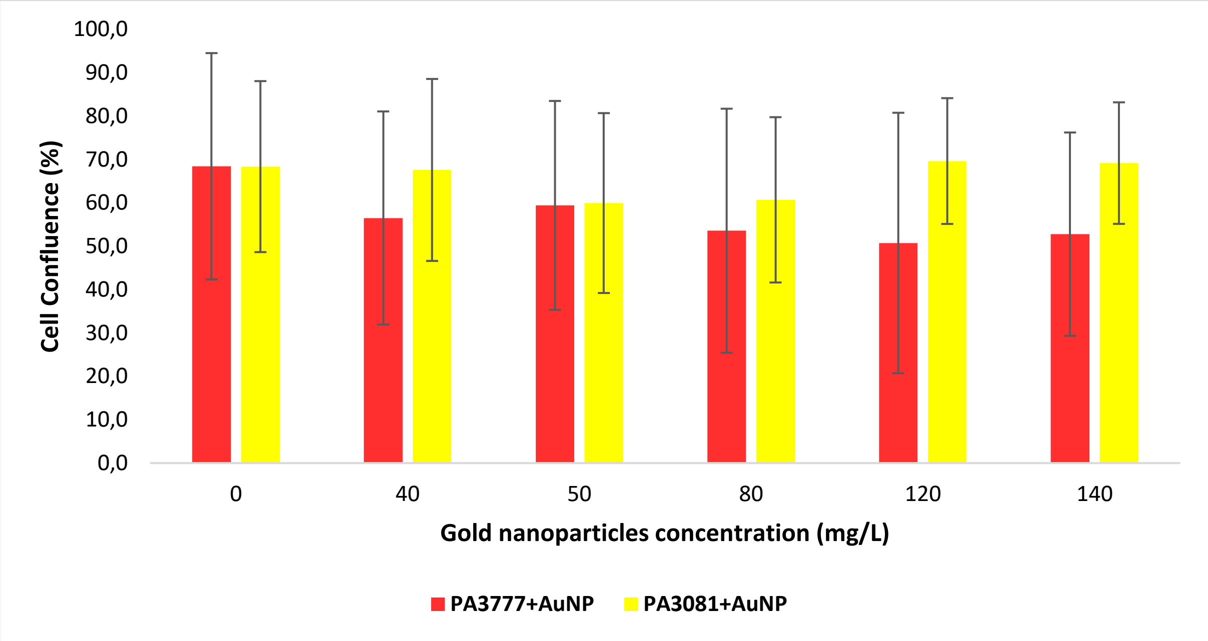

Figure 22 - Cell confluence percentage obtained by analysis in *Cellavista* for AuNP, with PA 3777 and PA 3081.

Analyzing Figure 21 and Figure 22, a higher cell confluence (well area percentage occupied by the biofilm) is observable for the experiments with the gold nanoparticles (for similar NPs concentrations), indicating that the silver nanoparticles seem to affect more the ability of the studied *P. aeruginosa* strains to form biofilms. Furthermore, when the CC values are normalized per suspended biomass, the normalized CC value for the AuNPs assays are almost twice the value than for the AgNPs assays (for similar NPs concentrations). This is in accordance with the higher toxicity effect of AgNPs found in section 10.1, hindering both the suspended growth of these bacteria and their ability to form biofilms. Keeping in mind the larger HSL concentrations obtained for the AgNPs (regarding similar AuNPs concentrations), contradictory to the cell aggregation values, only the study of the concentration of the specific autoinducers responsible for *P. aeruginosa* cell aggregation could bring further enlightenment with that regard.

Comparing the PA 3777 and PA 3081 strains, lower CC values were obtained for PA 3777 for AuNPs, but larger for AgNPs, and slightly larger CC values normalized per suspended biomass were obtained for the PA 3081. This seems to point out to a slight larger effect on the adhesion ability of the PA 3777 strain than on the PA 3081 strain. In fact, the behavior of the CC values, for increasing NPs concentrations, is more pronounced for the PA 3777 than for PA 3081. Furthermore, when comparing the CC values normalized per suspended biomass, for similar NPs concentrations, the values corresponding to the AuNPs were more than twice as large for the PA 3081, but only a third larger for the PA 3777. As a result, it could be observed that the larger effect on the adhesion abilities seems to be obtained by the use of AgNPs in the PA 3777 strain.

However, it should be kept in mind that, given the fact that the CC analysis only determines the area of the adhered biofilm, and not necessarily its volume (and mass), coupled to the inherent errors of this procedure, care should be taken in reaching definite conclusions.

9.2.2.2. CRYSTAL VIOLET ASSAY

CV dying is a method that stains both living and dead cells, including the extracellular matrix and other products in the biofilm. The experiment required to remove the supernatant content of the wells, with multiple washing steps, which increases the possibility of removing biofilm cells, thus potentially decreasing the resulting biomass contents when compared with the cell confluence registered by *Cellavista*.

The CV assay results for PA 3777 and PA 3081, cultivated in a shaker for 24 h, at 150 rpm, and 30ºC or 37 ºC, with AgNPs are presented in Figure 23.

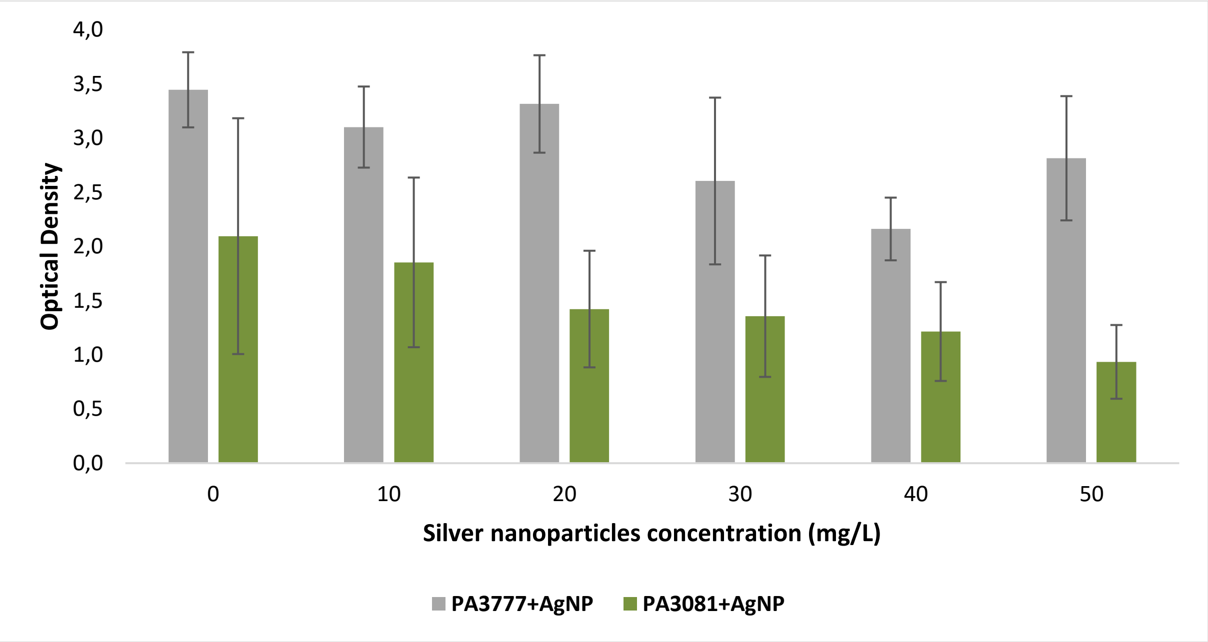

Figure 23 - Graphical representation of the CV assay, for PA3777 and PA3081, with Ag nanoparticles.

The CV assay results for PA 3777 and PA 3081, cultivated in a shaker for 24 h, at 150 rpm, and 30ºC or 37 ºC, with AuNPs are presented in Figure 24.

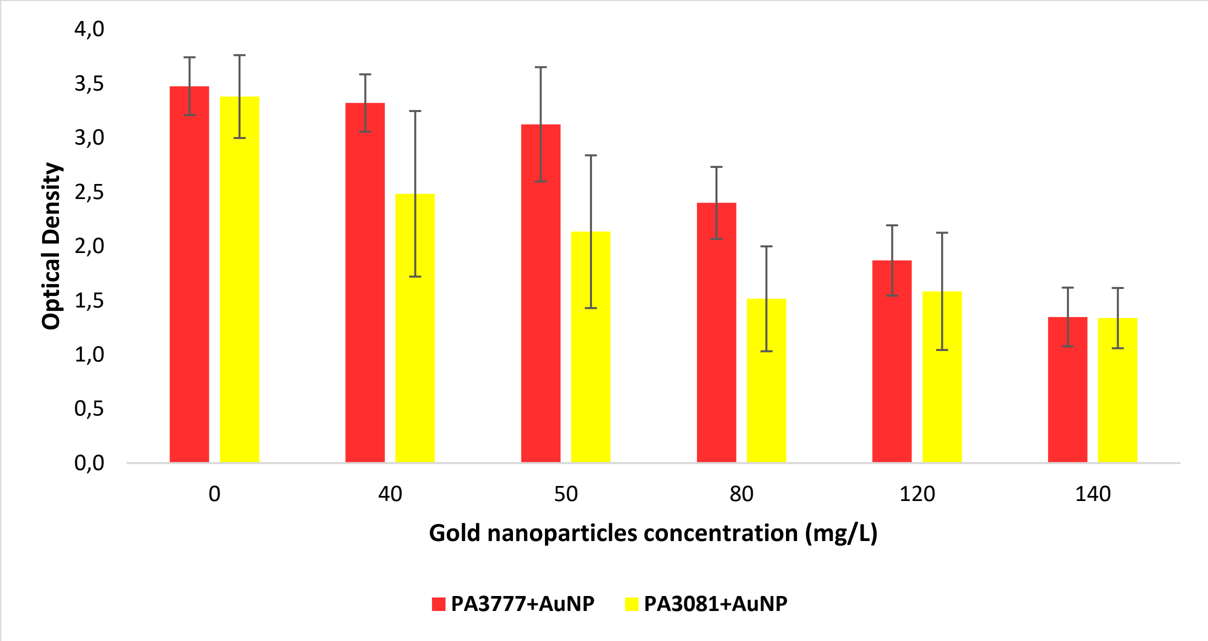

Figure 24 - Graphical representation of the CV assay, for PA3777 and PA3081, with Au nanoparticles.

Analyzing Figure 23 and Figure 24, apart from the results of PA 3777 strain with AgNP, which were somewhat oscillatory, the CV method allowed to establish a strong decreasing biofilm (including biomass and extracellular substances) trend with the increase of the nanoparticles concentration, meaning that less cells were attached to the bottom of the wells, and/or extracellular matrix components were produced. For both AgNPs and AuNPs, the PA 3777 strain presented larger CV values (both in absolute values and per suspended biomass) than the PA 3081 strain, meaning that the biofilm formation for this later was more affected. This result does not follow the obtained results for the CC analysis, especially regarding the use of AuNPs. However, it should be noticed though that the CC analysis determines the projected area of a biofilm, whereas the CV analysis depends on the volume (or mass) of the biofilm. Furthermore, it should be kept in mind that the bounding ability of the CV to the bacteria itself, and to the EPS, may be different and, thus, differences in the EPS composition and fraction within the biofilm may lead to different CV values. For all of the above reasons, it is difficult to select one of these two methods as the best biofilm assessment method and, therefore, both should be analyzed.

On the other hand, for similar NPs concentrations, AgNPs presented less CV values than AuNPs (in total and also per suspended biomass). As for the CC analysis, it could be concluded that the silver nanoparticles seem to affect more the ability of the studied *P. aeruginosa* strains to form biofilms. Again, a much larger difference was found between the AuNPs and AgNPs effect for the PA 3081 strain than for the PA 3777 strain. As a result, it could be observed that the larger effect on the adhesion abilities, by the CV method, seems to be obtained by the use of AgNPs in the PA 3081 strain, contrary to the PA 3777 strain observed in the CC method.

9.2.2.3. MTT ASSAY

The MTT assay was used with the intent of measuring the biofilm cell metabolic activity and, thus, indirectly the biofilm cell viability. When cells die, their dehydrogenase system becomes inactive and can no longer convert the MTT into formazan. Thus, the MTT assay signals only the viable cells (Riss *et al.*, 2013).

The MTT assay results for PA 3777 and PA 3081, cultivated in a shaker for 24 h, at 150 rpm, and 30ºC or 37 ºC, with AgNPs are presented in Figure 25.

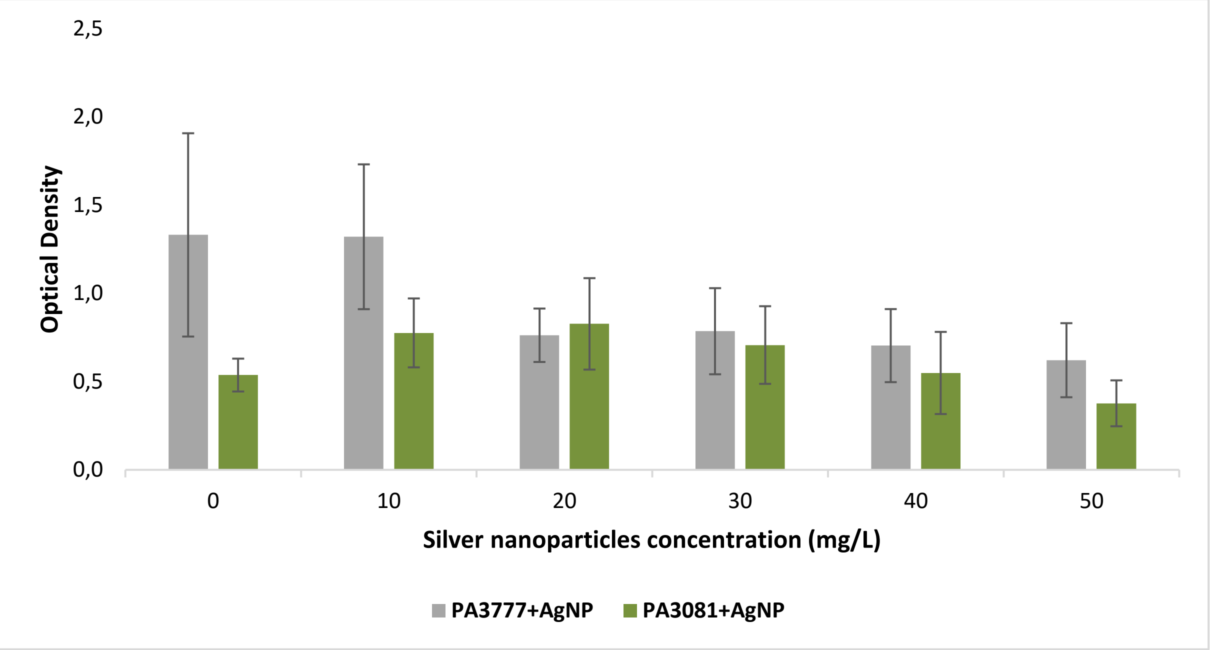

Figure 25 - Graphical representation of the MTT assay, for PA3777 and PA3081, with Ag nanoparticles.

The MTT assay results for PA 3777and PA 3081, cultivated in a shaker for 24 h, at 150 rpm, and 30ºC or 37 ºC, with AuNPs are presented in Figure 26.

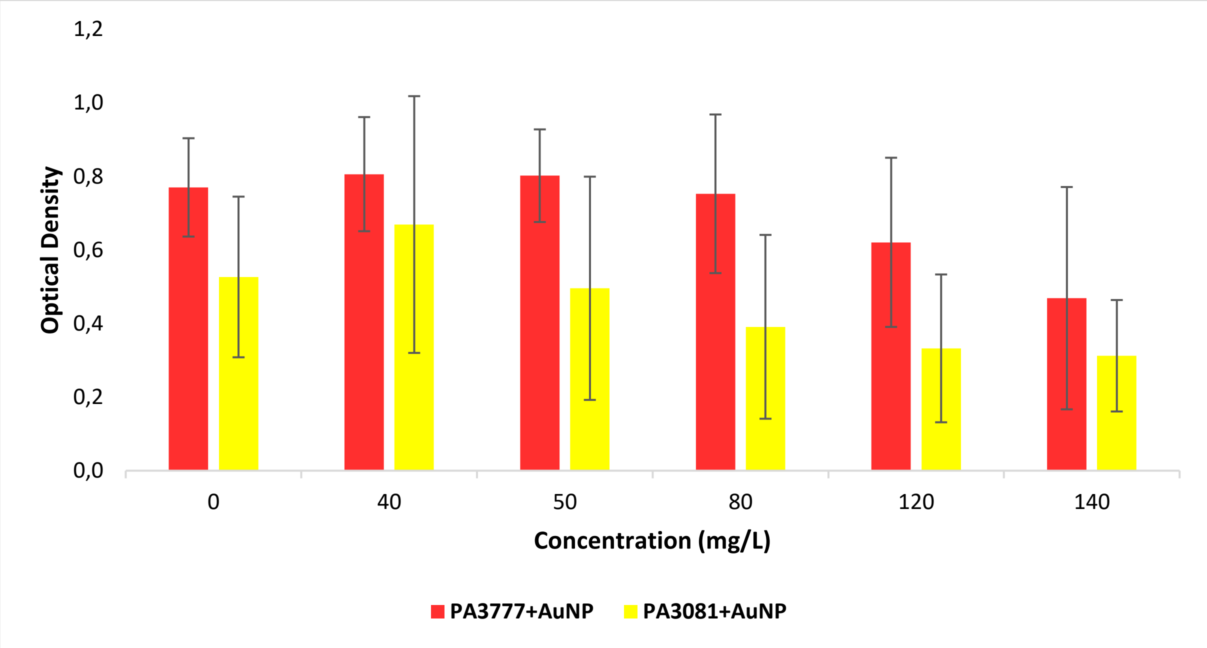

Figure 26 - Graphical representation of the MTT assay, for PA3777 and PA3081, with Au nanoparticles.

Analyzing Figure 25 and Figure 26 it can be observed that the PA 3777 strain presented larger absolute MTT values than the PA 3081 strain for both AgNPs and AuNPs. It could also be observed that the larger difference between the two strains was found for AuNPs. Furthermore, the PA 3777 strain also presented slightly larger MTT values normalized per CC than the PA 3081 strain. Therefore, it could be inferred that the biofilm metabolic activity of the PA 3081 strain seems to be more affected that the one of the PA 3777 strain. Thus, this fact is more in accordance with the CV results, pointing towards a lesser effect on the biofilm formation for PA 3777 than the CC results (which presented somewhat mixed results). However, a mixed behavior was found comparing the AuNPs effect, with larger MTT/CC values for the PA 3777 strain and the AgNPs with an opposite trend.

When comparing the effect of the two studied nanoparticles, and for similar NPs concentrations, the AgNPs presented slightly less MTT values than the AuNPs in absolute values, but larger when normalized by the adhered biomass (in terms of CC). This effect was more pronounced for the PA 3081 strain. Again, the absolute MTT results point towards an increased effect of the AgNPs, with respect to the AuNPs, in the studied *P. aeruginosa* strains. However, the normalized MTT results (per CC) were not in accordance with the absolute value trend, with AgNPs presenting an overall value 1.5 higher than AuNPs, and although, it should be kept in mind the limitations of the CC analysis.

It should be kept in mind that, as it was pointed out by Ulukaya *et al.* (2008), the MTT assay results can overestimate the cell viability, thus underestimating the NPs inhibition, due to the fact that, although dead, the cells still retain some formazan for a period of time.

Nonetheless, and although the limitations presented above for the MTT assay, this test has already been used by, among others, Kumar & Ganesan (2012) to determine the cell viability in a cytotoxicity assay of gold nanoparticles with different stabilizing agents (citrate, starch, and Arabic gum). These authors used three different assays (MTT, neutral red and lactate dehydrogenase assay) to determine cell viability at different times of exposure. The obtained results were similar in all the three assays, proving MTT as a reliable test for cell viability determination. Furthermore, they also concluded the decrease of the studied microorganisms' cell viability with the increase of the tested AuNP concentrations (20, 50, 80, 110, and 140 μg/mL).

10. CONCLUSION

In this work, five different methods of testing NPs (gold and silver) efficiency on reducing bacteria growth were performed: Bacterial suspension growth analysis in *Biosreen C* with a wideband between 420-580 over 24h, produced inducers (by the HSL method), biofilm formation by the cell confluence (CC) and crystal violet (CV) methods, and metabolic activity by the MTT method. To this end, *P. aeruginosa* 3777 and 3081 were grown in LB Media for 24 hours and then diluted to a 0.6 absorbance at 600 nm, achieving a bulk suspension, from where bacteria for the tests was taken. With these tests, it was proven, to a certain extent, the NPs toxicity effect on *P. aeruginosa* 3777 and 3081, both in suspension and in regarding biofilm formation.

Regarding the biomass suspension growth study, the PA 3777 strain seems to be more affected than the PA 3081, and thus more resistant to the NPs toxicity, presenting lower growth abilities in comparison. Furthermore, the silver nanoparticles demonstrated to be more effective than the gold nanoparticles, needing lower nanoparticles concentrations to obtain the same effect.

Regarding the biofilm studies, it was verified, for the CC results, a tendency for the PA 3777 to be more affected by the NPs presence, than PA 3081, being the biofilm covered area for the first slightly higher. On the contrary, the CV results, point towards the opposite, establishing a strong decreasing trend in the biofilm contents, stained by this method encompassing both strains, but especially PA3081. Given the obtained results, it is important to emphasize that the existing differences between these two methods, may account for such disparities between the two methods. Additionally, AgNPs, seemed to have higher effect on biofilm growth, in both assays (CV and CC), than AuNPs. Indeed, for both strains, there was a greater effect on the prevention of biofilm formation as a result of the presence of silver nanoparticles (for concentrations similar to gold NPs).

With respect to the HSL assay, the PA 3777 produced more autoinducers, both in absolute terms and per suspended biomass contents, than PA 3081. It was also noticeable a higher production of inductors as a result of the presence of silver nanoparticles (for similar concentrations to gold NPs). However, when normalized by the adhesion ability (in terms of CC), the results seem to indicate that PA 3777 may present a larger use of HSL communication for other purposes than just triggering adhesion. For similar concentrations, the AgNPs induced the production of larger amount of HSL, for both strains, in absolute terms. However, when these values were normalized per suspended biomass mixed results, regarding the two strains, were obtained.

The MTT assay, assessing the biofilm metabolic activity, indicated a higher production of formazan for PA 3777 than for PA 3081, mainly in absolute values. For similar concentrations, the AgNPs led to a slightly lower metabolic activity on both strains, in absolute terms. However, when these values were normalized by the adhered biomass (CC), a strong inversion occurred.

Further investigation would be necessary to completely elucidate why PA 3081 resistance to the silver and gold nanoparticles seems to be higher than PA 3777 in bulk medium growth, but contrary in biofilm formation (at least in terms of CV). Moreover, the complete role of the studied autoiducers, beyond the biofilm formation triggering, should also be further investigated, and thus account for their larger production in the performed analysis, which could not be attributed to bacterial adhesion. Additionally, it would also be interesting to determine if the CV stains more heavily bacteria than EPS, or otherwise, and to perfect this methodology in order to obtain a better assessment of the bacteria contents within the biofilm.

In conclusion, this work permitted further insight on the silver and gold NPs effect over two *P. aeruginosa* strains (3777 and 3081), and allowed to help understanding the importance of NPs in the modern world, and why there should be further research in this field.

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12. APPENDIX

The followed tables presented in this section permitted the elaboration of the chapters 9.1 and 9.2 . The tables provide the compilation of the data obtained during the experiments to verify the effect of gold and silver nanoparticles, studied for two different strains of *Pseudomonas aeruginosa*: 3777 and 3081.

12.1. BACTERIAL SUSPENSION GROWTH ANALYSIS

Table 7, Table 8, Table 9 and Table 10 present the volume of nanoparticles, inoculum and LB media, used for the different pretended concentrations, to analyse *Pseudomonas aeruginosa* bacterial suspension growth in the presence of different concentrations of silver and gold nanoparticles.

Table 7 - Volume of silver nanoparticles, PA 3777 inoculum and LB media, used for bacterial suspension growth to analyze the AgNPs efficiency.

Well Number	AgNP (mg/L)	AgNP (µL)	LB Media (µL)	PA 3777 Inoculum (µL)
1	0.0	0.0	290.0	30.0
2	0.0	0.0	290.0	30.0
3	0.0	0.0	290.0	30.0
4	0.0	0.0	290.0	30.0
5	0.0	0.0	290.0	30.0
6	10.0	31.4	258.6	30.0
7	10.0	31.4	258.6	30.0
8	10.0	31.4	258.6	30.0
9	10.0	31.4	258.6	30.0
10	10.0	31.4	258.6	30.0
11	13.0	40.8	249.2	30.0
12	13.0	40.8	249.2	30.0
13	13.0	40.8	249.2	30.0
14	13.0	40.8	249.2	30.0
15	13.0	40.8	249.2	30.0
16	16.0	50.2	239.8	30.0
17	16.0	50.2	239.8	30.0
18	16.0	50.2	239.8	30.0
19	16.0	50.2	239.8	30.0
20	16.0	50.2	239.8	30.0
21	19.0	59.6	230.4	30.0
22	19.0	59.6	230.4	30.0
23	19.0	59.6	230.4	30.0
24	19.0	59.6	230.4	30.0
25	19.0	59.6	230.4	30.0
26	22.0	69.0	221.0	30.0
27	22.0	69.0	221.0	30.0
28	22.0	69.0	221.0	30.0
29	22.0	69.0	221.0	30.0
30	22.0	69.0	221.0	30.0
31	25.0	78.4	211.6	30.0
32	25.0	78.4	211.6	30.0
33	25.0	78.4	211.6	30.0
34	25.0	78.4	211.6	30.0
35	25.0	78.4	211.6	30.0
36	28.0	87.8	202.2	30.0

Well Number	AgNP (mg/L)	AgNP (µL)	LB Media (µL)	PA 3777 Inoculum (µL)
37	28.0	87.8	202.2	30.0
38	28.0	87.8	202.2	30.0
39	28.0	87.8	202.2	30.0
40	28.0	87.8	202.2	30.0

Table 8 - Volume of silver nanoparticles, PA 3801 inoculum and LB media, used for bacterial growth to analyze AgNPs the efficiency.

Well Number	AgNP (mg/L)	AgNP (µL)	LB Media (µL)	PA 3081 Inoculum (µLl)
1	0.0	0.0	290.0	30.0
2	0.0	0.0	290.0	30.0
3	0.0	0.0	290.0	30.0
4	0.0	0.0	290.0	30.0
5	0.0	0.0	290.0	30.0
6	20.0	62.7	227.3	30.0
7	20.0	62.7	227.3	30.0
8	20.0	62.7	227.3	30.0
9	20.0	62.7	227.3	30.0
10	20.0	62.7	227.3	30.0
11	22.0	69.0	221.0	30.0
12	22.0	69.0	221.0	30.0
13	22.0	69.0	221.0	30.0
14	22.0	69.0	221.0	30.0
15	22.0	69.0	221.0	30.0
16	24.0	75.3	214.7	30.0
17	24.0	75.3	214.7	30.0
18	24.0	75.3	214.7	30.0
19	24.0	75.3	214.7	30.0
20	24.0	75.3	214.7	30.0
21	26.0	81.6	208.4	30.0
22	26.0	81.6	208.4	30.0
23	26.0	81.6	208.4	30.0
24	26.0	81.6	208.4	30.0
25	26.0	81.6	208.4	30.0
26	28.0	87.8	202.2	30.0
27	28.0	87.8	202.2	30.0
28	28.0	87.8	202.2	30.0
29	28.0	87.8	202.2	30.0
30	28.0	87.8	202.2	30.0
31	30.0	94.1	195.9	30.0
32	30.0	94.1	195.9	30.0
33	30.0	94.1	195.9	30.0
34	30.0	94.1	195.9	30.0
35	30.0	94.1	195.9	30.0
36	32.0	100.4	189.6	30.0

Well Number	AgNP (mg/L)	AgNP (µL)	LB Media (µL)	PA 3081 Inoculum (µLl)
37	32.0	100.4	189.6	30.0
38	32.0	100.4	189.6	30.0
39	32.0	100.4	189.6	30.0
40	32.0	100.4	189.6	30.0
41	34.0	106.7	183.3	30.0
42	34.0	106.7	183.3	30.0
43	34.0	106.7	183.3	30.0
44	34.0	106.7	183.3	30.0
45	34.0	106.7	183.3	30.0
46	36.0	112.9	177.1	30.0
47	36.0	112.9	177.1	30.0
48	36.0	112.9	177.1	30.0
49	36.0	112.9	177.1	30.0
50	36.0	112.9	177.1	30.0
51	38.0	119.2	170.8	30.0
52	38.0	119.2	170.8	30.0
53	38.0	119.2	170.8	30.0
54	38.0	119.2	170.8	30.0
55	38.0	119.2	170.8	30.0
56	40.0	125.5	164.5	30.0
57	40.0	125.5	164.5	30.0
58	40.0	125.5	164.5	30.0
59	40.0	125.5	164.5	30.0
60	40.0	125.5	164.5	30.0
61	42.0	131.8	158.2	30.0
62	42.0	131.8	158.2	30.0
63	42.0	131.8	158.2	30.0
64	42.0	131.8	158.2	30.0
65	42.0	131.8	158.2	30.0
66	44.0	138.0	152.0	30.0
67	44.0	138.0	152.0	30.0
68	44.0	138.0	152.0	30.0
69	44.0	138.0	152.0	30.0
70	44.0	138.0	152.0	30.0
71	46.0	144.3	145.7	30.0
72	46.0	144.3	145.7	30.0
73	46.0	144.3	145.7	30.0
74	46.0	144.3	145.7	30.0
75	46.0	144.3	145.7	30.0
76	48.0	150.6	139.4	30.0
77	48.0	150.6	139.4	30.0
78	48.0	150.6	139.4	30.0
79	48.0	150.6	139.4	30.0
80	48.0	150.6	139.4	30.0

Table 9 - Volume of gold nanoparticles, PA 3777 inoculum and LB media, used for bacterial growth to analyze the AuNPs efficiency.

Well Number	AuNP (mg/L)	AuNP (µL)	LB Media (µL)	PA 3777 Inoculum (µL)
1	0.0	0.0	290.0	30.0
2	0.0	0.0	290.0	30.0
3	0.0	0.0	290.0	30.0
4	0.0	0.0	290.0	30.0
5	0.0	0.0	290.0	30.0
6	5.0	4.4	285.6	30.0
7	5.0	4.4	285.6	30.0
8	5.0	4.4	285.6	30.0
9	5.0	4.4	285.6	30.0
10	5.0	4.4	285.6	30.0
11	8.0	7.1	282.9	30.0
12	8.0	7.1	282.9	30.0
13	8.0	7.1	282.9	30.0
14	8.0	7.1	282.9	30.0
15	8.0	7.1	282.9	30.0
16	10.0	8.9	281.1	30.0
17	10.0	8.9	281.1	30.0
18	10.0	8.9	281.1	30.0
19	10.0	8.9	281.1	30.0
20	10.0	8.9	281.1	30.0
21	12.0	10.7	279.3	30.0
22	12.0	10.7	279.3	30.0
23	12.0	10.7	279.3	30.0
24	12.0	10.7	279.3	30.0
25	12.0	10.7	279.3	30.0
26	15.0	13.3	276.7	30.0
27	15.0	13.3	276.7	30.0
28	15.0	13.3	276.7	30.0
29	15.0	13.3	276.7	30.0
30	15.0	13.3	276.7	30.0
31	20.0	17.8	272.2	30.0
32	20.0	17.8	272.2	30.0
33	20.0	17.8	272.2	30.0
34	20.0	17.8	272.2	30.0
35	20.0	17.8	272.2	30.0
36	22.0	19.6	270.4	30.0
37	22.0	19.6	270.4	30.0
38	22.0	19.6	270.4	30.0
39	22.0	19.6	270.4	30.0
40	22.0	19.6	270.4	30.0
41	24.0	21.3	268.7	30.0
42	24.0	21.3	268.7	30.0

Well Number	AuNP (mg/L)	AuNP (µL)	LB Media (µL)	PA 3777 Inoculum (µL)
43	24.0	21.3	268.7	30.0
44	24.0	21.3	268.7	30.0
45	24.0	21.3	268.7	30.0
46	26.0	23.1	266.9	30.0
47	26.0	23.1	266.9	30.0
48	26.0	23.1	266.9	30.0
49	26.0	23.1	266.9	30.0
50	26.0	23.1	266.9	30.0
51	28.0	24.9	265.1	30.0
52	28.0	24.9	265.1	30.0
53	28.0	24.9	265.1	30.0
54	28.0	24.9	265.1	30.0
55	28.0	24.9	265.1	30.0
56	30.0	26.7	263.3	30.0
57	30.0	26.7	263.3	30.0
58	30.0	26.7	263.3	30.0
59	30.0	26.7	263.3	30.0
60	30.0	26.7	263.3	30.0
61	32.0	28.4	261.6	30.0
62	32.0	28.4	261.6	30.0
63	32.0	28.4	261.6	30.0
64	32.0	28.4	261.6	30.0
65	32.0	28.4	261.6	30.0

Table 10 - Volume of gold nanoparticles, PA 3801 inoculum and LB media, used for bacterial growth to analyze the AuNPs efficiency.

Well Number	AuNP (mg/L)	AuNP (µL)	LB Media (µL)	PA 3081 Inoculum (µL)
1	0.0	0.0	290.0	30.0
2	0.0	0.0	290.0	30.0
3	0.0	0.0	290.0	30.0
4	0.0	0.0	290.0	30.0
5	0.0	0.0	290.0	30.0
6	80.0	71.1	218.9	30.0
7	80.0	71.1	218.9	30.0
8	80.0	71.1	218.9	30.0
9	80.0	71.1	218.9	30.0
10	80.0	71.1	218.9	30.0
11	83.0	73.8	216.2	30.0
12	83.0	73.8	216.2	30.0
13	83.0	73.8	216.2	30.0
14	83.0	73.8	216.2	30.0
15	83.0	73.8	216.2	30.0
16	86.0	76.4	213.6	30.0
17	86.0	76.4	213.6	30.0

Well Number	AuNP (mg/L)	AuNP (µL)	LB Media (µL)	PA 3081 Inoculum (µL)
18	86.0	76.4	213.6	30.0
19	86.0	76.4	213.6	30.0
20	86.0	76.4	213.6	30.0
21	89.0	79.1	210.9	30.0
22	89.0	79.1	210.9	30.0
23	89.0	79.1	210.9	30.0
24	89.0	79.1	210.9	30.0
25	89.0	79.1	210.9	30.0
26	92.0	81.8	208.2	30.0
27	92.0	81.8	208.2	30.0
28	92.0	81.8	208.2	30.0
29	92.0	81.8	208.2	30.0
30	92.0	81.8	208.2	30.0
31	95.0	84.4	205.6	30.0
32	95.0	84.4	205.6	30.0
33	95.0	84.4	205.6	30.0
34	95.0	84.4	205.6	30.0
35	95.0	84.4	205.6	30.0
36	98.0	87.1	202.9	30.0
37	98.0	87.1	202.9	30.0
38	98.0	87.1	202.9	30.0
39	98.0	87.1	202.9	30.0
40	98.0	87.1	202.9	30.0
41	101.0	89.8	200.2	30.0
42	101.0	89.8	200.2	30.0
43	101.0	89.8	200.2	30.0
44	101.0	89.8	200.2	30.0
45	101.0	89.8	200.2	30.0
46	104.0	92.4	197.6	30.0
47	104.0	92.4	197.6	30.0
48	104.0	92.4	197.6	30.0
49	104.0	92.4	197.6	30.0
50	104.0	92.4	197.6	30.0

Table 11, Table 12, Table 13 and Table 14 present the optical density data, measured between 420-580 nm, collected from *Bioscreen C*, used to analyze the efficiency of the nanoparticles in a suspension with PA, LB media and gold or silver NPs, every 30 minutes during 24-hour periods.

Table 11 – Optical density from *Bioscreen C* for *Pseudomonas aeruginosa* 3777 with silver nanoparticles.

Time (h)	0 mg/L	10 mg/L	13 mg/L	16 mg/L	19 mg/L	22 mg/L	25 mg/L	28 mg/L
0.0	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
0.5	0.215	0.194	0.195	0.192	0.189	0.192	0.195	0.197
1.0	0.219	0.194	0.194	0.193	0.188	0.193	0.195	0.194
1.5	0.221	0.191	0.197	0.193	0.190	0.193	0.195	0.195
2.0	0.237	0.191	0.195	0.191	0.188	0.193	0.194	0.194
2.5	0.261	0.191	0.195	0.191	0.187	0.192	0.194	0.195
3.0	0.288	0.186	0.190	0.187	0.188	0.191	0.192	0.195
3.5	0.324	0.187	0.189	0.187	0.187	0.192	0.193	0.195
4.0	0.365	0.196	0.188	0.185	0.185	0.190	0.191	0.191
4.5	0.408	0.192	0.188	0.184	0.182	0.189	0.190	0.190
5.0	0.456	0.191	0.187	0.183	0.182	0.188	0.190	0.187
5.5	0.504	0.196	0.192	0.184	0.181	0.190	0.190	0.188
6.0	0.553	0.200	0.193	0.184	0.181	0.190	0.191	0.189
6.5	0.609	0.202	0.193	0.183	0.182	0.189	0.190	0.187
7.0	0.635	0.208	0.195	0.183	0.180	0.189	0.189	0.185
7.5	0.662	0.214	0.198	0.183	0.179	0.190	0.190	0.186
8.0	0.690	0.226	0.200	0.184	0.178	0.189	0.191	0.187
8.5	0.717	0.238	0.199	0.183	0.176	0.187	0.190	0.183
9.0	0.729	0.250	0.200	0.181	0.177	0.187	0.188	0.181
9.5	0.744	0.273	0.202	0.182	0.176	0.188	0.189	0.180
10.0	0.754	0.292	0.206	0.183	0.176	0.188	0.188	0.181
10.5	0.746	0.317	0.212	0.181	0.176	0.186	0.188	0.179
11.0	0.753	0.350	0.222	0.181	0.174	0.186	0.188	0.178
11.5	0.744	0.379	0.238	0.184	0.175	0.186	0.189	0.181
12.0	0.737	0.409	0.255	0.183	0.174	0.187	0.188	0.178

Time (h)	0 mg/L	10 mg/L	13 mg/L	16 mg/L	19 mg/L	22 mg/L	25 mg/L	28 mg/L
12.5	0.735	0.434	0.276	0.182	0.171	0.184	0.185	0.177
13.0	0.733	0.457	0.293	0.183	0.173	0.186	0.188	0.177
13.5	0.743	0.492	0.315	0.183	0.172	0.186	0.187	0.176
14.0	0.744	0.529	0.339	0.181	0.171	0.186	0.187	0.173
14.5	0.756	0.552	0.364	0.182	0.169	0.184	0.185	0.176
15.0	0.761	0.596	0.389	0.181	0.166	0.180	0.182	0.170
15.5	0.766	0.624	0.412	0.177	0.163	0.177	0.180	0.167
16.0	0.773	0.655	0.440	0.180	0.169	0.183	0.182	0.169
16.5	0.788	0.669	0.465	0.181	0.171	0.184	0.186	0.171
17.0	0.792	0.695	0.500	0.183	0.166	0.180	0.184	0.171
17.5	0.798	0.705	0.527	0.177	0.162	0.176	0.179	0.165
18.0	0.803	0.699	0.547	0.178	0.162	0.175	0.180	0.164
18.5	0.809	0.720	0.593	0.180	0.165	0.178	0.182	0.166
19.0	0.814	0.724	0.619	0.185	0.167	0.182	0.185	0.171
19.5	0.817	0.722	0.642	0.184	0.163	0.177	0.181	0.167
20.0	0.827	0.724	0.661	0.183	0.162	0.175	0.178	0.162
20.5	0.823	0.717	0.673	0.188	0.162	0.175	0.178	0.162
21.0	0.826	0.718	0.688	0.190	0.160	0.174	0.178	0.159
21.5	0.832	0.722	0.701	0.194	0.163	0.177	0.181	0.162
22.0	0.828	0.715	0.709	0.198	0.162	0.175	0.180	0.161
22.5	0.834	0.713	0.708	0.198	0.158	0.173	0.178	0.157
23.0	0.826	0.713	0.705	0.205	0.163	0.181	0.183	0.162
23.5	0.823	0.708	0.704	0.211	0.160	0.175	0.180	0.160
24.0	0.817	0.710	0.696	0.212	0.156	0.170	0.176	0.154

Table 12 - Optical density from *Bioscreen C* for *Pseudomonas aeruginosa* 3081 with silver nanoparticles.

Time (h)	**0 mg/L**	**13 mg/L**	**15 mg/L**	**17 mg/L**	**18 mg/L**	**20 mg/L**	**22 mg/L**	**24 mg/L**	**26 mg/L**	**28 mg/L**	**30 mg/L**	**32 mg/L**	**34 mg/L**	**36 mg/L**	**38 mg/L**	**40 mg/L**
0.0	0.278	0.266	0.272	0.268	0.269	0.264	0.272	0.260	0.274	0.265	0.269	0.269	0.265	0.265	0.276	0.271
0.5	0.255	0.240	0.240	0.240	0.234	0.233	0.233	0.228	0.231	0.228	0.226	0.229	0.226	0.226	0.227	0.227
1.5	0.267	0.243	0.241	0.241	0.235	0.234	0.233	0.229	0.232	0.230	0.225	0.228	0.223	0.223	0.226	0.226
2.0	0.282	0.244	0.241	0.241	0.233	0.231	0.230	0.226	0.229	0.231	0.223	0.225	0.220	0.220	0.223	0.224
2.5	0.313	0.248	0.245	0.244	0.235	0.232	0.231	0.225	0.230	0.238	0.223	0.226	0.222	0.222	0.226	0.226
3.0	0.363	0.260	0.258	0.253	0.243	0.238	0.235	0.228	0.229	0.249	0.224	0.227	0.223	0.223	0.227	0.226
3.5	0.441	0.278	0.269	0.263	0.248	0.243	0.238	0.228	0.230	0.266	0.222	0.227	0.224	0.224	0.228	0.227
4.0	0.545	0.310	0.288	0.276	0.257	0.250	0.242	0.229	0.229	0.287	0.220	0.225	0.223	0.223	0.228	0.226
4.5	0.649	0.369	0.328	0.306	0.275	0.265	0.254	0.236	0.233	0.308	0.221	0.225	0.224	0.224	0.230	0.227
5.0	0.706	0.456	0.395	0.358	0.308	0.289	0.269	0.246	0.239	0.319	0.223	0.227	0.225	0.225	0.231	0.229
5.5	0.746	0.548	0.480	0.430	0.355	0.325	0.294	0.261	0.249	0.332	0.227	0.230	0.229	0.229	0.236	0.233
6.0	0.790	0.596	0.576	0.524	0.432	0.382	0.328	0.280	0.257	0.341	0.224	0.224	0.224	0.224	0.231	0.226
6.5	0.828	0.655	0.605	0.574	0.515	0.460	0.387	0.313	0.274	0.358	0.229	0.228	0.227	0.227	0.234	0.231
7.0	0.859	0.730	0.688	0.632	0.561	0.529	0.464	0.363	0.299	0.374	0.232	0.227	0.224	0.224	0.228	0.226
7.5	0.890	0.795	0.761	0.713	0.630	0.578	0.537	0.443	0.350	0.403	0.244	0.232	0.227	0.227	0.232	0.230
8.0	0.920	0.845	0.814	0.778	0.720	0.659	0.582	0.512	0.416	0.436	0.258	0.235	0.230	0.230	0.233	0.232
8.5	0.950	0.895	0.862	0.831	0.791	0.741	0.675	0.559	0.489	0.486	0.276	0.238	0.229	0.229	0.238	0.233
9.0	0.973	0.938	0.906	0.877	0.848	0.802	0.754	0.638	0.531	0.546	0.305	0.241	0.226	0.226	0.233	0.230
9.5	0.997	0.977	0.947	0.923	0.899	0.859	0.820	0.723	0.608	0.596	0.360	0.250	0.225	0.225	0.229	0.226
10.0	1.016	1.015	0.988	0.969	0.953	0.915	0.882	0.803	0.696	0.645	0.436	0.268	0.225	0.225	0.229	0.227
10.5	1.036	1.045	1.023	1.008	1.003	0.966	0.940	0.869	0.789	0.719	0.503	0.303	0.229	0.229	0.236	0.233
11.0	1.054	1.067	1.046	1.035	1.036	1.005	0.985	0.928	0.866	0.786	0.537	0.338	0.224	0.224	0.229	0.226
11.5	1.068	1.094	1.069	1.063	1.061	1.038	1.024	0.981	0.931	0.857	0.621	0.368	0.223	0.223	0.227	0.225
12.0	1.082	1.108	1.091	1.087	1.089	1.069	1.062	1.028	0.995	0.925	0.710	0.415	0.227	0.227	0.232	0.229
12.5	1.095	1.120	1.107	1.103	1.109	1.090	1.085	1.062	1.042	0.980	0.793	0.454	0.223	0.223	0.227	0.224

Time (h)	0 mg/L	13 mg/L	15 mg/L	17 mg/L	18 mg/L	20 mg/L	22 mg/L	24 mg/L	26 mg/L	28 mg/L	30 mg/L	32 mg/L	34 mg/L	36 mg/L	38 mg/L	40 mg/L
13.0	1.111	1.135	1.123	1.120	1.126	1.114	1.110	1.096	1.087	1.035	0.879	0.507	0.226	0.226	0.229	0.225
13.5	1.127	1.148	1.137	1.135	1.138	1.131	1.127	1.117	1.118	1.074	0.947	0.561	0.227	0.227	0.234	0.229
14.0	1.137	1.157	1.147	1.147	1.151	1.147	1.140	1.136	1.136	1.107	1.003	0.616	0.227	0.227	0.231	0.226
14.5	1.146	1.165	1.159	1.157	1.165	1.156	1.154	1.147	1.157	1.131	1.043	0.672	0.224	0.224	0.224	0.219
15.0	1.157	1.177	1.174	1.173	1.180	1.170	1.169	1.163	1.165	1.152	1.077	0.734	0.229	0.229	0.226	0.220
15.5	1.171	1.186	1.185	1.184	1.191	1.184	1.183	1.178	1.185	1.161	1.100	0.790	0.236	0.236	0.225	0.219
16.0	1.190	1.195	1.197	1.195	1.202	1.197	1.198	1.192	1.197	1.175	1.119	0.831	0.248	0.248	0.229	0.222
16.5	1.200	1.202	1.206	1.203	1.218	1.206	1.210	1.205	1.206	1.180	1.125	0.857	0.263	0.263	0.225	0.216
17.0	1.219	1.218	1.221	1.217	1.240	1.227	1.231	1.223	1.220	1.187	1.137	0.898	0.300	0.300	0.235	0.221
17.5	1.241	1.235	1.231	1.228	1.257	1.242	1.252	1.235	1.223	1.182	1.137	0.932	0.345	0.345	0.235	0.215
18.0	1.277	1.253	1.250	1.253	1.274	1.271	1.274	1.254	1.225	1.180	1.144	0.969	0.398	0.398	0.251	0.218
18.5	1.306	1.257	1.270	1.267	1.264	1.288	1.274	1.245	1.214	1.178	1.147	1.003	0.446	0.446	0.270	0.219
19.0	1.357	1.261	1.299	1.279	1.263	1.280	1.261	1.231	1.210	1.176	1.146	1.024	0.492	0.492	0.298	0.219
19.5	1.407	1.261	1.305	1.292	1.259	1.270	1.257	1.230	1.207	1.178	1.149	1.049	0.548	0.548	0.343	0.231
20.0	1.441	1.262	1.294	1.295	1.257	1.264	1.249	1.225	1.202	1.176	1.143	1.057	0.607	0.607	0.386	0.237
20.5	1.546	1.266	1.289	1.289	1.255	1.263	1.250	1.225	1.203	1.180	1.141	1.069	0.679	0.679	0.452	0.259
21.0	1.567	1.271	1.288	1.285	1.253	1.260	1.247	1.222	1.199	1.180	1.137	1.072	0.736	0.736	0.494	0.278
21.5	1.580	1.282	1.292	1.287	1.257	1.260	1.246	1.223	1.198	1.182	1.136	1.076	0.790	0.790	0.544	0.310
22.0	1.638	1.294	1.297	1.290	1.258	1.260	1.246	1.223	1.197	1.186	1.139	1.081	0.842	0.842	0.601	0.350
22.5	1.623	1.308	1.302	1.293	1.260	1.257	1.241	1.217	1.192	1.184	1.133	1.078	0.881	0.881	0.662	0.391
23.0	1.614	1.327	1.314	1.300	1.268	1.261	1.241	1.221	1.193	1.190	1.137	1.085	0.921	0.921	0.729	0.432
23.5	1.652	1.347	1.323	1.308	1.274	1.263	1.246	1.221	1.193	1.192	1.137	1.085	0.953	0.953	0.784	0.478
24.0	1.677	1.371	1.336	1.318	1.281	1.269	1.248	1.226	1.193	1.192	1.134	1.082	0.974	0.974	0.823	0.527
24.5	1.676	1.394	1.349	1.328	1.290	1.273	1.250	1.226	1.192	1.190	1.130	1.078	0.989	0.989	0.859	0.586

Table 13 - Optical density from *Bioscreen C* for *Pseudomonas aeruginosa* 3777 with gold nanoparticles.

Time (h)	0 mg/L	5 mg/L	6 mg/L	8 mg/L	10 mg/L	12 mg/L	15 mg/L	20 mg/L	22 mg/L	24 mg/L	26 mg/L	28 mg/L	30 mg/L	32 mg/L
0.0	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
0.5	0.102	0.102	0.105	0.104	0.109	0.117	0.108	0.118	0.127	0.116	0.117	0.125	0.123	0.207
1.0	0.115	0.112	0.114	0.111	0.117	0.122	0.114	0.123	0.132	0.118	0.117	0.127	0.124	0.204
1.5	0.138	0.130	0.131	0.126	0.131	0.136	0.126	0.133	0.141	0.125	0.124	0.134	0.132	0.207
2.0	0.156	0.143	0.145	0.141	0.145	0.153	0.142	0.146	0.155	0.136	0.133	0.141	0.139	0.214
2.5	0.175	0.131	0.128	0.125	0.131	0.137	0.125	0.131	0.142	0.124	0.119	0.131	0.130	0.222
3.0	0.207	0.180	0.179	0.172	0.177	0.177	0.164	0.168	0.166	0.154	0.147	0.161	0.159	0.233
3.5	0.253	0.218	0.217	0.203	0.206	0.204	0.187	0.190	0.185	0.171	0.164	0.178	0.176	0.247
4.0	0.303	0.258	0.256	0.238	0.240	0.238	0.218	0.214	0.208	0.194	0.184	0.198	0.196	0.263
4.5	0.350	0.297	0.292	0.271	0.271	0.262	0.239	0.233	0.224	0.208	0.195	0.213	0.210	0.280
5.0	0.403	0.340	0.337	0.312	0.308	0.298	0.269	0.258	0.246	0.228	0.215	0.232	0.227	0.302
5.5	0.452	0.383	0.379	0.352	0.346	0.336	0.301	0.282	0.269	0.248	0.235	0.249	0.243	0.318
6.0	0.490	0.414	0.410	0.381	0.372	0.362	0.323	0.298	0.284	0.261	0.249	0.261	0.254	0.339
6.5	0.526	0.441	0.438	0.408	0.398	0.390	0.346	0.318	0.301	0.278	0.264	0.277	0.270	0.356
7.0	0.565	0.479	0.474	0.437	0.423	0.416	0.370	0.339	0.321	0.296	0.280	0.294	0.287	0.367
7.5	0.618	0.533	0.531	0.486	0.467	0.452	0.403	0.363	0.343	0.318	0.300	0.314	0.308	0.376
8.0	0.670	0.590	0.592	0.543	0.518	0.499	0.443	0.391	0.364	0.337	0.320	0.331	0.324	0.394
8.5	0.724	0.649	0.652	0.603	0.577	0.558	0.496	0.433	0.398	0.364	0.343	0.352	0.344	0.412
9.0	0.779	0.704	0.710	0.660	0.632	0.620	0.550	0.479	0.440	0.401	0.375	0.380	0.369	0.416
9.5	0.827	0.751	0.756	0.706	0.678	0.668	0.595	0.518	0.475	0.433	0.407	0.408	0.393	0.431
10.0	0.873	0.794	0.799	0.748	0.720	0.712	0.636	0.555	0.511	0.465	0.440	0.436	0.419	0.432
10.5	0.914	0.830	0.835	0.783	0.755	0.748	0.672	0.588	0.540	0.492	0.469	0.462	0.446	0.441
11.0	0.955	0.864	0.867	0.814	0.787	0.781	0.704	0.615	0.568	0.518	0.496	0.487	0.472	0.451
11.5	0.954	0.857	0.859	0.808	0.782	0.775	0.695	0.611	0.563	0.514	0.492	0.481	0.466	0.455
12.0	1.042	0.940	0.930	0.876	0.852	0.846	0.764	0.674	0.623	0.573	0.552	0.541	0.525	0.460

Time (h)	0 mg/L	5 mg/L	6 mg/L	8 mg/L	10 mg/L	12 mg/L	15 mg/L	20 mg/L	22 mg/L	24 mg/L	26 mg/L	28 mg/L	30 mg/L	32 mg/L
12.5	1.079	0.964	0.960	0.903	0.879	0.872	0.788	0.701	0.647	0.600	0.580	0.565	0.552	0.462
13.0	1.121	0.997	0.990	0.930	0.907	0.896	0.811	0.725	0.671	0.623	0.607	0.588	0.575	0.463
13.5	1.158	1.034	1.028	0.956	0.940	0.929	0.841	0.754	0.702	0.658	0.645	0.617	0.599	0.466
14.0	1.184	1.057	1.053	0.987	0.965	0.954	0.863	0.774	0.721	0.683	0.661	0.633	0.611	0.468
14.5	1.212	1.082	1.080	1.014	0.996	0.987	0.894	0.801	0.745	0.701	0.677	0.652	0.626	0.468
15.0	1.240	1.103	1.103	1.036	1.023	1.018	0.920	0.817	0.763	0.715	0.691	0.668	0.642	0.472
15.5	1.265	1.123	1.124	1.057	1.044	1.042	0.943	0.831	0.777	0.727	0.700	0.677	0.643	0.473
16.0	1.295	1.143	1.150	1.081	1.067	1.067	0.965	0.850	0.796	0.739	0.712	0.688	0.652	0.474
16.5	1.311	1.157	1.166	1.101	1.083	1.081	0.980	0.863	0.807	0.744	0.717	0.692	0.653	0.474
17.0	1.330	1.175	1.183	1.123	1.104	1.095	0.995	0.874	0.814	0.748	0.721	0.695	0.654	0.477
17.5	1.353	1.196	1.204	1.146	1.124	1.114	1.009	0.885	0.823	0.753	0.727	0.699	0.658	0.478
18.0	1.370	1.211	1.218	1.163	1.137	1.121	1.014	0.886	0.825	0.751	0.728	0.696	0.656	0.474
18.5	1.385	1.219	1.234	1.180	1.154	1.135	1.024	0.894	0.830	0.753	0.732	0.698	0.659	0.478
19.0	1.399	1.223	1.241	1.193	1.162	1.142	1.029	0.898	0.830	0.752	0.733	0.697	0.661	0.475
19.5	1.425	1.230	1.250	1.204	1.173	1.155	1.036	0.903	0.835	0.754	0.736	0.701	0.668	0.470
20.0	1.434	1.238	1.256	1.208	1.174	1.159	1.037	0.903	0.834	0.751	0.734	0.700	0.667	0.468
20.5	1.451	1.253	1.267	1.217	1.175	1.166	1.041	0.907	0.836	0.751	0.736	0.702	0.672	0.462
21.0	1.461	1.262	1.275	1.224	1.175	1.175	1.047	0.913	0.842	0.754	0.738	0.707	0.678	0.454
21.5	1.465	1.266	1.271	1.223	1.167	1.174	1.048	0.913	0.842	0.751	0.737	0.708	0.677	0.452
22.0	1.473	1.273	1.278	1.218	1.157	1.174	1.051	0.915	0.842	0.754	0.740	0.713	0.682	0.448
22.5	1.474	1.278	1.281	1.213	1.152	1.169	1.051	0.913	0.840	0.752	0.738	0.713	0.681	0.443
23.0	1.482	1.284	1.292	1.215	1.154	1.166	1.055	0.914	0.846	0.756	0.742	0.717	0.684	0.435
23.5	1.488	1.290	1.298	1.219	1.157	1.164	1.057	0.918	0.846	0.760	0.745	0.721	0.685	0.436
24.0	1.497	1.293	1.298	1.223	1.160	1.158	1.058	0.918	0.849	0.766	0.750	0.726	0.687	0.429

Table 14 - Optical density from *Bioscreen C* for *Pseudomonas aeruginosa* 3081 with gold nanoparticles.

Time (h)	0 mg/L	80 mg/L	83 mg/L	86 mg/L	89 mg/L	92 mg/L	95 mg/L	98 mg/L	101 mg/L	104 mg/L
0.0	0.373	0.920	0.937	0.947	0.962	0.950	0.972	0.966	1.001	1.006
0.5	0.269	0.850	0.853	0.861	0.868	0.859	0.876	0.877	0.896	0.903
1.0	0.283	0.853	0.858	0.867	0.875	0.869	0.884	0.882	0.903	0.908
1.5	0.301	0.867	0.872	0.881	0.890	0.885	0.897	0.893	0.917	0.920
2.0	0.330	0.887	0.893	0.903	0.911	0.906	0.918	0.910	0.935	0.936
2.5	0.383	0.921	0.926	0.937	0.945	0.941	0.952	0.939	0.965	0.963
3.0	0.460	0.962	0.963	0.976	0.984	0.978	0.990	0.974	1.001	0.998
3.5	0.565	0.999	0.999	1.012	1.019	1.009	1.019	1.006	1.030	1.026
4.0	0.681	1.017	1.021	1.027	1.034	1.025	1.036	1.023	1.040	1.040
4.5	0.754	1.030	1.034	1.040	1.049	1.050	1.054	1.036	1.053	1.051
5.0	0.804	1.066	1.065	1.071	1.081	1.088	1.083	1.065	1.082	1.076
5.5	0.860	1.115	1.106	1.116	1.119	1.128	1.120	1.104	1.115	1.107
6.0	0.915	1.158	1.141	1.156	1.153	1.157	1.150	1.140	1.142	1.132
6.5	0.962	1.193	1.177	1.187	1.185	1.180	1.176	1.166	1.163	1.154
7.0	1.003	1.223	1.212	1.214	1.213	1.201	1.198	1.189	1.182	1.174
7.5	1.040	1.251	1.236	1.237	1.234	1.221	1.217	1.209	1.200	1.192
8.0	1.071	1.274	1.258	1.258	1.253	1.238	1.233	1.226	1.215	1.207
8.5	1.103	1.292	1.275	1.271	1.266	1.252	1.246	1.241	1.227	1.217
9.0	1.139	1.310	1.293	1.286	1.282	1.267	1.261	1.254	1.239	1.231
9.5	1.170	1.325	1.308	1.301	1.294	1.277	1.272	1.266	1.250	1.240
10.0	1.196	1.339	1.321	1.314	1.306	1.288	1.282	1.278	1.260	1.249
10.5	1.225	1.353	1.337	1.327	1.321	1.300	1.293	1.290	1.269	1.259
11.0	1.251	1.363	1.347	1.338	1.330	1.307	1.301	1.300	1.276	1.267
11.5	1.277	1.375	1.361	1.352	1.343	1.321	1.316	1.313	1.289	1.278
12.0	1.303	1.383	1.371	1.363	1.353	1.330	1.325	1.323	1.297	1.285
12.5	1.326	1.394	1.383	1.374	1.364	1.344	1.336	1.335	1.307	1.297
13.0	1.345	1.404	1.397	1.390	1.380	1.361	1.352	1.350	1.320	1.310
13.5	1.349	1.419	1.415	1.406	1.396	1.383	1.372	1.369	1.336	1.330
14.0	1.367	1.436	1.436	1.427	1.419	1.406	1.396	1.391	1.363	1.356
14.5	1.390	1.447	1.455	1.451	1.446	1.424	1.426	1.406	1.397	1.381
15.0	1.404	1.452	1.478	1.456	1.463	1.436	1.445	1.410	1.418	1.391
15.5	1.427	1.450	1.479	1.459	1.475	1.435	1.447	1.411	1.417	1.389
16.0	1.444	1.453	1.477	1.459	1.477	1.435	1.445	1.409	1.419	1.391
16.5	1.447	1.451	1.476	1.459	1.475	1.437	1.447	1.410	1.421	1.390
17.0	1.475	1.451	1.475	1.460	1.475	1.438	1.447	1.412	1.422	1.390
17.5	1.492	1.451	1.476	1.462	1.474	1.438	1.448	1.411	1.423	1.391
18.0	1.500	1.451	1.475	1.463	1.474	1.439	1.448	1.416	1.423	1.392
18.5	1.510	1.454	1.473	1.462	1.471	1.437	1.447	1.416	1.423	1.392
19.0	1.530	1.457	1.475	1.463	1.473	1.438	1.447	1.414	1.424	1.391
19.5	1.550	1.458	1.473	1.463	1.470	1.438	1.448	1.412	1.416	1.391
20.0	1.571	1.464	1.482	1.468	1.475	1.445	1.453	1.418	1.425	1.397

Time (h)	**0 mg/L**	**80 mg/L**	**83 mg/L**	**86 mg/L**	**89 mg/L**	**92 mg/L**	**95 mg/L**	**98 mg/L**	**101 mg/L**	**104 mg/L**
20.5	1.589	1.467	1.475	1.465	1.474	1.444	1.443	1.416	1.423	1.393
21.0	1.611	1.470	1.479	1.471	1.474	1.452	1.451	1.419	1.420	1.393
21.5	1.629	1.477	1.478	1.471	1.474	1.451	1.447	1.419	1.423	1.394
22.0	1.650	1.486	1.487	1.477	1.476	1.455	1.450	1.421	1.426	1.397
22.5	1.663	1.494	1.493	1.482	1.482	1.457	1.453	1.425	1.432	1.398
23.0	1.679	1.508	1.499	1.489	1.493	1.463	1.461	1.430	1.431	1.404
23.5	1.691	1.510	1.507	1.496	1.494	1.470	1.466	1.435	1.432	1.406
24.0	1.703	1.522	1.518	1.503	1.504	1.479	1.472	1.445	1.436	1.414

12.2. SUPERNATANT ANALISYS

Table 15, Table 16, Table 17 and Table 18 present the optical density values obtained for the different OD600 trials and biomass concentrations, corrected with the dilution factor, the correspondent average and standard deviation, for each *Pseudomonas aeruginosa* strain, nanoparticles and respective concentrations.

The final suspended biomass concentrations were obtained, using the calibration curve of Equation 2, also present in Figure 27, where x is the OD at 600 nm and y the PA concentration in mg/mL.

$$y = 2.0087 \times \mathrm{x} + 0.0764 \qquad (2)$$

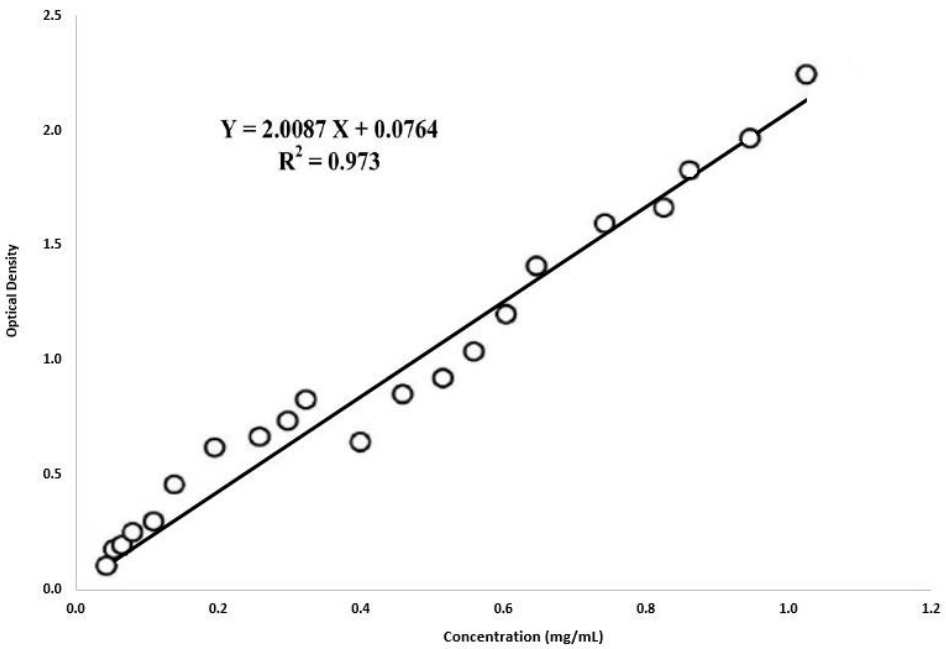

Figure 27 - Calibration curve for conversion of the OD into the final suspended biomass concentration in mg/mL.

Table 15 - OD600 data and biomass concentrations, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AgNP.

Concentration (mg/L)	**Trial OD Values**		**Biomass Concentration Values (mg/L)**		**Final Biomass Average**	**Standard Deviation**
	1	**2**	**1**	**2**		
0	0.8032	0.9837	1689.79	2052.36	2232.40	284.76
	0.9325	1.0227	1949.51	2130.70		
	0.9574	1.0373	1999.53	2160.02		
	0.9754	1.0474	2035.69	2180.31		
	1.0609	1.0972	2207.43	2280.35		
	1.0983	1.1201	2282.56	2326.34		
	1.2629	1.1414	2613.19	2369.13		
	1.4406	1.1923	2970.13	2471.37		
10	0.9690	0.9280	2022.83	1940.47	2105.93	110.33
	0.9842	0.9453	2053.36	1975.22		
	0.9851	0.9541	2055.17	1992.90		
	1.0194	0.9725	2124.07	2029.86		
	1.0308	0.9732	2146.97	2031.27		
	1.0337	1.0151	2152.79	2115.43		
	1.0921	1.0474	2270.10	2180.31		
	1.1132	1.1028	2312.48	2291.59		
20	0.8726	0.8608	1829.19	1805.49	1921.15	67.61
	0.8841	0.8641	1852.29	1812.12		
	0.9271	0.8945	1938.67	1873.18		
	0.9312	0.9073	1946.90	1898.89		
	0.9455	0.9329	1975.63	1950.32		
	0.9496	0.9431	1983.86	1970.80		
	0.9633	0.9491	2011.38	1982.86		
	1.0409*	0.9505	-	1985.67		
30	0.7450	0.7192	1572.88	1521.06	1657.50	102.42
	0.7520	0.7385	1586.94	1559.82		
	0.8010	0.7416	1685.37	1566.05		
	0.8014	0.7487	1686.17	1580.31		
	0.8154	0.7490	1714.29	1580.92		
	0.8220	0.7691	1727.55	1621.29		
	0.8466	0.7701	1776.97	1623.30		
	0.8848	0.8896	1853.70	1863.34		
40	0.6468	0.5799	1375.63	1241.25	1417.02	95.37
	0.6541	0.6211	1390.29	1324.00		
	0.6543	0.6322	1390.69	1346.30		
	0.6658	0.6370	1413.79	1355.94		
	0.6714	0.6479	1425.04	1377.84		
	0.6821	0.6545	1446.53	1391.09		
	0.6925	0.7599	1467.42	1602.81		
	0.7010	0.7780	1484.50	1639.17		

Concentration (mg/L)	Trial OD Values		Biomass Concentration Values (mg/L)		Final Biomass Average	Standard Deviation
	1	2	1	2		
50	0.5042	0.4588	1089.19	997.99	1247.71	125.49
	0.5720	0.5279	1225.38	1136.79		
	0.5771	0.5500	1235.62	1181.19		
	0.5805	0.5557	1242.45	1192.63		
	0.5936	0.5687	1268.76	1218.75		
	0.6072	0.5809	1296.08	1243.25		
	0.6442	0.5980	1370.40	1277.60		
	0.7263	0.6848	1535.32	1451.96		

*Outlier rejected with Dixon's Q test.

Table 16 - OD600 data and biomass concentrations, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AuNP.

Concentration (mg/L)	**Trial OD Values**				**Biomass Concentration Values (mg/L)**				**Final Biomass Average**	**Standard Deviation**
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**		
0	1.0461	0.9774	0.9628	0.8868	2177.70	2039.70	2010.38	1857.72	2275.38	137.83
	1.0799	1.0640	1.0524	1.0443	2245.60	2213.66	2190.36	2174.09		
	1.1634	1.0669	1.0662	1.0619	2413.32	2219.48	2218.08	2209.44		
	1.1710	1.0830	1.0777	1.0664	2428.59	2251.82	2241.18	2218.48		
	1.1802	1.1006	1.0955	1.0702	2447.07	2287.18	2276.93	2226.11		
	1.1865	1.1208	1.1225	1.0779	2459.72	2327.75	2331.17	2241.58		
	1.1941	1.1605	1.1272	1.0872	2474.99	2407.50	2340.61	2260.26		
	1.2042	1.1648	1.1502	1.1187	2495.28	2416.13	2386.81	2323.53		
40	0.6334	0.6179	0.5632	0.5523	1348.71	1317.58	1207.70	1185.80	1633.93	193.16
	0.6591	0.6352	0.6015	0.5653	1400.33	1352.33	1284.63	1211.92		
	0.6977	0.6533	0.6088	0.6011	1477.87	1388.68	1299.30	1283.83		
	0.7201	0.7218	0.6473	0.6294	1522.86	1526.28	1376.63	1340.68		
	0.7231	0.7256	0.6502	0.6789	1528.89	1533.91	1382.46	1440.11		
	0.7371	0.7794	0.6546	0.7176	1557.01	1641.98	1391.30	1517.84		
	0.8272	0.7869	0.6774	0.7210	1738.00	1657.05	1437.09	1524.67		
	0.8643	0.9395	0.6899	0.6423	1812.52	1963.57	1462.20	1366.65		
50	0.5907	0.6375	0.4731	0.4954	1262.94	1356.95	1026.72	1071.51	1615.25	235.16
	0.6269	0.7061	0.4833	0.5420	1335.65	1494.74	1047.20	1165.12		
	0.6503	0.7642	0.5893	0.5597	1382.66	1611.45	1260.13	1200.67		
	0.6517	0.7715	0.5901	0.5754	1385.47	1626.11	1261.73	1232.21		
	0.6587	0.8135	0.6013	0.5870	1399.53	1710.48	1284.23	1255.51		
	0.7020	0.8170	0.6136	0.6145	1486.51	1717.51	1308.94	1310.75		
	0.7111	0.8271	0.6161	0.6266	1504.79	1737.80	1313.96	1335.05		
	0.7293	0.8297	0.6520	0.8323	1541.34	1743.02	1386.07	1748.24		

Concentration (mg/L)	Trial OD Values				Biomass Concentration Values (mg/L)				Final Biomass Average	Standard Deviation
	1	2	3	4	1	2	3	4		
80	0.4138	0.6609	0.5635	0.4748	907.60	1403.95	1208.30	1030.13	1689.45	242.42
	0.4274	0.6864	0.5861	0.4972	934.92	1455.17	1253.70	1075.13		
	0.4949	0.6884	0.5942	0.5353	1070.51	1459.19	1269.97	1151.66		
	0.5301	0.7158	0.6051	0.5671	1141.21	1514.23	1291.86	1215.53		
	0.6037	0.7493	0.6134	0.5803	1289.05	1581.52	1308.54	1242.05		
	0.6052	0.7514	0.6327	0.6080	1292.07	1585.74	1347.30	1297.69		
	0.6202	0.7859	0.6603	0.6377	1322.20	1655.04	1402.74	1357.35		
	0.7120	0.7917	0.6705	0.6596	1506.59	1666.69	1423.23	1401.34		
120	0.3084	0.4009	0.5274	0.5331	695.88	881.69	1135.79	1147.24	1452.51	171.72
	0.4972	0.4657	0.5355	0.5691	1075.13	1011.85	1152.06	1219.55		
	0.5371	0.4915	0.5672	0.5826	1155.27	1063.68	1215.73	1246.67		
	0.5575	0.5446	0.5724	0.5838	1196.25	1170.34	1226.18	1249.08		
	0.6228	0.5696	0.6110	0.5974	1327.42	1220.56	1303.72	1276.40		
	0.6891	0.7845	0.6175	0.5993	1460.60	1652.23	1316.77	1280.21		
	0.7395	0.8675	0.6244	0.6275	1561.83	1818.95	1330.63	1336.86		
	0.7926	0.9675	0.6570	0.6560	1668.50	2019.82	1396.12	-		
140	0.2987	0.2796	0.4616	0.4797	676.40	638.03	1003.62	1039.97	1208.26	202.28
	0.4455	0.4062	0.5521	0.5307	971.28	892.33	1185.40	1142.42		
	0.5313	0.5404	0.5822	0.5818	1143.62	1161.90	1245.87	1245.06		
	0.5490	0.5475	0.5827	0.5819	1179.18	1176.16	1246.87	1245.26		
	0.5685	0.5592	0.6427	0.6024	1218.35	1199.67	1367.39	1286.44		
	0.5823	0.6253	0.6450	0.6288	1246.07	1332.44	1372.01	1339.47		
	0.6505	0.6729	0.6549	0.6391	1383.06	1428.05	1391.90	1360.16		
	0.7358	1.0053*	0.6612	0.6484	1554.40	-	1404.55	1378.84		

*Outlier rejected with Dixon's Q test.

Table 17 - OD600 data and biomass concentrations, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AgNP.

Concentration (mg/L)	Trial OD Values				Biomass Concentration Values (mg/L)				Final Biomass Average	Standard Deviation
	1	2	3	4	1	2	3	4		
0	1.3922	1.3596	0.7695	0.7144	2872.91	2807.43	1622.09	1511.42	2238.99	669.48
	1.3444	1.4203	0.7461	0.6584	2776.90	2929.36	1575.09	1398.93		
	1.4945	1.4023	0.7791	0.7093	3078.40	2893.20	1641.38	1501.17		
	1.3469	1.4452	0.7521	0.7827	2781.92	2979.37	1587.14	1648.61		
	1.2514	1.3839	0.7621	0.8472	2590.09	2856.24	1607.23	1778.17		
	1.6229	1.4202	0.7667	0.7411	3336.32	2929.16	1616.47	1565.05		
	1.3673	1.3984	0.7343	0.7352	2822.90	2885.37	1551.39	1553.20		
	1.2954	1.5059	0.7933	0.7093	2678.47	3101.30	1669.90	1501.17		
10	1.1044	1.4087	0.6509	0.6258	2294.89	2906.06	1383.86	1333.44	2179.18	794.03
	1.3135	1.2664	0.6501	0.5096	2714.83	2620.22	1382.26	1100.03		
	1.2495	1.2303	0.6485	0.6002	2586.27	2547.70	1379.04	1282.02		
	1.4224	1.2238	0.5100	0.5803	2933.58	2534.65	1100.81	1242.05		
	1.1892	1.2844	0.6325	0.5648	2465.15	2656.37	1346.90	1210.91		
	1.2660	1.3391	0.6100	0.5630	2619.41	2766.25	1301.71	1207.30		
	1.3731	1.4637	0.6182	0.5377	2834.55	3016.53	1318.18	1156.48		
	1.1923	1.3059	0.5602	0.6021	2471.37	2699.56	1201.67	1285.84		
20	1.1924	1.0807	0.5671	0.4702	2471.57	2247.20	1215.53	1020.89	2186.0	889.95
	1.1548	1.1191	0.4998	0.5446	2396.05	2324.34	1080.35	1170.34		
	1.4864	1.1599	0.4509	0.4633	3062.13	2406.29	982.12	1007.03		
	1.1735	1.1021	0.5776	0.4058	2433.61	2290.19	1236.63	891.53		
	1.1549	1.0969	0.6101	0.3962	2396.25	2279.74	1301.91	872.25		
	1.1014	1.2086	0.4596	0.4402	2288.78	2504.11	999.60	960.63		
	1.3327	1.0934	0.4527	0.4570	2753.39	2272.71	985.74	994.38		

Concentration (mg/L)	Trial OD Values				Biomass Concentration Values (mg/L)				Final Biomass Average	Standard Deviation
	1	2	3	4	1	2	3	4		
30	1.2917	1.1728	0.4794	0.5864	2671.04	2432.20	1039.37	1254.30	2290.67	992.33
	1.0332	1.3390	0.4551	0.4433	2151.79	2766.05	990.56	966.86		
	1.0113	0.7861	0.4669	0.5955	2107.80	1655.44	1014.26	1272.58		
	0.9199	1.1036	0.4085	0.4462	1924.20	2293.20	896.95	972.68		
	1.5664	0.9781	0.4336	0.3457	3222.83	2041.11	947.37	770.81		
	1.0640	1.0702	0.3989	0.4466	2213.66	2226.11	877.67	973.49		
	1.1173	1.2565	0.4862	0.4605	2320.72	2600.33	1053.03	1001.41		
	1.1165	1.1099	0.4121	0.4358	2319.11	2305.86	904.19	951.79		
	0.9589	1.0332	0.4121	0.4351	2002.54	2151.79	904.19	950.39		
40	0.7861	0.8426	0.3633	0.3631	1655.44	1768.93	806.16	805.76	1965.62	716.21
	0.9166	0.7873	0.4879	0.3206	1917.57	1657.85	1056.44	720.39		
	1.2187	0.9051	0.3760	0.3557	2524.40	1894.47	831.67	790.89		
	1.0571	0.9294	0.4343	0.3182	2199.80	1943.29	948.78	715.57		
	1.0365	0.8551	0.3875	0.3601	2158.42	1794.04	854.77	799.73		
	1.0470	1.1018	0.4093	0.3060	2179.51	2289.59	898.56	691.06		
	0.9147	0.7804	0.3527	0.3906	1913.76	1643.99	784.87	861.00		
	0.9453	0.7447	0.3335	0.3764	1975.22	1572.28	746.30	832.47		
50	0.8329	1.0467	0.3327	0.5442	1749.45	2178.91	744.69	1169.53	1182.32	454.10
	0.8531	0.8937	0.2624	0.3534	1790.02	1871.58	603.48	786.27		
	0.6108	0.7445	0.2749	0.3063	1303.31	1571.88	628.59	691.66		
	0.7575	0.6654	0.2807	0.2876	1597.99	1412.99	640.24	654.10		
	0.8231	0.5993	0.3057	0.3669	1729.76	1280.21	690.46	813.39		
	0.7960	0.7738	0.2920	0.4742	1675.33	1630.73	662.94	1028.93		
	0.6534	0.6971	0.3335	0.4353	1388.88	1476.66	746.30	950.79		
	0.6225	0.7001	0.3192	0.3792	1326.82	1482.69	717.58	838.10		

Table 18 - OD600 data and biomass concentrations, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AuNP.

Concentration (mg/L)	Trial OD Values				Biomass Concentration Values (mg/L)				Final Biomass Average	Standard Deviation
	1	2	3	4	1	2	3	4		
0	0.7463	0.7195	0.7608	0.8304	1575.49	1521.66	1604.62	1744.42	1555.87	179.35
	0.8474	0.7527	0.7880	0.6004	1778.57	1588.35	1659.26	1282.42		
	0.8804	0.6842	0.6158	0.6085	1844.86	1450.75	1313.36	1298.69		
	0.8509	0.7355	0.5580	0.7829	1785.60	1553.80	1197.25	1649.01		
	0.8515	0.6979	0.6694	0.8320	1786.81	1478.27	1421.02	1747.64		
	0.8275	0.7413	0.7034	0.8815	1738.60	1565.45	1489.32	1847.07		
	0.7341	0.6657	0.7078	0.6717	1550.99	1413.59	1498.16	1425.64		
	0.5776	0.7054	0.6903	0.8502	1236.63	1493.34	1463.01	1784.20		
40	0.7625	0.5731	0.6504	0.4200	1608.03	1227.59	1382.86	920.05	1353.28	248.10
	0.6088	0.5835	0.4840	0.3929	1299.30	1248.48	1048.61	865.62		
	0.5999	0.4939	0.4024	0.4860	1281.42	1068.50	884.70	1052.63		
	0.5206	0.8072	0.4662	0.5260	1122.13	1697.82	1012.86	1132.98		
	0.5264	0.7323	0.5636	0.4330	1133.78	1547.37	1208.50	946.17		
	0.4806	0.5663	0.8240	0.5294	1041.78	1213.93	1731.57	1139.81		
	0.6244	0.6275	0.5726	0.5975	1330.63	1336.86	1226.58	1276.60		
	0.5793	0.5634	0.3694	0.5809	1240.04	1208.10	818.41	1243.25		
50	0.6058	0.4205	0.4775	0.6797	1293.27	921.06	1035.55	1441.71	1310.39	299.92
	0.5228	0.4678	0.3561	0.4054	1126.55	1016.07	791.70	890.73		
	0.3738	0.6702	0.4719	0.8281	827.25	1422.63	1024.31	1739.80		
	0.4964	0.5590	0.4297	0.8805	1073.52	1199.26	939.54	1845.06		
	0.6262	0.6433	0.4770	0.4514	1334.25	1368.60	1034.55	983.13		
	0.5350	0.7439	0.3983	0.3747	1151.05	1570.67	876.47	829.06		
	0.5530	0.5511	0.4951	0.3881	1187.21	1183.39	1070.91	855.98		

Concentration (mg/L)	Trial OD Values				Biomass Concentration Values (mg/L)				Final Biomass Average	Standard Deviation
	1	2	3	4	1	2	3	4		
80	0.4767	0.5282	0.4843	0.3857	1033.95	1137.40	1049.21	851.16	1274.99	251.63
	0.3940	0.4943	0.3133	0.4535	867.83	1069.30	705.73	987.35		
	0.6780	0.4684	0.4791	0.3873	1438.30	1017.28	1038.77	854.37		
	0.5169	0.4752	0.4970	0.4163	1114.70	1030.93	1074.72	912.62		
	0.5105	0.6587	0.4362	0.3878	1101.84	1399.53	952.59	855.37		
	0.5291	0.4713	0.5072	0.3192	1139.20	1023.10	1095.21	717.58		
	0.4889	0.4819	0.3387	0.3243	1058.45	1044.39	756.75	727.82		
	0.5219	0.5043	0.3463	0.3080	1124.74	1089.39	772.01	695.08		
	0.5513	0.6367	0.3603	0.3299	1183.80	1355.34	800.13	739.07		
120	0.5502	0.4526	0.3696	0.4761	1.1816	0.9855	0.8188	1.0327	1203.03	220.56
	0.5084	0.5145	0.3441	0.3800	1.0976	1.1099	0.7676	0.8397		
	0.4859	0.3678	0.5215	0.4688	1.0524	0.8152	1.1239	1.0181		
	0.4960	0.4761	0.3543	0.5050	1.0727	1.0327	0.7881	1.0908		
	0.5026	0.5983	0.5706	0.3519	1.0860	1.2782	1.2226	0.7833		
	0.6119	0.5121	0.4311	0.8920	1.3055	1.1051	0.9424	1.8682		
	0.4993	0.6635	0.5222	0.5101	1.0793	1.4092	1.1253	1.1010		
	0.7742	0.4979	0.6550	1.1963*	1.6315	1.0765	1.3921	-		
140	0.5637	0.5946	0.4387	0.3994	1.2087	1.2708	0.9576	0.8787	1156.03	330.47
	0.8711	0.4967	0.3832	0.3868	1.8262	1.0741	0.8461	0.8534		
	0.6784	0.4142	0.8689	0.6244	1.4391	0.9084	1.8218	1.3306		
	0.5944	1.0525	0.5618	0.4308	1.2704	2.1906	1.2049	0.9417		
	0.5001	0.8161	0.4556	0.4520	1.0810	1.7157	0.9916	0.9843		
	0.3311	0.5793	0.6013	0.4188	0.7415	1.2400	1.2842	0.9176		
	0.5456	0.5089	0.3471	0.4022	1.1723	1.0986	0.7736	0.8843		
	0.5198	0.4867	0.3778	0.4972	1.1205	1.0540	0.8353	1.0751		

*Outlier rejected with Dixon's Q test.

To determine the concentration of autoinducers (HSL) present in the Quorum sensing assay, it was necessary to construct three calibration curves, for the different room temperature incubation time (60, 90 and 120 min). The OD data collected, at 660 nm, is represented in Table 19, Table 20 and Table 21, and the calibration curves are represented in Figure 28, Figure 29 and Figure 30.

Table 19 - Data collected from the standard HSL concentrations, after 60 min of incubation, used to construct Figure 28.

HSL Standard Concentration (mg/L)	Average OD After 60 min
0.10	0.2514
0.25	0.3327
0.50	0.4467
0.75	0.4847
1.00	0.5664
1.25	0.6410

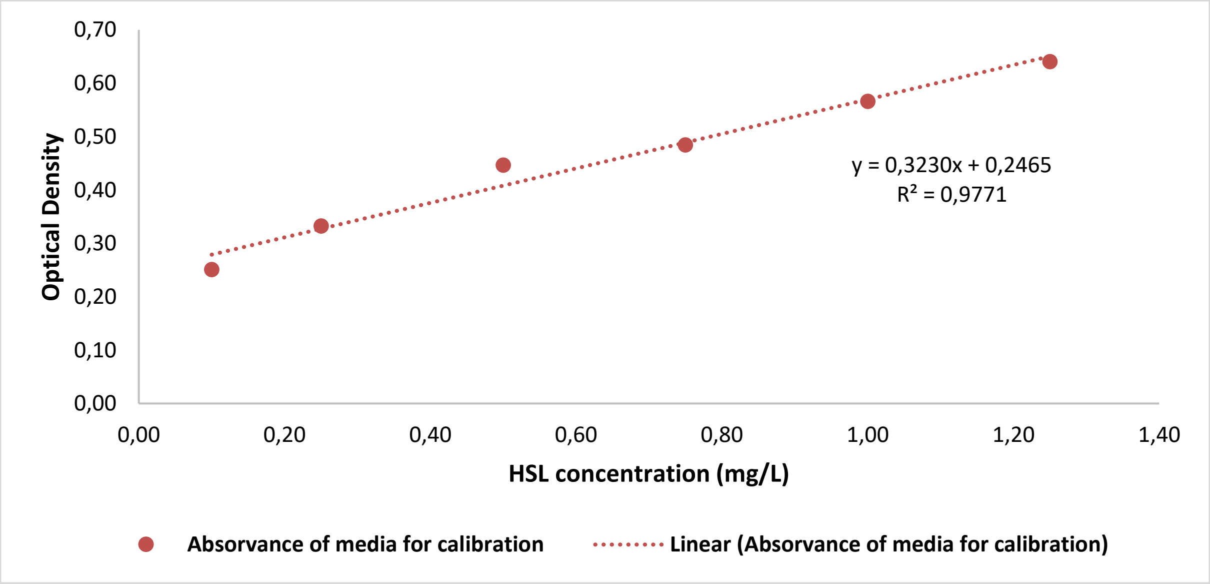

Figure 28 - Concentration calibration curve after 60 min of incubation.

Table 20 - Data collected from the standard HSL concentrations, after 90 min of incubation, used to construct Figure 29.

HSL Standard Concentration (mg/L)	Average OD After 90 min
0.10	0.2648
0.25	0.3879
0.50	0.5206
0.75	0.5763
1.00	0.6500
1.25	0.7726

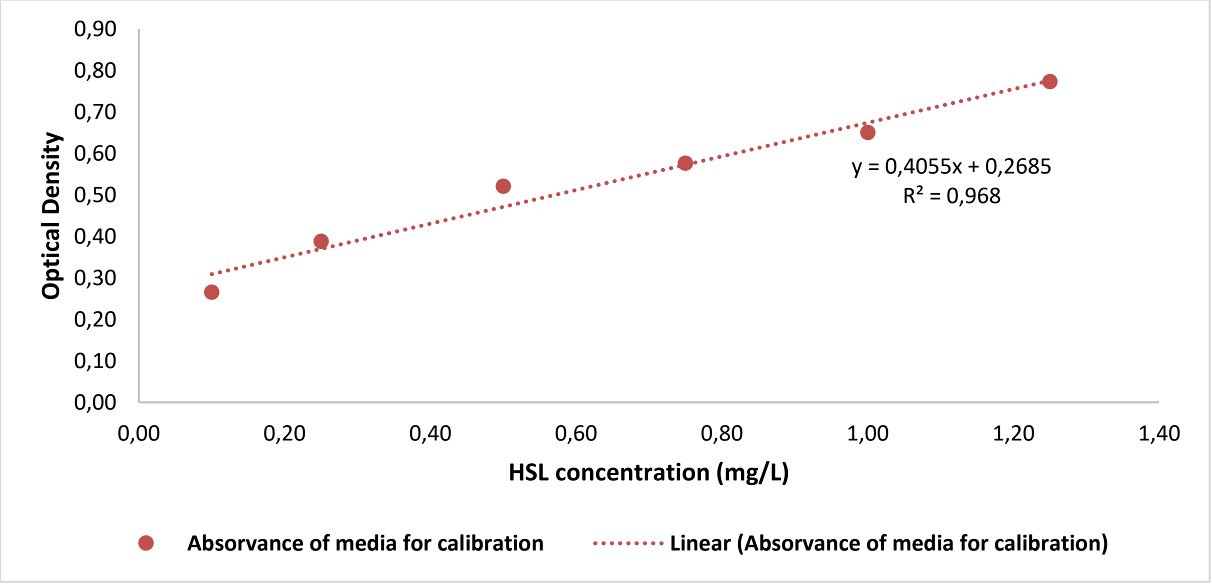

Figure 29 - Concentration calibration curve after 90 min of incubation.

Table 21 - Data collected from the standard HSL concentrations, after 60 min of incubation, used to construct Figure 30.

HSL Standard Concentration (mg/L)	Average OD After 120 min
0.10	0.2858
0.25	0.4158
0.50	0.5450
0.75	0.6122
1.00	0.6815
1.25	0.7882

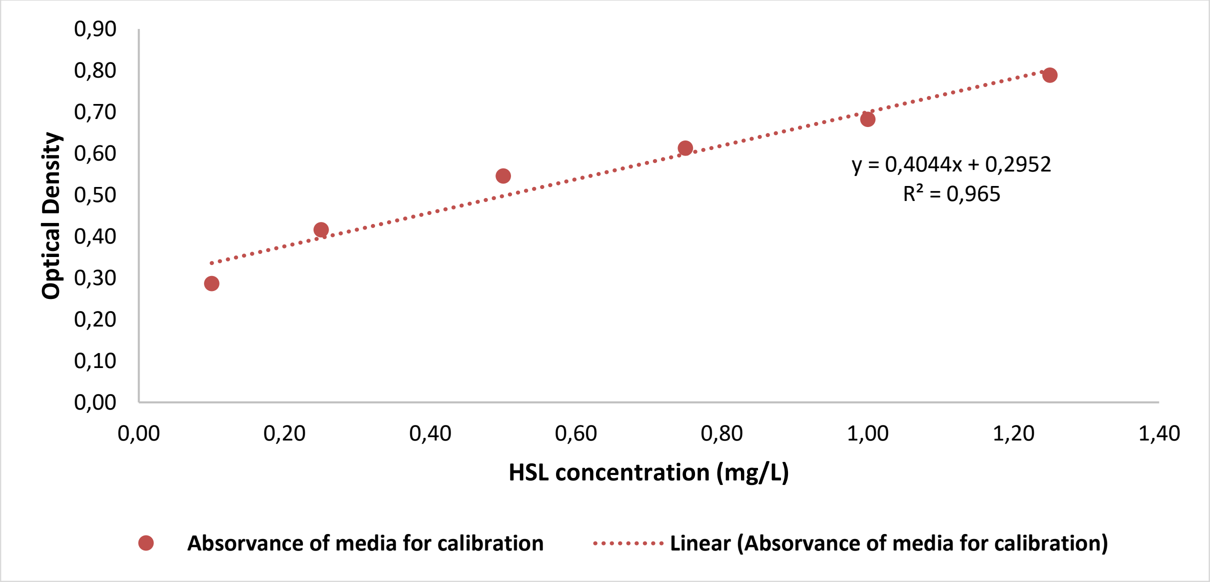

Figure 30 - Concentration calibration curve after 120 min of incubation.

Table 22, Table 23, Table 24 and Table 25 present the optical density values obtained for the different HSL trials, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for each *Pseudomonas aeruginosa* strain, nanoparticles and respective concentrations.

Table 22 – Optical density measured at 660nm for the HSL trials, and respective concentrations, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3777 with AgNP.

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
0	1	0.6631	0.7786	0.8061	1.2898	1.2580	1.2634	1.4472	0.6150
		0.6241	0.7156	0.7531	1.1690	1.1026	1.1323		
		0.4570	0.5109	0.5350	0.6517	0.5978	0.5930		
		0.8912	1.0805	1.1585	1.9960	2.0025	2.1348		
		0.2575	0.2741	0.2796	0.0341	0.0138	0.0000		
		0.4344	0.5239	0.5606	0.5817	0.6298	0.6563		
		0.8518	0.9609	1.0054	1.8740	1.7075	1.7562		
		0.6298	0.7211	0.7371	1.1867	1.1162	1.0927		
	2	0.7476	0.9641	1.0188	1.5514	1.7154	1.7893		
		0.8689	1.0166	1.0852	1.9269	1.8449	1.9535		
		0.9372	1.0872	1.1701	2.1384	2.0190	2.1635		
		0.9127	1.0542	1.1165	2.0625	1.9376	2.0309		
		0.9074	1.0628	1.1227	2.0461	1.9588	2.0462		
		0.8925	1.0109	1.0760	2.0000	1.8308	1.9308		
		0.8589	0.9863	1.0534	1.8960	1.7702	1.8749		
		0.6028	0.6794	0.7085	1.1031	1.0133	1.0220		
10	1	0.6665	0.8189	0.8843	1.3003	1.3573	1.4567	1.5999	0.3672
		0.6502	0.7494	0.7880	1.2498	0.0000	1.2186		
		0.7256	0.8079	0.8591	1.4833	1.3302	1.3944		
		0.6183	0.7108	0.7518	1.1511	1.0908	1.1291		
		0.6911	0.7978	0.8418	1.3765	1.3053	1.3516		
		0.7878	0.8905	0.9353	1.6759	1.5339	1.5828		
		0.6995	0.7827	0.8297	1.4025	1.2681	1.3217		
		0.8542	0.9679	1.0036	1.8814	1.7248	1.7517		
	2	0.7140	0.9198	0.9991	1.4474	1.6062	1.7406		
		0.7673	0.9454	1.0123	1.6124	1.6693	1.7732		
		0.8212	0.9595	1.0298	1.7793	1.7041	1.8165		
		0.8805	1.0239	1.0831	1.9628	1.8629	1.9483		
		0.8823	1.0316	1.1015	1.9684	1.8819	1.9938		
		0.9018	1.0458	1.1108	2.0288	1.9169	2.0168		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
		0.9145	1.0566	1.1137	2.0681	1.9435	2.0240		
		0.8760	1.0088	1.0712	1.9489	1.8256	1.9189		
20	1	0.7905	0.9741	1.0273	1.6842	1.7401	1.8103	1.7989	0.4236
		0.3302	0.3933	0.4316	0.2591	0.3078	0.3373		
		0.9562	1.1220	1.1905	2.1972	2.1048	2.2139		
		0.8902	1.0700	1.1272	1.9929	1.9766	2.0574		
		0.8626	1.0224	1.0995	1.9074	1.8592	1.9889		
		0.9367	1.0911	1.1521	2.1368	2.0286	2.1189		
		0.8414	0.9962	1.0532	1.8418	1.7946	1.8744		
		0.8966	1.0308	1.0914	2.0127	1.8799	1.9688		
	2	0.6678	0.8622	0.9243	1.3043	1.4641	1.5556		
		0.8393	0.9708	1.0432	1.8353	1.7319	1.8497		
		0.8319	0.9941	1.0534	1.8124	1.7894	1.8749		
		0.8838	1.0253	1.0934	1.9731	1.8663	1.9738		
		0.8402	0.9670	1.0282	1.8381	1.7226	1.8126		
		0.9446	1.0849	1.1469	2.1613	2.0133	2.1061		
		0.8887	1.0232	1.0922	1.9882	1.8612	1.9708		
		0.8802	1.0125	1.0844	1.9619	1.8348	1.9515		
30	1	0.7467	0.9469	1.0257	1.5486	1.6730	1.8064	1.7939	0.1334
		0.8452	1.0096	1.0667	1.8536	1.8276	1.9078		
		0.7964	0.9581	1.0197	1.7025	1.7006	1.7915		
		0.8622	1.0235	1.0669	1.9062	1.8619	1.9083		
		0.8699	1.0287	1.1006	1.9300	1.8747	1.9916		
		0.8386	0.9735	1.0291	1.8331	1.7386	1.8148		
		0.8937	1.0454	1.1078	2.0037	1.9159	2.0094		
		0.9182	1.0544	1.1210	2.0796	1.9381	2.0420		
	2	0.8008	1.0000	1.0857	1.7161	1.8039	1.9547		
		0.7697	0.9061	0.9702	1.6198	1.5724	1.6691		
		0.7602	0.9138	0.9732	1.5904	1.5914	1.6766		
		0.7863	0.9162	0.9739	1.6712	1.5973	1.6783		
		0.7982	0.9364	1.0195	1.7080	1.6471	1.7910		
		0.8463	0.9913	1.0731	1.8570	1.7825	1.9236		
		0.8141	0.9364	0.9991	1.7573	1.6471	1.7406		
		0.8483	0.9748	1.0423	1.8632	1.7418	1.8474		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
40	1	0.6690	0.8837	0.9221	1.3080	1.5171	1.5502	1.8549	0.2227
		0.8889	1.0936	1.1592	1.9889	2.0348	2.1365		
		0.8595	1.0231	1.0882	1.8978	1.8609	1.9609		
		1.0261	1.2119	1.2822	2.4136	2.3265	2.4407		
		0.9874	1.1514	1.2108	2.2938	2.1773	2.2641		
		0.7934	0.9478	1.0221	1.6932	1.6752	1.7975		
		0.8103	0.9631	1.0277	1.7455	1.7129	1.8113		
		0.8376	0.9894	1.0523	1.8300	1.7778	1.8722		
	2	0.7768	0.9837	1.0859	1.6418	1.7637	1.9552		
		0.8052	0.9794	1.0618	1.7297	1.7531	1.8956		
		0.7866	0.9557	1.0491	1.6721	1.6947	1.8642		
		0.8233	0.9928	1.0607	1.7858	1.7862	1.8929		
		0.8453	0.9937	1.0834	1.8539	1.7884	1.9491		
		0.7825	0.9514	1.0384	1.6594	1.6841	1.8378		
		0.8133	0.9613	1.0250	1.7548	1.7085	1.8046		
		0.8312	0.9839	1.0627	1.8102	1.7642	1.8979		
50	1	0.6950	0.9146	0.9724	1.3885	1.5933	1.6746	1.7108	0.2163
		0.9515	1.1482	1.2157	2.1827	2.1694	2.2762		
		0.7286	0.8866	0.9436	1.4926	1.5243	1.6034		
		0.8003	0.9634	1.0161	1.7146	1.7137	1.7826		
		0.8540	1.0196	1.0736	1.8808	1.8523	1.9248		
		0.8548	0.9975	1.0659	1.8833	1.7978	1.9058		
		0.8461	1.0079	1.0598	1.8563	1.8234	1.8907		
		0.6864	0.8011	0.8558	1.3619	1.3134	1.3863		
	2	0.6523	0.8355	0.9274	1.2563	1.3983	1.5633		
		0.7975	0.9637	1.0505	1.7059	1.7144	1.8677		
		0.7565	0.9270	1.0034	1.5789	1.6239	1.7512		
		0.7189	0.8586	0.9197	1.4625	1.4552	1.5443		
		0.8257	0.9804	1.0621	1.7932	1.7556	1.8964		
		0.7810	0.9407	1.0124	1.6548	1.6577	1.7735		
		0.7944	0.9403	1.0144	1.6963	1.6567	1.7784		
		0.8395	0.9971	1.0661	1.8359	1.7968	1.9063		

Table 23 - Optical density measured at 660nm for the HSL trials, and respective concentrations, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3777 with AuNP.

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
0	1	0.8770	1.0328	1.0671	1.9520	1.8848	1.9088	1.5795	0.8080
		0.7603	0.8797	0.9962	1.5907	1.5073	1.7334		
		0.2888	0.3138	0.3286	0.1310	0.1117	0.0826		
		0.2857	0.2966	0.3102	0.1214	0.0693	0.0371		
		1.0416	1.2052	1.2586	2.4616	2.3100	2.3823		
		1.0297	1.1687	1.1928	2.4248	2.2200	2.2196		
		1.0562	1.1617	1.1882	2.5068	2.2027	2.2082		
		1.0120	1.1310	1.0931	2.3700	2.1270	1.9730		
	2	1.0367	1.2109	1.2620	2.4464	2.3240	2.3907		
		0.7494	0.8833	0.9269	1.5570	1.5162	1.5621		
		0.2732	0.3083	0.3253	0.0827	0.0982	0.0744		
		0.8719	1.0236	1.0438	1.9362	1.8621	1.8511		
		0.9433	1.0751	1.1181	2.1573	1.9891	2.0349		
		0.8715	1.0087	1.0464	1.9350	1.8254	1.8576		
		0.7990	0.9172	0.9661	1.7105	1.5998	1.6590		
		0.8344	0.9930	1.0182	1.8201	1.7867	1.7878		
	3	0.8501	0.9317	0.9851	1.8687	1.6355	1.7060		
		0.6352	0.6995	0.7234	1.2034	1.0629	1.0589		
		0.3001	0.3642	0.4036	0.1659	0.2360	0.2681		
		0.3452	0.4197	0.4507	0.3056	0.3729	0.3845		
		1.0110	1.1740	1.3410	2.3669	2.2330	2.5861		
		0.9874	1.0950	1.2240	2.2938	2.0382	2.2967		
		1.0470	1.1010	1.2894	2.4783	2.0530	2.4585		
		0.9456	0.9741	1.0450	2.1644	1.7401	1.8541		
	4	0.8452	0.8654	0.9017	1.8536	1.4720	1.4998		
		1.2389	1.4216	1.6540	3.0724	2.8436	3.3600		
		0.8426	0.8794	0.8964	1.8455	1.5065	1.4866		
		0.9123	0.9674	0.9934	2.0613	1.7236	1.7265		
		0.4287	0.4429	0.4752	0.5641	0.4301	0.4451		
		0.3698	0.3970	0.4136	0.3817	0.3169	0.2928		

		0.7415	0.7652	0.7859	1.5325	1.2249	1.2134		
		0.9334	0.9637	0.9857	2.1266	1.7144	1.7075		
Concentration (mg/L)	**Trial**	**OD After 60 min**	**OD After 90 min**	**OD After 120 min**	**Concentration (mg/L) After 60 min**	**Concentration (mg/L) After 90 min**	**Concentration (mg/L) After 120 min**	**Average Concentration (mg/L)**	**Standard Deviation**
40	1	0.2651	0.2805	0.2755	0.0576	0.0296	0.0000	1.2235	0.8302
		0.2591	0.2646	0.2766	0.0390	0.0000	0.0000		
		0.9027	1.0575	1.0899	2.0316	1.9457	1.9651		
		0.7675	0.9272	0.9955	1.6130	1.6244	1.7317		
		0.8647	0.9817	1.0393	1.9139	1.7588	1.8400		
		0.9119	1.0459	1.1098	2.0601	1.9171	2.0143		
		0.8259	0.9853	1.0500	1.7938	1.7677	1.8665		
		0.9302	1.1452	1.1455	2.1167	2.1620	2.1026		
	2	0.2854	0.2978	0.3000	0.1204	0.0723	0.0119		
		0.7831	0.9030	0.9603	1.6613	1.5647	1.6447		
		0.7405	0.8732	0.9235	1.5294	1.4912	1.5537		
		0.7656	0.9007	0.9464	1.6071	1.5591	1.6103		
		0.3375	0.3576	0.3860	0.2817	0.2197	0.2245		
		0.3075	0.3178	0.3330	0.1889	0.1216	0.0935		
		0.2751	0.2802	0.2808	0.0885	0.0289	0.0000		
		0.9002	1.0519	1.0733	2.0238	1.9319	1.9241		
	3	0.4232	0.4350	0.4624	0.5471	0.4106	0.4135		
		0.3423	0.3517	0.3869	0.2966	0.2052	0.2268		
		0.8452	0.9001	0.9374	1.8536	1.5576	1.5880		
		0.7012	0.7365	0.7634	1.4077	1.1541	1.1578		
		0.8001	0.8997	0.9251	1.7139	1.5566	1.5576		
		1.3250	1.4120	1.6040	3.3390	2.8200	3.2364		
		0.8021	0.8225	0.8499	1.7201	1.3662	1.3717		
		0.9745	0.9913	1.1036	2.2539	1.7825	1.9990		
	4	0.3014	0.3349	0.3650	0.1700	0.1637	0.1726		
		0.2887	0.3169	0.3324	0.1307	0.1194	0.0920		
		0.7014	0.7401	0.7698	1.4084	1.1630	1.1736		
		0.6589	0.6783	0.7064	1.2768	1.0106	1.0168		
		0.8965	0.9136	0.9452	2.0125	1.5910	1.6073		
		0.4558	0.4867	0.5039	0.6480	0.5381	0.5161		
		0.9521	0.9766	1.0960	2.1845	1.7462	1.9802		
		0.9002	0.9251	0.9647	2.0238	1.6192	1.6555		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
50	1	0.2772	0.2817	0.2825	0.0950	0.0326	0.0000	0.8687	0.8262
		0.5846	0.6454	0.7012	1.0467	0.9295	1.0040		
		0.9819	1.1532	1.1838	2.2768	2.1818	2.1973		
		0.2918	0.3015	0.3113	0.1402	0.0814	0.0398		
		0.2756	0.2776	0.2806	0.0901	0.0224	0.0000		
		1.0273	1.1911	1.2318	2.4173	2.2752	2.3160		
		1.0049	1.1491	1.2062	2.3480	2.1716	2.2527		
		0.9530	1.1397	1.1494	2.1873	2.1485	2.1123		
	2	0.2839	0.2940	0.2924	0.1158	0.0629	0.0000		
		0.3676	0.4059	0.4370	0.3749	0.3388	0.3506		
		0.2818	0.2830	0.2873	0.1093	0.0358	0.0000		
		0.2774	0.2860	0.2847	0.0957	0.0432	0.0000		
		0.8492	0.9863	1.0276	1.8659	1.7702	1.8111		
		0.2689	0.2723	0.2769	0.0693	0.0094	0.0000		
		0.2786	0.2881	0.2922	0.0994	0.0483	0.0000		
		0.3017	0.3144	0.3148	0.1709	0.1132	0.0485		
	3	0.3125	0.3411	0.3526	0.2043	0.1790	0.1419		
		0.6785	0.6858	0.7124	1.3375	1.0291	1.0317		
		0.6232	0.6534	0.6825	1.1663	0.9492	0.9577		
		0.3456	0.3820	0.4102	0.3068	0.2799	0.2844		
		0.3045	0.3319	0.3604	0.1796	0.1564	0.1612		
		1.1450	1.2270	1.3890	2.7817	2.3637	2.7047		
		0.8521	0.8698	0.9041	1.8749	1.4829	1.5057		
		0.9547	0.9815	1.0230	2.1926	1.7583	1.7997		
	4	0.6104	0.6327	0.6597	1.1266	0.8982	0.9013		
		0.3672	0.3945	0.4215	0.3737	0.3107	0.3123		
		0.4260	0.4468	0.4796	0.5557	0.4397	0.4560		
		0.5874	0.6138	0.6304	1.0554	0.8515	0.8289		
		0.7412	0.7566	0.7749	1.5316	1.2037	1.1862		
		0.5692	0.5987	0.6266	0.9991	0.8143	0.8195		
		0.5758	0.6014	0.6278	1.0195	0.8210	0.8225		
		0.4109	0.4364	0.4632	0.5090	0.4141	0.4154		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
80	1	0.9012	1.1090	1.1763	2.0269	2.0727	2.1788	1.6457	0.7089
		0.8872	1.0624	1.0928	1.9836	1.9578	1.9723		
		0.9741	1.1119	1.1957	2.2526	2.0799	2.2268		
		0.9044	1.0355	1.0856	2.0368	1.8915	1.9545		
		0.9941	1.1388	1.1711	2.3146	2.1462	2.1659		
		0.8687	1.0260	1.0723	1.9263	1.8681	1.9216		
		0.8798	1.0098	1.0649	1.9607	1.8281	1.9033		
		0.2691	0.2713	0.2788	0.0700	0.0069	0.0000		
	2	0.2842	0.2891	0.2961	0.1167	0.0508	0.0022		
		0.9862	1.1569	1.1928	2.2901	2.1909	2.2196		
		0.9936	1.1610	1.1988	2.3130	2.2010	2.2344		
		0.8630	1.0213	1.0645	1.9087	1.8565	1.9023		
		0.9042	1.0869	1.1209	2.0362	2.0182	2.0418		
		0.9437	1.0850	1.1146	2.1585	2.0136	2.0262		
		0.7579	0.9184	0.9708	1.5833	1.6027	1.6706		
		0.7972	0.9421	0.9510	1.7050	1.6612	1.6217		
	3	1.1258	1.2290	1.3742	2.7223	2.3687	2.6682		
		0.8125	0.8441	0.8629	1.7523	1.4195	1.4038		
		0.9415	0.9748	1.0040	2.1517	1.7418	1.7527		
		0.4561	0.4924	0.5204	0.6489	0.5521	0.5569		
		0.9945	1.1700	1.2379	2.3158	2.2232	2.3311		
		0.7645	0.7759	0.8032	1.6037	1.2513	1.2562		
		0.8214	0.8412	0.8599	1.7799	1.4123	1.3964		
		0.5968	0.6428	0.6902	1.0845	0.9231	0.9768		
	4	0.4120	0.4306	0.4525	0.5124	0.3998	0.3890		
		0.3147	0.3459	0.3769	0.2111	0.1909	0.2020		
		0.9997	1.2060	1.3870	2.3319	2.3120	2.6998		
		0.8124	0.8460	0.8965	1.7520	1.4242	1.4869		
		0.9356	0.9547	0.9745	2.1334	1.6922	1.6798		
		0.9124	0.9366	0.9635	2.0616	1.6476	1.6526		
		0.6478	0.6706	0.7014	1.2424	0.9916	1.0045		
		1.0220	1.2280	1.4010	2.4009	2.3662	2.7344		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
120	1	0.2665	0.2702	0.2713	0.0619	0.0042	0.0000	1.4987	0.6233
		0.6994	0.8407	0.8942	1.4022	1.4111	1.4812		
		0.7650	0.9051	0.9562	1.6053	1.5699	1.6345		
		0.2924	0.2986	0.3005	0.1421	0.0742	0.0131		
		0.8742	1.0601	1.1081	1.9433	1.9522	2.0101		
		0.8835	1.0228	1.0643	1.9721	1.8602	1.9018		
		0.9191	1.0336	1.1050	2.0824	1.8868	2.0025		
		0.8944	1.0661	1.1037	2.0059	1.9670	1.9993		
	2	1.0213	1.2141	1.2727	2.3988	2.3319	2.4172		
		0.9579	1.1163	1.1358	2.2025	2.0908	2.0786		
		0.8374	1.0693	1.1132	1.8294	1.9748	2.0227		
		0.7904	0.9521	0.9997	1.6839	1.6858	1.7421		
		0.9763	1.2372	1.2744	2.2594	2.3889	2.4214		
		0.8861	0.9841	0.9921	1.9802	1.7647	1.7233		
		0.7759	0.8688	0.9383	1.6390	1.4804	1.5903		
		0.7718	1.0500	1.0812	1.6263	1.9273	1.9436		
	3	0.3255	0.3471	0.3611	0.2446	0.1938	0.1630		
		0.7423	0.7752	0.7992	1.5350	1.2496	1.2463		
		0.8423	0.8849	0.9214	1.8446	1.5201	1.5485		
		0.3256	0.3564	0.3896	0.2449	0.2168	0.2334		
		0.7412	0.7790	0.7985	1.5316	1.2589	1.2446		
		0.8991	0.9247	0.9536	2.0204	1.6182	1.6281		
		0.9452	0.9636	0.9864	2.1632	1.7142	1.7091		
		0.8127	0.8394	0.8745	1.7529	1.4079	1.4325		
	4	0.5008	0.5327	0.5684	0.7873	0.6515	0.6756		
		0.9024	0.9344	0.9612	2.0307	1.6422	1.6469		
		0.8123	0.8364	0.8569	1.7517	1.4005	1.3890		
		0.7415	0.7741	0.8021	1.5325	1.2469	1.2535		
		0.9015	0.9247	0.9366	2.0279	1.6182	1.5861		
		0.8657	0.8863	0.9064	1.9170	1.5236	1.5114		
		0.8010	0.8346	0.8694	1.7167	1.3961	1.4199		
		0.6398	0.6702	0.7124	1.2176	0.9906	1.0317		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
140	1	0.2956	0.3051	0.3081	0.1520	0.0903	0.0319	1.1146	0.7730
		0.2947	0.3034	0.3163	0.1492	0.0861	0.0522		
		0.9058	1.0654	1.1048	2.0412	1.9652	2.0020		
		0.8936	1.0796	1.1196	2.0034	2.0002	2.0386		
		0.8120	1.0055	1.0512	1.7508	1.8175	1.8694		
		0.8355	0.9899	1.0407	1.8235	1.7790	1.8435		
		0.8608	1.0441	1.0492	1.9019	1.9127	1.8645		
		0.8273	0.9382	0.9628	1.7981	1.6515	1.6508		
	2	0.3637	0.4026	0.4155	0.3628	0.3307	0.2975		
		1.0316	1.1696	1.2168	2.4307	2.2222	2.2789		
		0.4087	0.4504	0.5012	0.5022	0.4486	0.5094		
		0.5242	0.6597	0.7153	0.8598	0.9647	1.0388		
		1.0065	1.1641	1.2387	2.3529	2.2086	2.3331		
		0.4330	0.5393	0.5774	0.5774	0.6678	0.6978		
		0.3955	0.4504	0.4974	0.4613	0.4486	0.5000		
		0.4796	0.5776	0.5724	0.7217	0.7623	0.6855		
	3	0.4210	0.4527	0.4952	0.5402	0.4543	0.4946		
		0.2248	0.2845	0.3251	0.0000	0.0395	0.0739		
		0.8856	0.9125	0.9521	1.9786	1.5882	1.6244		
		0.8199	0.8309	0.8527	1.7752	1.3869	1.3786		
		0.7469	0.7699	0.7994	1.5492	1.2365	1.2468		
		0.4342	0.4426	0.4620	0.5811	0.4293	0.4125		
		0.4563	0.4751	0.5045	0.6495	0.5095	0.5176		
		0.8563	0.8769	0.8974	1.8879	1.5004	1.4891		
	4	0.3175	0.3321	0.3741	0.2198	0.1568	0.1951		
		0.8459	0.8654	0.8821	1.8557	1.4720	1.4513		
		0.5200	0.5562	0.5891	0.8467	0.7095	0.7268		
		0.3785	0.3912	0.4436	0.4087	0.3026	0.3670		
		1.0780	1.2475	1.4260	2.5743	2.4143	2.7962		
		0.9450	0.9655	0.9964	2.1625	1.7189	1.7339		
		0.3952	0.4396	0.4536	0.4604	0.4219	0.3917		
		0.4118	0.4364	0.4613	0.5118	0.4141	0.4107		

Table 24 – Optical density measured at 660nm for the HSL trials, and respective concentrations, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3081 with AgNP.

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
0	1	0.4363	0.3154	0.3248	0.5876	0.1157	0.0732	0.5084	0.1301
		0.3813	0.5144	0.5917	0.4173	0.6064	0.7332		
		0.3950	0.4642	0.5126	0.4598	0.4826	0.5376		
		0.3871	0.5199	0.5827	0.4353	0.6200	0.7109		
		0.3910	0.4369	0.4877	0.4474	0.4153	0.4760		
		0.3947	0.4714	0.5274	0.4588	0.5004	0.5742		
		0.3802	0.4462	0.4950	0.4139	0.4382	0.4941		
		0.3930	0.5204	0.5757	0.4536	0.6212	0.6936		
	2	0.3641	0.4560	0.5124	0.3641	0.4624	0.5371		
		0.3231	0.4844	0.5529	0.2372	0.5324	0.6372		
		0.3579	0.4622	0.5155	0.3449	0.4777	0.5448		
		0.3168	0.4736	0.5260	0.2176	0.5058	0.5707		
		0.3642	0.4767	0.5345	0.3644	0.5134	0.5917		
		0.3680	0.5065	0.5628	0.3762	0.5869	0.6617		
		0.3502	0.4679	0.5240	0.3211	0.4917	0.5658		
		0.3967	0.5315	0.5847	0.4650	0.6486	0.7159		
	3	0.3666	0.4624	0.5083	0.3718	0.4782	0.5270		
		0.3834	0.4739	0.5128	0.4238	0.5065	0.5381		
		0.4261	0.5254	0.5551	0.5560	0.6335	0.6427		
		0.3570	0.4148	0.4506	0.3421	0.3608	0.3843		
		0.4421	0.5115	0.5728	0.6056	0.5993	0.6864		
		0.4177	0.5048	0.5420	0.5300	0.5827	0.6103		
		0.4047	0.4916	0.5417	0.4898	0.5502	0.6095		
		0.3871	0.4527	0.4949	0.4353	0.4543	0.4938		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	4	0.3621	0.4014	0.4542	0.3579	0.3277	0.3932		
		0.4137	0.4709	0.5234	0.5176	0.4991	0.5643		
		0.4436	0.5349	0.5819	0.6102	0.6570	0.7090		
		0.4132	0.4824	0.5008	0.5161	0.5275	0.5084		
		0.3961	0.4384	0.4748	0.4632	0.4190	0.4441		
		0.3895	0.4347	0.4733	0.4427	0.4099	0.4404		
		0.4304	0.4951	0.5411	0.5693	0.5588	0.6081		
		0.4750	0.5869	0.6434	0.7074	0.7852	0.8610		
10	1	0.3570	0.4631	0.5269	0.3421	0.4799	0.0000	0.4732	0.1600
		0.3634	0.4451	0.4894	0.3619	0.0000	0.0000		
		0.3729	0.4537	0.4984	0.3913	0.4567	0.5025		
		0.3383	0.5292	0.5967	0.2842	0.6429	0.7455		
		0.3694	0.6433	0.7508*	0.3805	0.9243	-		
		0.3591	0.4759	0.5293	0.3486	0.5115	0.5789		
		0.3587	0.5394	0.5987	0.3474	0.6681	0.7505		
		0.3446	0.4923	0.5449	0.3037	0.5519	0.6175		
	2	0.3817	0.4449	0.5126	0.4186	0.4350	0.5376		
		0.3686	0.5238	0.5851	0.3780	0.6296	0.7169		
		0.3556	0.4911	0.5490	0.3378	0.5490	0.6276		
		0.3523	0.5199	0.5791	0.3276	0.6200	0.7020		
		0.3511	0.5102	0.6139	0.3238	0.5961	0.7881		
		0.4032	0.4907	0.5629	0.4851	0.5480	0.6620		
		0.4137	0.4936	0.5415	0.5176	0.5551	0.0000		
		0.4492	0.5313	0.5942	0.6276	0.6481	0.7394		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	3	0.3690	0.4394	0.4776	0.3793	0.4215	0.4510		
		0.4023	0.4673	0.5222	0.4824	0.4903	0.5613		
		0.3770	0.4350	0.4683	0.4040	0.4106	0.4280		
		0.3691	0.4260	0.4649	0.3796	0.3884	0.4196		
		0.3721	0.4250	0.4535	0.3889	0.3859	0.3914		
		0.3874	0.4492	0.4939	0.4362	0.4456	0.4913		
		0.3876	0.4451	0.4813	0.4368	0.4355	0.4602		
		0.3318	0.3474	0.3574	0.2641	0.1946	0.1538		
	4	0.3882	0.4459	0.5013	0.4387	0.4375	0.5096		
		0.4005	0.4644	0.5103	0.4768	0.4831	0.5319		
		0.4211	0.4837	0.5257	0.5406	0.5307	0.5700		
		0.4171	0.4868	0.5277	0.5282	0.5383	0.5749		
		0.4198	0.4762	0.5161	0.5365	0.5122	0.5462		
		0.4051	0.4671	0.5118	0.4910	0.4898	0.5356		
		0.3886	0.4536	0.4914	0.4399	0.4565	0.4852		
		0.4151	0.4839	0.5328	0.5220	0.5312	0.5875		
20	1	0.3593	0.4316	0.5020	0.3492	0.4022	0.0000	0.4102	0.1558
		0.3620	0.4981	0.5576	0.3576	0.5662	0.6489		
		0.3867	0.4390	0.4840	0.4341	0.4205	0.4669		
		0.3389	0.5353	0.6089	0.2861	0.6580	0.7757		
		0.3693	0.3991	0.4441	0.3802	0.3221	0.0000		
		0.3699	0.4580	0.5014	0.3820	0.4673	0.5099		
		0.3763	0.5003	0.5407	0.4019	0.5716	0.6071		
		0.3347	0.4739	0.5234	0.2731	0.5065	0.5643		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	2	0.3794	0.4802	0.5359	0.4115	0.5221	0.0000		
		0.2888	0.4659	0.5176	0.1310	0.4868	0.5500		
		0.3776	0.5396	0.5950	0.4059	0.6686	0.0000		
		0.3654	0.5121	0.5557	0.3681	0.6007	0.0000		
		0.3528	0.4742	0.5265	0.3291	0.5073	0.5720		
		0.2853	0.4961	0.5605	0.1201	0.5613	0.0000		
		0.3794	0.4751	0.5185	0.4115	0.5095	0.0000		
		0.3450	0.4596	0.5157	0.3050	0.4713	0.5453		
	3	0.3363	0.3820	0.4219	0.2780	0.2799	0.3133		
		0.3650	0.4261	0.4657	0.3669	0.3887	0.4216		
		0.3513	0.4097	0.4481	0.3245	0.3482	0.3781		
		0.3774	0.4468	0.4826	0.4053	0.4397	0.4634		
		0.3797	0.4378	0.4729	0.4124	0.4175	0.4394		
		0.3745	0.4310	0.4625	0.3963	0.4007	0.4137		
		0.3563	0.4119	0.4439	0.3399	0.3536	0.3677		
		0.3943	0.4530	0.4917	0.4576	0.4550	0.4859		
	4	0.4104	0.5190	0.5938	0.5074	0.6178	0.7384		
		0.3791	0.4387	0.4919	0.4105	0.4197	0.4864		
		0.3979	0.4589	0.5090	0.4687	0.4695	0.5287		
		0.3846	0.4423	0.4868	0.4276	0.4286	0.4738		
		0.3932	0.4331	0.4687	0.4542	0.4059	0.4290		
		0.3823	0.4286	0.4620	0.4204	0.3948	0.4125		
		0.3966	0.4660	0.5032	0.4647	0.4871	0.5143		
		0.3768	0.4324	0.4723	0.4034	0.4042	0.4379		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
30	1	0.3792	0.4142	0.4883	0.4108	0.3593	0.4775	0.4129	0.1040
		0.3871	0.4766	0.5439	0.4353	0.5132	0.6150		
		0.4022	0.3108	0.3192	0.4820	0.1043	0.0593		
		0.3902	0.4340	0.4863	0.4449	0.4081	0.4726		
		0.4050	0.4455	0.4915	0.4907	0.4365	0.4854		
		0.4328	0.4762	0.5223	0.5768	0.5122	0.5616		
		0.3623	0.3814	0.4849	0.3585	0.2784	0.4691		
		0.3684	0.5007	0.5589	0.3774	0.5726	0.0000		
	2	0.3602	0.4026	0.4516	0.3520	0.3307	0.3867		
		0.4290	0.4781	0.5393	0.5650	0.5169	0.6036		
		0.3597	0.4711	0.5265	0.3505	0.4996	0.5720		
		0.3355	0.4809	0.5239	0.2755	0.5238	0.5655		
		0.4005	0.3855	0.4262	0.4768	0.2885	0.3239		
		0.3598	0.4313	0.4734	0.3508	0.4015	0.4407		
		0.3851	0.4390	0.4853	0.4291	0.4205	0.4701		
		0.3663	0.4399	0.4756	0.3709	0.4227	0.4461		
	3	0.3587	0.4154	0.4603	0.3474	0.3623	0.4083		
		0.3739	0.4313	0.4719	0.3944	0.4015	0.4369		
		0.3458	0.3861	0.4113	0.3074	0.2900	0.2871		
		0.3538	0.3991	0.4318	0.3322	0.3221	0.3378		
		0.4144	0.4754	0.4950	0.5198	0.5102	0.4941		
		0.3808	0.4390	0.4725	0.4158	0.4205	0.4384		
		0.3764	0.4303	0.4616	0.4022	0.3990	0.4115		
		0.3568	0.3712	0.3788	0.3415	0.2533	0.2067		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	4	0.3504	0.3999	0.4578	0.3217	0.3240	0.4021		
		0.3838	0.4568	0.5154	0.4251	0.4644	0.5445		
		0.3822	0.4405	0.4834	0.4201	0.4242	0.4654		
		0.3963	0.4596	0.5011	0.4638	0.4713	0.5091		
		0.3875	0.4254	0.4561	0.4365	0.3869	0.3979		
		0.3760	0.4165	0.4482	0.4009	0.3650	0.3783		
		0.3883	0.4333	0.4683	0.4390	0.4064	0.4280		
		0.4041	0.4520	0.4951	0.4879	0.4525	0.4943		
40	1	0.4086	0.5681	0.6553	0.5019	0.7388	0.0000	0.4618	0.1279
		0.3939	0.4880	0.5541	0.4563	0.5413	0.6402		
		0.3964	0.5240	0.5881	0.4641	0.6301	0.7243		
		0.4183	0.4250	0.4715	0.5319	0.3859	0.4360		
		0.3942	0.4265	0.4721	0.4573	0.3896	0.4374		
		0.3899	0.4710	0.5252	0.4440	0.4994	0.5687		
		0.4092	0.4553	0.4803	0.5037	0.4607	0.4577		
		0.3659	0.4768	0.5302	0.3697	0.5137	0.5811		
	2	0.3813	0.3923	0.4726	0.4173	0.3053	0.4387		
		0.4106	0.4125	0.4947	0.5080	0.3551	0.4933		
		0.3824	0.4320	0.5046	0.4207	0.4032	0.5178		
		0.4923	0.4454	0.5100	0.7610	0.4363	0.5312		
		0.4464	0.4461	0.4413	0.6189	0.4380	0.3613		
		0.3676	0.4469	0.5553	0.3749	0.4400	0.6432		
		0.3579	0.4669	0.4717	0.3449	0.4893	0.4364		
		0.3924	0.4800	0.5057	0.4517	0.5216	0.5205		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	3	0.3728	0.4261	0.4880	0.3910	0.3887	0.4768		
		0.3549	0.4001	0.4322	0.3356	0.3245	0.3388		
		0.3753	0.4383	0.4810	0.3988	0.4187	0.4594		
		0.3770	0.4396	0.4735	0.4040	0.4219	0.4409		
		0.4163	0.5047	0.5408	0.5257	0.5825	0.6073		
		0.3757	0.4545	0.4829	0.4000	0.4587	0.4641		
		0.3474	0.3992	0.4303	0.3124	0.3223	0.3341		
		0.5347	0.6057	0.6509	0.8923	0.8316	0.8796		
	4	0.3386	0.3852	0.4517	0.2851	0.2878	0.3870		
		0.3570	0.4047	0.4475	0.3421	0.3359	0.3766		
		0.3730	0.4283	0.4741	0.3916	0.3941	0.4424		
		0.4104	0.4825	0.5413	0.5074	0.5277	0.6086		
		0.3735	0.4133	0.4539	0.3932	0.3571	0.3924		
		0.3902	0.4364	0.4762	0.4449	0.4141	0.4476		
		0.3984	0.4424	0.4782	0.4703	0.4289	0.4525		
		0.3930	0.4313	0.4661	0.4536	0.4015	0.4226		
50	1	0.4006	0.4360	0.5001	0.4771	0.4131	0.5067	0.4471	0.1202
		0.3891	0.4445	0.5020	0.4415	0.4340	0.5114		
		0.3905	0.4424	0.4997	0.4458	0.4289	0.5057		
		0.3946	0.4485	0.5010	0.4585	0.4439	0.5089		
		0.3850	0.3567	0.4072	0.4288	0.2175	0.2770		
		0.3693	0.4380	0.4973	0.3802	0.4180	0.4998		
		0.3842	0.3871	0.4228	0.4263	0.2925	0.3155		
		0.3781	0.4530	0.4963	0.4074	0.4550	0.4973		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	2	0.4022	0.4770	0.5661	0.4820	0.5142	0.6699		
		0.3653	0.4979	0.5772	0.3678	0.5657	0.6973		
		0.3708	0.4923	0.5672	0.3848	0.5519	0.6726		
		0.3507	0.5000	0.5842	0.3226	0.5709	0.7146		
		0.3868	0.4991	0.5766	0.4344	0.5687	0.6958		
		0.3777	0.4793	0.5623	0.4062	0.5199	0.6605		
		0.4007	0.4957	0.5705	0.4774	0.5603	0.6808		
		0.2865	0.5894	0.6983*	0.1238	0.7914	-		
	3	0.3771	0.4855	0.5165	0.4043	0.5351	0.5472		
		0.3425	0.3868	0.4220	0.0000	0.2917	0.3136		
		0.3706	0.4158	0.4560	0.3842	0.3633	0.3976		
		0.3584	0.4022	0.4356	0.3464	0.3297	0.3472		
		0.3803	0.4348	0.4760	0.4142	0.4101	0.4471		
		0.3932	0.4476	0.4775	0.4542	0.4417	0.4508		
		0.3685	0.4150	0.4461	0.3777	0.3613	0.3731		
		0.4057	0.4550	0.4754	0.4929	0.4599	0.4456		
	4	0.3443	0.4059	0.4875	0.3028	0.3388	0.4755		
		0.3636	0.4387	0.5427	0.3625	0.4197	0.6120		
		0.3864	0.4554	0.5486	0.4331	0.4609	0.6266		
		0.3797	0.4590	0.5438	0.4124	0.4698	0.6147		
		0.3705	0.4133	0.4709	0.3839	0.3571	0.4345		
		0.3799	0.4272	0.4828	0.4130	0.3914	0.4639		
		0.3742	0.4141	0.4638	0.3954	0.3591	0.4169		
		0.3869	0.4333	0.4860	0.4347	0.4064	0.4718		

*Outlier rejected with Dixon's Q test.

Table 25 - Optical density measured at 660nm for the HSL trials, and respective concentrations, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3081 with AuNP.

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
0	1	0.5002	0.5656	0.5789	0.7854	0.7327	0.7015	0.7045	0.1937
		0.5485	0.5941	0.6101	0.9350	0.8030	0.7787		
		0.5423	0.5999	0.6128	0.9158	0.8173	0.7854		
		0.5687	0.6174	0.6277	0.9975	0.8604	0.8222		
		0.5694	0.6202	0.6328	0.9997	0.8673	0.8348		
		0.5403	0.5889	0.5915	0.9096	0.7901	0.7327		
		0.5078	0.5779	0.5880	0.8090	0.7630	0.7240		
		0.5579	0.6002	0.6158	0.9641	0.8180	0.7928		
	2	0.4395	0.5002	0.5314	0.5975	0.5714	0.5841		
		0.4512	0.5012	0.5326	0.6337	0.5739	0.5870		
		0.4441	0.4988	0.5283	0.6118	0.5679	0.5764		
		0.4178	0.4769	0.5027	0.5303	0.5139	0.5131		
		0.4158	0.4702	0.5033	0.5241	0.4974	0.5146		
		0.4289	0.5003	0.5303	0.5647	0.5716	0.5814		
		0.4302	0.4951	0.5239	0.5687	0.5588	0.5655		
		0.4107	0.4885	0.5122	0.5084	0.5425	0.5366		
	3	0.5201	0.6031	0.6440	0.8471	0.8252	0.8625		
		0.5518	0.6427	0.6903	0.9452	0.9228	0.9770		
		0.5678	0.6575	0.7036	0.9947	0.9593	1.0099		
		0.5578	0.6389	0.6844	0.9638	0.9134	0.9624		
		0.5433	0.6254	0.6558	0.9189	0.8801	0.8917		
		0.5725	0.6730	0.7008	1.0093	0.9975	1.0030		
		0.5511	0.6298	0.6668	0.9430	0.8910	0.9189		
		0.5652	0.6479	0.6820	0.9867	0.9356	0.9565		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	4	0.3925	0.4577	0.5014	0.4520	0.4666	0.5099		
		0.3906	0.4580	0.4945	0.4461	0.4673	0.4928		
		0.3833	0.4341	0.4738	0.4235	0.4084	0.4416		
		0.3908	0.4579	0.5014	0.4467	0.4671	0.5099		
		0.4111	0.4901	0.5212	0.5096	0.5465	0.5589		
		0.4006	0.4667	0.5037	0.4771	0.4888	0.5156		
		0.3985	0.4801	0.5103	0.4706	0.5218	0.5319		
		0.4133	0.4863	0.5282	0.5164	0.5371	0.5762		
40	1	0.4187	0.4865	0.4982	0.5331	0.5376	0.0000	0.2956	0.1267
		0.4451	0.5012	0.5207	0.6149	0.0000	0.0000		
		0.3698	0.4236	0.4386	0.3817	0.3825	0.3546		
		0.4652	0.5014	0.5179	0.6771	0.5744	0.5507		
		0.3998	0.4563	0.4714	0.4746	0.4631	0.4357		
		0.3845	0.4367	0.4495	0.4272	0.4148	0.3816		
		0.3697	0.4126	0.4284	0.3814	0.3554	0.3294		
		0.4015	0.4617	0.4788	0.4799	0.4764	0.4540		
	2	0.3841	0.4136	0.4301	0.4260	0.3578	0.3336		
		0.3659	0.4002	0.4234	0.3697	0.3248	0.3170		
		0.3458	0.4099	0.4267	0.3074	0.3487	0.3252		
		0.3654	0.3972	0.4116	0.3681	0.3174	0.2878		
		0.3994	0.4361	0.4487	0.4734	0.4133	0.3796		
		0.3647	0.4001	0.4203	0.3659	0.3245	0.3093		
		0.3496	0.3862	0.4017	0.3192	0.2903	0.0000		
		0.3433	0.3899	0.4019	0.2997	0.2994	0.2638		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	3	0.3192	0.3426	0.3677	0.2251	0.1827	0.1793		
		0.3431	0.3802	0.4073	0.2991	0.2755	0.2772		
		0.3321	0.3674	0.3927	0.2650	0.2439	0.2411		
		0.3304	0.3653	0.3924	0.2598	0.2387	0.2404		
		0.3306	0.3616	0.3886	0.2604	0.2296	0.2310		
		0.3106	0.3432	0.3698	0.1985	0.1842	0.1845		
		0.3269	0.3625	0.3884	0.2489	0.2318	0.2305		
		0.3331	0.3656	0.3903	0.2681	0.2395	0.2352		
	4	0.3517	0.3900	0.4095	0.3257	0.2996	0.2826		
		0.3346	0.3687	0.3922	0.2728	0.2471	0.2399		
		0.3466	0.3772	0.4048	0.3099	0.2681	0.2710		
		0.3306	0.3641	0.3861	0.2604	0.2358	0.2248		
		0.3112	0.3383	0.3556	0.2003	0.1721	0.1494		
		0.3064	0.3255	0.3510	0.1854	0.1406	0.1380		
		0.3181	0.3416	0.3620	0.2217	0.1803	0.1652		
		0.3008	0.3214	0.3414	0.1681	0.1305	0.1142		
50	1	0.3154	0.3240	0.3311	0.2133	0.1369	0.0000	0.1612	0.0888
		0.3684	0.3704	0.3814	0.3774	0.2513	0.2132		
		0.3945	0.4011	0.4103	0.4582	0.3270	0.2846		
		0.3415	0.4175	0.4269	0.2941	0.3674	0.3257		
		0.3887	0.3912	0.4089	0.4402	0.3026	0.0000		
		0.3224	0.3302	0.3416	0.2350	0.1522	0.1147		
		0.3267	0.3324	0.3456	0.2483	0.1576	0.1246		
		0.3014	0.3119	0.3227	0.1700	0.1070	0.0680		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	2	0.2823	0.3002	0.3124	0.1108	0.0782	0.0000		
		0.3214	0.3317	0.3436	0.2319	0.1559	0.1197		
		0.3224	0.3312	0.3440	0.2350	0.1546	0.0000		
		0.2987	0.3075	0.3171	0.1616	0.0962	0.0000		
		0.2936	0.3089	0.3196	0.1458	0.0996	0.0603		
		0.3004	0.3126	0.3235	0.1669	0.1088	0.0000		
		0.3126	0.3227	0.3224	0.2046	0.1337	0.0000		
		0.3140	0.3294	0.3401	0.2090	0.1502	0.1110		
	3	0.2979	0.3167	0.3291	0.1591	0.1189	0.0838		
		0.3088	0.3239	0.3331	0.1929	0.1366	0.0937		
		0.3087	0.3235	0.3296	0.1926	0.1356	0.0851		
		0.3069	0.3221	0.3353	0.1870	0.1322	0.0992		
		0.3076	0.3267	0.3435	0.1892	0.1435	0.1194		
		0.3299	0.3500	0.3639	0.2582	0.2010	0.1699		
		0.3069	0.3197	0.3325	0.1870	0.1263	0.0922		
		0.3178	0.3323	0.3423	0.2207	0.1573	0.1165		
	4	0.2798	0.3100	0.3199	0.1031	0.1023	0.0611		
		0.3327	0.3605	0.3825	0.2669	0.2269	0.2159		
		0.3161	0.3389	0.3536	0.2155	0.1736	0.1444		
		0.3040	0.3283	0.3450	0.1780	0.1475	0.1231		
		0.2912	0.3070	0.3209	0.1384	0.0949	0.0636		
		0.3169	0.3362	0.3512	0.2180	0.1670	0.1385		
		0.3172	0.3385	0.3523	0.2189	0.1726	0.1412		
		0.3108	0.3282	0.3438	0.1991	0.1472	0.1202		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
80	1	0.3001	0.3107	0.3148	0.1659	0.1041	0.0485	0.1221	0.0504
		0.3145	0.3199	0.3287	0.2105	0.1268	0.0828		
		0.3224	0.3313	0.3429	0.2350	0.1549	0.1180		
		0.2879	0.2940	0.3149	0.1282	0.0629	0.0487		
		0.2994	0.3178	0.3284	0.1638	0.1216	0.0821		
		0.2899	0.3014	0.3121	0.1344	0.0811	0.0418		
		0.3122	0.3204	0.3368	0.2034	0.1280	0.1029		
		0.2916	0.3089	0.3201	0.1396	0.0996	0.0000		
	2	0.3014	0.3108	0.3228	0.1700	0.1043	0.0682		
		0.2987	0.3089	0.3204	0.1616	0.0996	0.0623		
		0.2975	0.3084	0.3198	0.1579	0.0984	0.0608		
		0.3015	0.3121	0.3229	0.1703	0.1075	0.0685		
		0.3004	0.3128	0.3246	0.1669	0.1092	0.0727		
		0.2997	0.3102	0.3234	0.1647	0.1028	0.0697		
		0.2993	0.3122	0.3260	0.1635	0.1078	0.0762		
		0.3036	0.3151	0.3284	0.1768	0.1149	0.0821		
	3	0.3009	0.3080	0.3127	0.1684	0.0974	0.0433		
		0.3122	0.3197	0.3332	0.2034	0.1263	0.0940		
		0.2980	0.3081	0.3109	0.1594	0.0977	0.0388		
		0.3055	0.3152	0.3239	0.1827	0.1152	0.0710		
		0.2997	0.3089	0.3143	0.1647	0.0996	0.0472		
		0.3054	0.3172	0.3290	0.1824	0.1201	0.0836		
		0.3192	0.3290	0.3391	0.2251	0.1492	0.1086		
		0.3348	0.3476	0.3593	0.2734	0.1951	0.1585		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	4	0.3074	0.3229	0.3314	0.1885	0.1342	0.0895		
		0.2938	0.3089	0.3175	0.1464	0.0996	0.0551		
		0.3083	0.3220	0.3316	0.1913	0.1319	0.0900		
		0.3090	0.3189	0.3330	0.1935	0.1243	0.0935		
		0.2925	0.3147	0.3127	0.1424	0.1139	0.0433		
		0.3029	0.3194	0.3265	0.1746	0.1255	0.0774		
		0.3020	0.3152	0.3275	0.1718	0.1152	0.0799		
		0.3059	0.3219	0.3363	0.1839	0.1317	0.1016		
120	1	0.3045	0.3123	0.3213	0.1796	0.1080	0.0000	0.1085	0.0529
		0.3114	0.3205	0.3317	0.2009	0.1282	0.0903		
		0.2987	0.3154	0.3276	0.1616	0.1157	0.0801		
		0.3174	0.3257	0.3378	0.2195	0.1411	0.1053		
		0.3162	0.3229	0.3334	0.2158	0.1342	0.0945		
		0.3003	0.3102	0.3229	0.1666	0.1028	0.0685		
		0.2946	0.3172	0.3299	0.1489	0.1201	0.0858		
		0.2904	0.3056	0.3171	0.1359	0.0915	0.0542		
	2	0.3011	0.3115	0.3237	0.1690	0.1060	0.0705		
		0.3095	0.3190	0.3314	0.1950	0.1245	0.0895		
		0.2856	0.2964	0.3073	0.1211	0.0688	0.0299		
		0.3002	0.3109	0.3227	0.1663	0.1046	0.0680		
		0.2996	0.3101	0.3231	0.1644	0.1026	0.0690		
		0.2865	0.2994	0.3129	0.1238	0.0762	0.0438		
		0.2834	0.2990	0.3148	0.1142	0.0752	0.0485		
		0.3001	0.3114	0.3210	0.1659	0.1058	0.0638		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	3	0.3078	0.3166	0.3211	0.1898	0.1186	0.0640		
		0.3115	0.3236	0.3316	0.2012	0.1359	0.0900		
		0.3077	0.3186	0.3257	0.1895	0.1236	0.0754		
		0.3121	0.3242	0.3329	0.2031	0.1374	0.0932		
		0.3079	0.3101	0.3153	0.1901	0.1026	0.0497		
		0.2986	0.3119	0.3151	0.1613	0.1070	0.0492		
		0.3037	0.3089	0.3163	0.1771	0.0996	0.0522		
		0.2654	0.2769	0.2831	0.0585	0.0207	0.0000		
	4	0.3062	0.3330	0.3412	0.1848	0.1591	0.1137		
		0.3081	0.3271	0.3298	0.1907	0.1445	0.0856		
		0.2791	0.2937	0.2989	0.1009	0.0621	0.0091		
		0.3022	0.3161	0.3239	0.1724	0.1174	0.0710		
		0.2836	0.2970	0.3053	0.1149	0.0703	0.0250		
		0.2784	0.2880	0.2960	0.0988	0.0481	0.0020		
		0.2829	0.2935	0.3035	0.1127	0.0617	0.0205		
		0.3003	0.3108	0.3238	0.1666	0.1043	0.0707		
140	1	0.2974	0.3078	0.3184	0.1576	0.0969	0.0574	0.1187	0.1024
		0.3012	0.3127	0.3229	0.1693	0.1090	0.0685		
		0.2911	0.3016	0.3140	0.1381	0.0816	0.0465		
		0.2915	0.3024	0.3163	0.1393	0.0836	0.0522		
		0.3014	0.3128	0.3234	0.1700	0.1092	0.0697		
		0.2901	0.3014	0.3137	0.1350	0.0811	0.0457		
		0.2974	0.3198	0.3308	0.1576	0.1265	0.0880		
		0.2997	0.3117	0.3247	0.1647	0.1065	0.0729		

Concentration (mg/L)	Trial	OD After 60 min	OD After 90 min	OD After 120 min	Concentration (mg/L) After 60 min	Concentration (mg/L) After 90 min	Concentration (mg/L) After 120 min	Average Concentration (mg/L)	Standard Deviation
	2	0.3014	0.3116	0.3243	0.1700	0.1063	0.0720		
		0.2840	0.2997	0.3138	0.1161	0.0769	0.0460		
		0.2648	0.2802	0.2908	0.0567	0.0289	0.0000		
		0.2774	0.2849	0.2983	0.0957	0.0404	0.0077		
		0.3140	0.3214	0.3364	0.2090	0.1305	0.1019		
		0.3024	0.3129	0.3237	0.1731	0.1095	0.0705		
		0.3142	0.3237	0.3345	0.2096	0.1361	0.0972		
		0.2851	0.2964	0.3161	0.1195	0.0688	0.0517		
	3	0.2879	0.2955	0.3025	0.1282	0.0666	0.0181		
		0.2914	0.2995	0.3034	0.0000	0.0764	0.0203		
		0.2903	0.3071	0.3093	0.1356	0.0952	0.0349		
		0.2971	0.3053	0.3105	0.1567	0.0908	0.0378		
		0.2908	0.2983	0.3019	0.1372	0.0735	0.0166		
		0.3011	0.3070	0.3107	0.1690	0.0949	0.0383		
		0.3027	0.3204	0.3305	0.1740	0.1280	0.0873		
		0.3133	0.3245	0.3318	0.2068	0.1381	0.0905		
	4	0.3250	0.3244	0.3324	0.2430	0.1379	0.0920		
		0.2778	0.2888	0.2949	0.0969	0.0501	0.0000		
		0.2442	0.4920	0.3397	0.0000	0.5512	0.1100		
		0.2817	0.3391	0.3353	0.1090	0.1741	0.0992		
		0.3744	0.4155	0.3092	0.3960	0.3625	0.0346		
		0.3302	0.2515	0.2456	0.2591	0.0000	0.0000		
		0.3550	0.3523	0.5369	0.3359	0.2067	0.5977		
		0.2568	0.4180	0.3379	0.0319	0.3687	0.1056		

Table 26, Table 27, Table 28 and Table 29 present the optical density of the supernatant values obtained from de OD600 trials, and respective concentrations, for 60, 90 and 120 min of room temperature incubation, and correspondent mass of HSL per mass of biomass, average and standard deviation, for each *Pseudomonas aeruginosa* strain and nanoparticles.

Table 26 - Optical density measured at 600nm, respective biomass concentration, and mg of HSL per mg of biomass, for the HSL trials, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3777 with AgNP.

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
0	1	0.9574	1999.5294	0.00065	0.00063	0.00063	0.00067	0.00030
		0.9325	1949.5128	0.00060	0.00057	0.00058		
		0.9754	2035.6859	0.00032	0.00029	0.00029		
		0.8032	1689.7879	0.00118	0.00119	0.00126		
		1.4406	2970.1333	0.00001	0.00000	0.00000		
		1.2629	2613.1872	0.00022	0.00024	0.00025		
		1.0983	2282.5552	0.00082	0.00075	0.00077		
		1.0609	2207.4298	0.00054	0.00051	0.00050		
	2	1.0972	2280.3457	0.00068	0.00075	0.00078		
		1.1923	2471.3729	0.00078	0.00075	0.00079		
		1.0227	2130.6974	0.00100	0.00095	0.00102		
		1.1414	2369.1301	0.00087	0.00082	0.00086		
		0.9837	2052.3581	0.00100	0.00095	0.00100		
		1.0474	2180.3124	0.00092	0.00084	0.00089		
		1.1201	2326.3449	0.00082	0.00076	0.00081		
		1.0373	2160.0245	0.00051	0.00047	0.00047		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
10	1	0.9690	2022.8303	0.00064	0.00067	0.00072	0.00077	0.00019
		1.1132	2312.4847	0.00054	0.00000	0.00053		
		1.0194	2124.0688	0.00070	0.00063	0.00066		
		1.0921	2270.1013	0.00051	0.00048	0.00050		
		1.0337	2152.7932	0.00064	0.00061	0.00063		
		1.0308	2146.9679	0.00078	0.00071	0.00074		
		0.9842	2053.3625	0.00068	0.00062	0.00064		
		0.9851	2055.1703	0.00092	0.00084	0.00085		
	2	0.9453	1975.2241	0.00073	0.00081	0.00088		
		1.0474	2180.3124	0.00074	0.00077	0.00081		
		1.0151	2115.4314	0.00084	0.00081	0.00086		
		0.9280	1940.4735	0.00101	0.00096	0.00100		
		0.9541	1992.9007	0.00099	0.00094	0.00100		
		1.1028	2291.5944	0.00089	0.00084	0.00088		
		0.9725	2029.8608	0.00102	0.00096	0.00100		
		0.9732	2031.2669	0.00096	0.00090	0.00094		
20	1	1.0409	2167.2558	0.00078	0.00080	0.00084	0.00092	0.00023
		0.9455	1975.6259	0.00013	0.00016	0.00017		
		0.9496	1983.8615	0.00111	0.00106	0.00112		
		0.8726	1829.1917	0.00109	0.00108	0.00112		
		0.9633	2011.3807	0.00095	0.00092	0.00099		
		0.9271	1938.6658	0.00110	0.00105	0.00109		
		0.9312	1946.9015	0.00095	0.00092	0.00096		
		-						

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	0.9505	1985.6694	0.00066	0.00074	0.00078		
		0.8945	1873.1822	0.00098	0.00092	0.00099		
		0.8641	1812.1176	0.00100	0.00099	0.00103		
		0.8608	1805.4890	0.00109	0.00103	0.00109		
		0.9073	1898.8935	0.00097	0.00091	0.00095		
		0.9491	1982.8572	0.00109	0.00102	0.00106		
		0.9329	1950.3163	0.00102	0.00095	0.00101		
		0.9431	1970.8049	0.00100	0.00093	0.00099		
30	1	0.7525	1587.9467	0.00098	0.00105	0.00114	0.00109	0.00009
		0.8154	1714.2940	0.00108	0.00107	0.00111		
		0.8220	1727.5515	0.00099	0.00098	0.00104		
		0.7447	1572.2789	0.00121	0.00118	0.00121		
		0.8010	1685.3687	0.00115	0.00111	0.00118		
		0.8014	1686.1722	0.00109	0.00103	0.00108		
		0.8466	1776.9654	0.00113	0.00108	0.00113		
		0.8848	1853.6978	0.00112	0.00105	0.00110		
	2	0.7490	1580.9163	0.00109	0.00114	0.00124		
		0.7487	1580.3137	0.00102	0.00099	0.00106		
		0.7691	1621.2912	0.00098	0.00098	0.00103		
		0.8896	1863.3395	0.00090	0.00086	0.00090		
		0.7416	1566.0519	0.00109	0.00105	0.00114		
		0.7385	1559.8249	0.00119	0.00114	0.00123		
		0.7701	1623.2999	0.00108	0.00101	0.00107		
		0.7192	1521.0571	0.00122	0.00115	0.00121		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
40	1	0.6468	1375.6271	0.00095	0.00110	0.00113	0.00131	0.00018
		0.6541	1390.2907	0.00143	0.00146	0.00154		
		0.6925	1467.4247	0.00129	0.00127	0.00134		
		0.6543	1390.6924	0.00174	0.00167	0.00175		
		0.6821	1446.5343	0.00159	0.00151	0.00157		
		0.6714	1425.0412	0.00119	0.00118	0.00126		
		0.7010	1484.4987	0.00118	0.00115	0.00122		
		0.6658	1413.7924	0.00129	0.00126	0.00132		
	2	0.6211	1324.0036	0.00124	0.00133	0.00148		
		0.6545	1391.0942	0.00124	0.00126	0.00136		
		0.6370	1355.9419	0.00123	0.00125	0.00137		
		0.6479	1377.8367	0.00130	0.00130	0.00137		
		0.5799	1241.2452	0.00149	0.00144	0.00157		
		0.6322	1346.3001	0.00123	0.00125	0.00137		
		0.7780	1639.1686	0.00107	0.00104	0.00110		
		0.7599	1602.8111	0.00113	0.00110	0.00118		
50	1	0.5771	1235.6207	0.00112	0.00129	0.00136	0.00139	0.00023
		0.6072	1296.0827	0.00168	0.00167	0.00176		
		0.6442	1370.4046	0.00109	0.00111	0.00117		
		0.5936	1268.7643	0.00135	0.00135	0.00141		
		0.5805	1242.4504	0.00151	0.00149	0.00155		
		0.5720	1225.3765	0.00154	0.00147	0.00156		
		0.5042	1089.1865	0.00170	0.00167	0.00174		
		0.7263	1535.3188	0.00089	0.00086	0.00090		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	0.5687	1218.7477	0.00103	0.00115	0.00128		
		0.6848	1451.9578	0.00117	0.00118	0.00129		
		0.5809	1243.2539	0.00127	0.00131	0.00141		
		0.5500	1181.1850	0.00124	0.00123	0.00131		
		0.5557	1192.6346	0.00150	0.00147	0.00159		
		0.5279	1136.7927	0.00146	0.00146	0.00156		
		0.4588	997.9915	0.00170	0.00166	0.00178		
		0.5980	1277.6026	0.00144	0.00141	0.00149		

*Outlier rejected with Dixon's Q test.

Table 27 - Optical density measured at 600nm, respective biomass concentration, and mg of HSL per mg of biomass, for the HSL trials, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3777 with AuNP.

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
0	1	1.2042	2495.27660	0.00078	0.00076	0.00076	0.00069	0.00035
		1.1802	2447.06770	0.00065	0.00062	0.00071		
		1.0799	2245.59518	0.00006	0.00005	0.00004		
		1.1634	2413.32169	0.00005	0.00003	0.00002		
		1.1865	2459.72246	0.00100	0.00094	0.00097		
		1.0461	2177.70111	0.00111	0.00102	0.00102		
		1.1941	2474.98871	0.00101	0.00089	0.00089		
		1.1710	2428.58771	0.00098	0.00088	0.00081		
	2	1.1605	2407.49645	0.00102	0.00097	0.00099		
		1.1006	2287.17523	0.00068	0.00066	0.00068		
		1.1648	2416.13386	0.00003	0.00004	0.00003		
		1.0640	2213.65682	0.00087	0.00084	0.00084		
		1.1208	2327.75100	0.00093	0.00085	0.00087		
		1.0830	2251.82199	0.00086	0.00081	0.00082		
		1.0669	2219.48206	0.00077	0.00072	0.00075		
		0.9774	2039.70339	0.00089	0.00088	0.00088		
	3	1.0524	2190.35587	0.00085	0.00075	0.00078		
		1.1502	2386.80676	0.00050	0.00045	0.00044		
		1.0777	2241.17603	0.00007	0.00011	0.00012		
		0.9628	2010.37641	0.00015	0.00019	0.00019		
		1.1225	2331.16564	0.00102	0.00096	0.00111		
		1.1272	2340.60665	0.00098	0.00087	0.00098		
		1.0955	2276.93083	0.00109	0.00090	0.00108		
		1.0662	2218.07598	0.00098	0.00078	0.00084		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	4	1.0779	2241.57783	0.00083	0.00066	0.00067		
		1.0872	2260.25873	0.00136	0.00126	0.00149		
		1.0702	2226.11067	0.00083	0.00068	0.00067		
		1.0664	2218.47778	0.00093	0.00078	0.00078		
		1.0619	2209.43857	0.00026	0.00019	0.00020		
		1.1187	2323.53275	0.00016	0.00014	0.00013		
		1.0443	2174.08533	0.00070	0.00056	0.00056		
		0.8868	1857.71514	0.00114	0.00092	0.00092		
40	1	0.7371	1557.01278	0.00004	0.00002	0.00000	0.00084	0.00058
		0.8272	1737.99663	0.00002	0.00000	0.00000		
		0.6334	1348.71063	0.00151	0.00144	0.00146		
		0.6591	1400.33416	0.00115	0.00116	0.00124		
		0.6977	1477.87004	0.00130	0.00119	0.00125		
		0.7201	1522.86484	0.00135	0.00126	0.00132		
		0.8643	1812.51944	0.00099	0.00098	0.00103		
		0.7231	1528.89098	0.00138	0.00141	0.00138		
	2	0.6179	1317.57576	0.00009	0.00005	0.00001		
		0.7218	1526.27972	0.00109	0.00103	0.00108		
		0.9395	1963.57360	0.00078	0.00076	0.00079		
		0.7794	1641.98076	0.00098	0.00095	0.00098		
		0.7869	1657.04600	0.00017	0.00013	0.00014		
		0.6533	1388.68368	0.00014	0.00009	0.00007		
		0.6352	1352.32629	0.00007	0.00002	0.00000		
		0.7256	1533.91273	0.00132	0.00126	0.00125		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	3	0.6015	1284.63300	0.00043	0.00032	0.00032		
		0.5632	1207.69983	0.00025	0.00017	0.00019		
		0.6088	1299.29655	0.00143	0.00120	0.00122		
		0.6774	1437.09337	0.00098	0.00080	0.00081		
		0.6502	1382.45676	0.00124	0.00113	0.00113		
		0.6546	1391.29507	0.00240	0.00203	0.00233		
		0.6899	1462.20209	0.00118	0.00093	0.00094		
		0.6473	1376.63152	0.00164	0.00129	0.00145		
	4	0.5653	1211.91809	0.00014	0.00014	0.00014		
		0.7176	1517.84310	0.00009	0.00008	0.00006		
		0.5523	1185.80496	0.00119	0.00098	0.00099		
		0.7336	1549.98236	0.00082	0.00065	0.00066		
		0.7210	1524.67273	0.00132	0.00104	0.00105		
		0.6789	1440.10644	0.00045	0.00037	0.00036		
		0.6011	1283.82963	0.00170	0.00136	0.00154		
		0.6294	1340.67579	0.00151	0.00121	0.00123		
50	1	0.7111	1504.78653	0.00006	0.00002	0.00000	0.00066	0.00063
		0.6503	1382.65766	0.00076	0.00067	0.00073		
		0.7020	1486.50744	0.00153	0.00147	0.00148		
		0.6517	1385.46983	0.00010	0.00006	0.00003		
		0.7293	1541.34495	0.00006	0.00001	0.00000		
		0.6269	1335.65406	0.00181	0.00170	0.00173		
		0.6587	1399.53067	0.00168	0.00155	0.00161		
		0.5907	1262.93903	0.00173	0.00170	0.00167		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	0.7715	1626.11203	0.00007	0.00004	0.00000		
		0.7061	1494.74304	0.00025	0.00023	0.00023		
		0.7642	1611.44848	0.00007	0.00002	0.00000		
		0.8297	1743.01838	0.00005	0.00002	0.00000		
		0.8135	1710.47742	0.00109	0.00103	0.00106		
		0.6375	1356.94623	0.00005	0.00001	0.00000		
		0.8170	1717.50784	0.00006	0.00003	0.00000		
		0.8271	1737.79573	0.00010	0.00007	0.00003		
	3	0.4731	1026.71598	0.00020	0.00017	0.00014		
		0.6520	1386.07242	0.00096	0.00074	0.00074		
		0.6013	1284.23131	0.00091	0.00074	0.00075		
		0.5901	1261.73385	0.00024	0.00022	0.00023		
		0.6136	1308.93835	0.00014	0.00012	0.00012		
		0.6161	1313.96010	0.00212	0.00180	0.00206		
		0.5893	1260.12686	0.00149	0.00118	0.00119		
		0.4833	1047.20471	0.00209	0.00168	0.00172		
	4	0.4954	1071.51000	0.00105	0.00084	0.00084		
		0.5870	1255.50692	0.00030	0.00025	0.00025		
		0.8323	1748.24102	0.00032	0.00025	0.00026		
		0.6266	1335.05147	0.00079	0.00064	0.00062		
		0.6145	1310.74612	0.00117	0.00092	0.00090		
		0.5754	1232.20597	0.00081	0.00066	0.00067		
		0.5420	1165.11539	0.00088	0.00070	0.00071		
		0.5597	1200.66941	0.00042	0.00034	0.00035		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
80	1	0.6037	1289.05215	0.00157	0.00161	0.00169	0.00126	0.00057
		0.7120	1506.59442	0.00132	0.00130	0.00131		
		0.6052	1292.06523	0.00174	0.00161	0.00172		
		0.5301	1141.21185	0.00178	0.00166	0.00171		
		0.6202	1322.19570	0.00175	0.00162	0.00164		
		0.4949	1070.50561	0.00180	0.00175	0.00180		
		0.4274	934.91837	0.00210	0.00196	0.00204		
		0.4138	907.60006	0.00008	0.00001	0.00000		
	2	0.7514	1585.73717	0.00007	0.00003	0.00000		
		0.7859	1655.03732	0.00138	0.00132	0.00134		
		0.7917	1666.68780	0.00139	0.00132	0.00134		
		0.6884	1459.18902	0.00131	0.00127	0.00130		
		0.6609	1403.94982	0.00145	0.00144	0.00145		
		0.6864	1455.17167	0.00148	0.00138	0.00139		
		0.7493	1581.51892	0.00100	0.00101	0.00106		
		0.7158	1514.22743	0.00113	0.00110	0.00107		
	3	0.5635	1208.30242	0.00225	0.00196	0.00221		
		0.6327	1347.30454	0.00130	0.00105	0.00104		
		0.6603	1402.74464	0.00153	0.00124	0.00125		
		0.6051	1291.86432	0.00050	0.00043	0.00043		
		0.5861	1253.69903	0.00185	0.00177	0.00186		
		0.6705	1423.23331	0.00113	0.00088	0.00088		
		0.5942	1269.96957	0.00140	0.00111	0.00110		
		0.6134	1308.53654	0.00083	0.00071	0.00075		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	4	0.4972	1075.12566	0.00048	0.00037	0.00036		
		0.5803	1242.04856	0.00017	0.00015	0.00016		
		0.6080	1297.68956	0.00180	0.00178	0.00208		
		0.6596	1401.33856	0.00125	0.00102	0.00106		
		0.5671	1215.53375	0.00176	0.00139	0.00138		
		0.6377	1357.34803	0.00152	0.00121	0.00122		
		0.4748	1030.13074	0.00121	0.00096	0.00098		
		0.5353	1151.65714	0.00208	0.00205	0.00237		
120	1	0.3084	695.88309	0.00009	0.00001	0.00000	0.00117	0.00053
		0.7395	1561.83362	0.00090	0.00090	0.00095		
		0.4972	1075.12566	0.00149	0.00146	0.00152		
		0.5371	1155.27280	0.00012	0.00006	0.00001		
		0.7926	1668.49557	0.00116	0.00117	0.00120		
		0.6228	1327.41835	0.00149	0.00140	0.00143		
		0.6891	1460.59522	0.00143	0.00129	0.00137		
		0.5575	1196.25026	0.00168	0.00164	0.00167		
	2	0.5446	1170.33804	0.00205	0.00199	0.00207		
		0.4915	1063.67603	0.00207	0.00197	0.00195		
		0.8675	1818.94726	0.00101	0.00109	0.00111		
		0.4009	881.68784	0.00191	0.00191	0.00198		
		0.7845	1652.22516	0.00137	0.00145	0.00147		
		0.4657	1011.85159	0.00196	0.00174	0.00170		
		0.5696	1220.55549	0.00134	0.00121	0.00130		
		0.9675	2019.81719	0.00081	0.00095	0.00096		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	3	0.6110	1303.71570	0.00019	0.00015	0.00012		
		0.6175	1316.77226	0.00117	0.00095	0.00095		
		0.6244	1330.63232	0.00139	0.00114	0.00116		
		0.5355	1152.05883	0.00021	0.00019	0.00020		
		0.6570	1396.11591	0.00110	0.00090	0.00089		
		0.5274	1135.78841	0.00178	0.00142	0.00143		
		0.5672	1215.73465	0.00178	0.00141	0.00141		
		0.5724	1226.17983	0.00143	0.00115	0.00117		
	4	0.5993	1280.21396	0.00061	0.00051	0.00053		
		0.5826	1246.66862	0.00163	0.00132	0.00132		
		0.5838	1249.07910	0.00140	0.00112	0.00111		
		0.6560	1394.10724	0.00110	0.00089	0.00090		
		0.5331	1147.23799	0.00177	0.00141	0.00138		
		0.5691	1219.55121	0.00157	0.00125	0.00124		
		0.5974	1276.39740	0.00134	0.00109	0.00111		
		0.6275	1336.85925	0.00091	0.00074	0.00077		
140	1	0.5685	1218.34591	0.00012	0.00007	0.00003	0.00097	0.00072
		0.5823	1246.06602	0.00012	0.00007	0.00004		
		0.4455	971.27582	0.00210	0.00202	0.00206		
		0.7358	1554.40152	0.00129	0.00129	0.00131		
		0.5490	1179.17635	0.00148	0.00154	0.00159		
		0.5313	1143.62233	0.00159	0.00156	0.00161		
		0.2987	676.39870	0.00281	0.00283	0.00276		
		0.6505	1383.05935	0.00130	0.00119	0.00119		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	0.4062	892.33392	0.00041	0.00037	0.00033		
		0.6729	1428.05427	0.00170	0.00156	0.00160		
		0.5592	1199.66502	0.00042	0.00037	0.00042		
		0.2796	638.03251	0.00135	0.00151	0.00163		
		0.6253	1332.44009	0.00177	0.00166	0.00175		
		-						
		0.5475	1176.16328	0.00039	0.00038	0.00043		
		0.5404	1161.90154	0.00062	0.00066	0.00059		
	3	0.6450	1372.01146	0.00039	0.00033	0.00036		
		0.6612	1404.55241	0.00000	0.00003	0.00005		
		0.6427	1367.39152	0.00145	0.00116	0.00119		
		0.6549	1391.89766	0.00128	0.00100	0.00099		
		0.5822	1245.86512	0.00124	0.00099	0.00100		
		0.5827	1246.86952	0.00047	0.00034	0.00033		
		0.5521	1185.40328	0.00055	0.00043	0.00044		
		0.4616	1003.61593	0.00188	0.00149	0.00148		
	4	0.5819	1245.26253	0.00018	0.00013	0.00016		
		0.6024	1286.44089	0.00144	0.00114	0.00113		
		0.6391	1360.16020	0.00062	0.00052	0.00053		
		0.4797	1039.97339	0.00039	0.00029	0.00035		
		0.5307	1142.41715	0.00225	0.00211	0.00245		
		0.6288	1339.47051	0.00161	0.00128	0.00129		
		0.6484	1378.84110	0.00033	0.00031	0.00028		
		0.5818	1245.06163	0.00041	0.00033	0.00033		

Table 28 - Optical density measured at 600nm, respective biomass concentration, and mg of HSL per mg of biomass, for the HSL assay trials, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3081 with AgNP.

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
0	1	1.3922	2872.9121	0.00020	0.00004	0.00003	0.00025	0.00011
		1.3444	2776.8964	0.00015	0.00022	0.00026		
		1.4945	3078.4022	0.00015	0.00016	0.00017		
		1.3469	2781.9180	0.00016	0.00022	0.00026		
		1.2514	2590.0872	0.00017	0.00016	0.00018		
		1.6229	3336.3192	0.00014	0.00015	0.00017		
		1.3673	2822.8956	0.00015	0.00016	0.00018		
		1.2954	2678.4700	0.00017	0.00023	0.00026		
	2	1.3596	2807.4284	0.00013	0.00016	0.00019		
		1.4203	2929.3566	0.00008	0.00018	0.00022		
		1.4023	2893.2000	0.00012	0.00017	0.00019		
		1.4452	2979.3732	0.00007	0.00017	0.00019		
		1.3839	2856.2400	0.00013	0.00018	0.00021		
		1.4202	2929.1557	0.00013	0.00020	0.00023		
		1.3984	2885.3660	0.00011	0.00017	0.00020		
		1.5059	3101.3014	0.00015	0.00021	0.00023		
	3	0.7695	1622.0947	0.00023	0.00029	0.00032		
		0.7461	1575.0911	0.00027	0.00032	0.00034		
		0.7791	1641.3782	0.00034	0.00039	0.00039		
		0.7521	1587.1433	0.00022	0.00023	0.00024		
		0.7621	1607.2302	0.00038	0.00037	0.00043		
		0.7667	1616.4703	0.00033	0.00036	0.00038		
		0.7343	1551.3884	0.00032	0.00035	0.00039		
		0.7933	1669.9017	0.00026	0.00027	0.00030		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	4	0.7144	1511.4153	0.00024	0.00022	0.00026		
		0.6584	1398.9281	0.00037	0.00036	0.00040		
		0.7093	1501.1709	0.00041	0.00044	0.00047		
		0.7827	1648.6095	0.00031	0.00032	0.00031		
		0.8472	1778.1706	0.00026	0.00024	0.00025		
		0.7411	1565.0476	0.00028	0.00026	0.00028		
		0.7352	1553.1962	0.00037	0.00036	0.00039		
		0.7093	1501.1709	0.00047	0.00052	0.00057		
10	1	1.1044	2294.8084	0.00015	0.00021	0.00000	0.00027	0.00012
		1.3135	2714.8275	0.00013	0.00000	0.00000		
		1.2495	2586.2707	0.00015	0.00018	0.00019		
		1.4224	2933.5749	0.00010	0.00022	0.00025		
		1.1892	2465.1461	0.00015	0.00037	0.00000		
		1.2660	2619.4143	0.00013	0.00020	0.00022		
		1.3731	2834.5461	0.00012	0.00024	0.00026		
		1.1923	2471.3729	0.00012	0.00022	0.00025		
	2	1.4087	2906.0557	0.00014	0.00015	0.00018		
		1.2664	2620.2176	0.00014	0.00024	0.00027		
		1.2303	2547.7035	0.00013	0.00022	0.00025		
		1.2238	2534.6469	0.00013	0.00024	0.00028		
		1.2844	2656.3743	0.00012	0.00022	0.00030		
		1.3391	2766.2502	0.00018	0.00020	0.00024		
		1.4637	3016.5343	0.00017	0.00018	0.00000		
		1.3059	2699.5613	0.00023	0.00024	0.00027		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	3	0.6509	1383.8628	0.00027	0.00030	0.00033		
		0.6501	1382.2559	0.00035	0.00035	0.00041		
		0.6485	1379.0420	0.00029	0.00030	0.00031		
		0.5100	1100.8370	0.00034	0.00035	0.00038		
		0.6325	1346.9027	0.00029	0.00029	0.00029		
		0.6100	1301.7070	0.00034	0.00034	0.00038		
		0.6182	1318.1783	0.00033	0.00033	0.00035		
		0.5602	1201.6737	0.00022	0.00016	0.00013		
	4	0.6258	1333.4445	0.00033	0.00033	0.00038		
		0.5096	1100.0335	0.00043	0.00044	0.00048		
		0.6002	1282.0217	0.00042	0.00041	0.00044		
		0.5803	1242.0486	0.00043	0.00043	0.00046		
		0.5648	1210.9138	0.00044	0.00042	0.00045		
		0.5630	1207.2981	0.00041	0.00041	0.00044		
		0.5377	1156.4780	0.00038	0.00039	0.00042		
		0.6021	1285.8383	0.00041	0.00041	0.00046		
20	1	1.1924	2471.5738	0.00014	0.00016	0.00000	0.00028	0.00015
		1.1548	2396.0469	0.00015	0.00024	0.00027		
		1.4864	3062.1317	0.00014	0.00014	0.00015		
		1.1735	2433.6093	0.00012	0.00027	0.00032		
		1.1549	2396.2475	0.00016	0.00013	0.00000		
		1.1014	2288.7822	0.00017	0.00020	0.00022		
		1.3327	2753.3945	0.00015	0.00021	0.00022		
		1.2917	2671.0378	0.00010	0.00019	0.00021		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	1.0807	2247.2022	0.00018	0.00023	0.00000		
		1.1191	2324.3361	0.00006	0.00021	0.00024		
		1.1599	2406.2910	0.00017	0.00028	0.00000		
		1.1021	2290.1883	0.00016	0.00026	0.00000		
		1.0969	2279.7430	0.00014	0.00022	0.00025		
		1.2086	2504.1149	0.00005	0.00022	0.00000		
		1.0934	2272.7126	0.00018	0.00022	0.00000		
		1.1728	2432.2032	0.00013	0.00019	0.00022		
	3	0.5671	1215.5337	0.00023	0.00023	0.00026		
		0.4998	1080.3483	0.00034	0.00036	0.00039		
		0.4509	982.1228	0.00033	0.00035	0.00038		
		0.5776	1236.6251	0.00033	0.00036	0.00037		
		0.6101	1301.9078	0.00032	0.00032	0.00034		
		0.4596	999.5985	0.00040	0.00040	0.00041		
		0.4527	985.7385	0.00034	0.00036	0.00037		
		0.4794	1039.3708	0.00044	0.00044	0.00047		
	4	0.4702	1020.8907	0.00050	0.00061	0.00072		
		0.5446	1170.3380	0.00035	0.00036	0.00042		
		0.4633	1007.0307	0.00047	0.00047	0.00052		
		0.4058	891.5305	0.00048	0.00048	0.00053		
		0.3962	872.2469	0.00052	0.00047	0.00049		
		0.4402	960.6297	0.00044	0.00041	0.00043		
		0.4570	994.3759	0.00047	0.00049	0.00052		
		0.5864	1254.3016	0.00032	0.00032	0.00035		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
30	1	1.0332	2151.7889	0.00019	0.00017	0.00022	0.00031	0.00014
		1.0113	2107.7982	0.00021	0.00024	0.00029		
		0.9199	1924.2031	0.00025	0.00005	0.00003		
		1.5664	3222.8278	0.00014	0.00013	0.00015		
		1.0640	2213.6568	0.00022	0.00020	0.00022		
		1.1173	2320.7206	0.00025	0.00022	0.00024		
		1.1165	2319.1136	0.00015	0.00012	0.00020		
		0.9589	2002.5424	0.00019	0.00029	0.00000		
	2	1.3390	2766.0493	0.00013	0.00012	0.00014		
		0.7861	1655.4390	0.00034	0.00031	0.00036		
		1.1036	2293.2014	0.00015	0.00022	0.00025		
		0.9781	2041.1095	0.00013	0.00026	0.00028		
		1.0702	2226.1107	0.00021	0.00013	0.00015		
		1.2565	2600.3316	0.00013	0.00015	0.00017		
		1.1099	2305.8561	0.00019	0.00018	0.00020		
		1.0332	2151.7889	0.00017	0.00020	0.00021		
	3	0.4551	990.5594	0.00035	0.00037	0.00041		
		0.4669	1014.2620	0.00039	0.00040	0.00043		
		0.4085	896.9539	0.00034	0.00032	0.00032		
		0.4336	947.3723	0.00035	0.00034	0.00036		
		0.3989	877.6704	0.00059	0.00058	0.00056		
		0.4862	1053.0300	0.00039	0.00040	0.00042		
		0.4121	904.1852	0.00044	0.00044	0.00046		
		0.4121	904.1852	0.00038	0.00028	0.00023		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	4	0.4433	966.8567	0.00033	0.00034	0.00042		
		0.5955	1272.5808	0.00033	0.00036	0.00043		
		0.4462	972.6820	0.00043	0.00044	0.00048		
		0.3457	770.8076	0.00060	0.00061	0.00066		
		0.4466	973.4854	0.00045	0.00040	0.00041		
		0.4605	1001.4064	0.00040	0.00036	0.00038		
		0.4358	951.7914	0.00046	0.00043	0.00045		
		0.4351	950.3853	0.00051	0.00048	0.00052		
40	1	0.7861	1655.4390	0.00030	0.00045	0.00000	0.00040	0.00021
		0.9166	1917.5744	0.00024	0.00028	0.00033		
		1.2187	2524.4028	0.00018	0.00025	0.00029		
		1.0571	2199.7969	0.00024	0.00018	0.00020		
		1.0365	2158.4175	0.00021	0.00018	0.00020		
		1.0470	2179.5090	0.00020	0.00023	0.00026		
		0.9147	1913.7578	0.00026	0.00024	0.00024		
		0.9453	1975.2241	0.00019	0.00026	0.00029		
	2	0.8426	1768.9306	0.00024	0.00017	0.00025		
		0.7873	1657.8495	0.00031	0.00021	0.00030		
		0.9051	1894.4743	0.00022	0.00021	0.00027		
		0.9294	1943.2858	0.00039	0.00022	0.00027		
		0.8551	1794.0393	0.00034	0.00024	0.00020		
		1.1018	2289.5856	0.00016	0.00019	0.00028		
		0.7804	1643.9894	0.00021	0.00030	0.00027		
		0.7447	1572.2789	0.00029	0.00033	0.00033		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	3	0.3633	806.1607	0.00049	0.00048	0.00059		
		0.4879	1056.4447	0.00032	0.00031	0.00032		
		0.3760	831.6712	0.00048	0.00050	0.00055		
		0.4343	948.7784	0.00043	0.00044	0.00046		
		0.3875	854.7712	0.00062	0.00068	0.00071		
		0.4093	898.5609	0.00045	0.00051	0.00052		
		0.3527	784.8685	0.00040	0.00041	0.00043		
		0.3335	746.3014	0.00120	0.00111	0.00118		
	4	0.3631	805.7590	0.00035	0.00036	0.00048		
		0.3206	720.3892	0.00047	0.00047	0.00052		
		0.3557	790.8946	0.00050	0.00050	0.00056		
		0.3182	715.5683	0.00071	0.00074	0.00085		
		0.3601	799.7329	0.00049	0.00045	0.00049		
		0.3060	691.0622	0.00064	0.00060	0.00065		
		0.3906	860.9982	0.00055	0.00050	0.00053		
		0.3764	832.4747	0.00054	0.00048	0.00051		
50	1	0.8329	1749.4462	0.00027	0.00024	0.00029	0.00043	0.00018
		0.8531	1790.0220	0.00025	0.00024	0.00029		
		0.6108	1303.3140	0.00034	0.00033	0.00039		
		0.7575	1597.9902	0.00029	0.00028	0.00032		
		0.8231	1729.7609	0.00025	0.00013	0.00016		
		0.7960	1675.3252	0.00023	0.00025	0.00030		
		0.6534	1388.8846	0.00031	0.00021	0.00023		
		0.6225	1326.8158	0.00031	0.00034	0.00037		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	1.0467	2178.9063	0.00022	0.00024	0.00031		
		0.8937	1871.5752	0.00020	0.00030	0.00037		
		0.7445	1571.8771	0.00024	0.00035	0.00043		
		0.6654	1412.9890	0.00023	0.00040	0.00051		
		0.5993	1280.2140	0.00034	0.00044	0.00054		
		0.7738	1630.7321	0.00025	0.00032	0.00041		
		0.6971	1476.6647	0.00032	0.00038	0.00046		
		0.7001	1482.6909	0.00008	0.00053	0.00000		
	3	0.3327	744.6945	0.00054	0.00072	0.00073		
		0.2624	603.4829	0.00000	0.00048	0.00052		
		0.2749	628.5916	0.00061	0.00058	0.00063		
		0.2807	640.2421	0.00054	0.00051	0.00054		
		0.3057	690.4596	0.00060	0.00059	0.00065		
		0.2920	662.9404	0.00069	0.00067	0.00068		
		0.3335	746.3014	0.00051	0.00048	0.00050		
		0.3192	717.5771	0.00069	0.00064	0.00062		
	4	0.5442	1169.5345	0.00026	0.00029	0.00041		
		0.3534	786.2746	0.00046	0.00053	0.00078		
		0.3063	691.6648	0.00063	0.00067	0.00091		
		0.2876	654.1021	0.00063	0.00072	0.00094		
		0.3669	813.3920	0.00047	0.00044	0.00053		
		0.4742	1028.9256	0.00040	0.00038	0.00045		
		0.4353	950.7871	0.00042	0.00038	0.00044		
		0.3792	838.0991	0.00052	0.00048	0.00056		

Table 29 - Optical density measured at 600nm, respective biomass concentration, and mg of HSL per mg of biomass, for the HSL trials, for 60, 90 and 120 min of room temperature incubation, with the correspondent average and standard deviation, for PA3081 with AuNP.

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
0	1	0.7463	1575.49277	0.00050	0.00047	0.00045	0.00046	0.00015
		0.8474	1778.57240	0.00053	0.00045	0.00044		
		0.8804	1844.85948	0.00050	0.00044	0.00043		
		0.8509	1785.60282	0.00056	0.00048	0.00046		
		0.8515	1786.80800	0.00056	0.00049	0.00047		
		0.8275	1738.59922	0.00052	0.00045	0.00042		
		0.7341	1550.98664	0.00052	0.00049	0.00047		
		0.5776	1236.62512	0.00078	0.00066	0.00064		
	2	0.7195	1521.65966	0.00039	0.00038	0.00038		
		0.7527	1588.34843	0.00040	0.00036	0.00037		
		0.6842	1450.75252	0.00042	0.00039	0.00040		
		0.7355	1553.79881	0.00034	0.00033	0.00033		
		0.6979	1478.27172	0.00035	0.00034	0.00035		
		0.7413	1565.44928	0.00036	0.00037	0.00037		
		0.6657	1413.59163	0.00040	0.00040	0.00040		
		0.7054	1493.33696	0.00034	0.00036	0.00036		
	3	0.7608	1604.61897	0.00053	0.00051	0.00054		
		0.7880	1659.25558	0.00057	0.00056	0.00059		
		0.6158	1313.35751	0.00076	0.00073	0.00077		
		0.5580	1197.25466	0.00080	0.00076	0.00080		
		0.6694	1421.02373	0.00065	0.00062	0.00063		
		0.7034	1489.31961	0.00068	0.00067	0.00067		
		0.7078	1498.15780	0.00063	0.00059	0.00061		
		0.6903	1463.00559	0.00067	0.00064	0.00065		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	4	0.8304	1744.42446	0.00026	0.00027	0.00029		
		0.6004	1282.42342	0.00035	0.00036	0.00038		
		0.6085	1298.69396	0.00033	0.00031	0.00034		
		0.7829	1649.01118	0.00027	0.00028	0.00031		
		0.8320	1747.63843	0.00029	0.00031	0.00032		
		0.8815	1847.06906	0.00026	0.00026	0.00028		
		0.6717	1425.64379	0.00033	0.00037	0.00037		
		0.8502	1784.19673	0.00029	0.00030	0.00032		
40	1	0.7625	1608.03373	0.00033	0.00033	0.00000	0.00025	0.00011
		0.6088	1299.29655	0.00047	0.00000	0.00000		
		0.5999	1281.41914	0.00030	0.00030	0.00028		
		0.5206	1122.12926	0.00060	0.00051	0.00049		
		0.5264	1133.77974	0.00042	0.00041	0.00038		
		0.4806	1041.78122	0.00041	0.00040	0.00037		
		0.6244	1330.63232	0.00029	0.00027	0.00025		
		0.5793	1240.03988	0.00039	0.00038	0.00037		
	2	0.5731	1227.58591	0.00035	0.00029	0.00027		
		0.5835	1248.47651	0.00030	0.00026	0.00025		
		0.4939	1068.49693	0.00029	0.00033	0.00030		
		0.8072	1697.82267	0.00022	0.00019	0.00017		
		0.7323	1547.37098	0.00031	0.00027	0.00025		
		0.5663	1213.92676	0.00030	0.00027	0.00025		
		0.6275	1336.85925	0.00024	0.00022	0.00000		
		0.5634	1208.10152	0.00025	0.00025	0.00022		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	3	0.6504	1382.85845	0.00016	0.00013	0.00013		
		0.4840	1048.61079	0.00029	0.00026	0.00026		
		0.4024	884.70085	0.00030	0.00028	0.00027		
		0.4662	1012.85593	0.00026	0.00024	0.00024		
		0.5636	1208.50333	0.00022	0.00019	0.00019		
		0.8240	1731.56880	0.00011	0.00011	0.00011		
		0.5726	1226.58163	0.00020	0.00019	0.00019		
		0.3694	818.41377	0.00033	0.00029	0.00029		
	4	0.4200	920.05397	0.00035	0.00033	0.00031		
		0.3929	865.61821	0.00032	0.00029	0.00028		
		0.4860	1052.62820	0.00029	0.00025	0.00026		
		0.5260	1132.97625	0.00023	0.00021	0.00020		
		0.4330	946.16710	0.00021	0.00018	0.00016		
		0.5294	1139.80576	0.00016	0.00012	0.00012		
		0.5975	1276.59830	0.00017	0.00014	0.00013		
		0.5809	1243.25386	0.00014	0.00010	0.00009		
50	1	0.6058	1293.27041	0.00016	0.00011	0.00000	0.00015	0.00009
		0.5228	1126.54842	0.00034	0.00022	0.00019		
		0.3738	827.25208	0.00055	0.00040	0.00034		
		0.4964	1073.51868	0.00027	0.00034	0.00030		
		0.6262	1334.24798	0.00033	0.00023	0.00000		
		0.5350	1151.05455	0.00020	0.00013	0.00010		
		0.5530	1187.21105	0.00021	0.00013	0.00010		
		0.4767	1033.94731	0.00016	0.00010	0.00007		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	0.4205	921.05837	0.00012	0.00008	0.00000		
		0.4678	1016.06984	0.00023	0.00015	0.00012		
		0.6702	1422.63072	0.00017	0.00011	0.00000		
		0.5590	1199.26333	0.00013	0.00008	0.00000		
		0.6433	1368.59670	0.00011	0.00007	0.00004		
		0.7439	1570.67193	0.00011	0.00007	0.00000		
		0.5511	1183.39460	0.00017	0.00011	0.00000		
		0.5282	1137.39528	0.00018	0.00013	0.00010		
	3	0.4775	1035.55423	0.00015	0.00011	0.00008		
		0.3561	791.69806	0.00024	0.00017	0.00012		
		0.4719	1024.30550	0.00019	0.00013	0.00008		
		0.4297	939.53836	0.00020	0.00014	0.00011		
		0.4770	1034.54990	0.00018	0.00014	0.00012		
		0.3983	876.46519	0.00029	0.00023	0.00019		
		0.4951	1070.90735	0.00017	0.00012	0.00009		
		0.4843	1049.21339	0.00021	0.00015	0.00011		
	4	0.6797	1441.71342	0.00007	0.00007	0.00004		
		0.4054	890.72700	0.00030	0.00025	0.00024		
		0.8281	1739.80452	0.00012	0.00010	0.00008		
		0.8805	1845.06039	0.00010	0.00008	0.00007		
		0.4514	983.12720	0.00014	0.00010	0.00006		
		0.3747	829.05991	0.00026	0.00020	0.00017		
		0.3881	855.97647	0.00026	0.00020	0.00016		
		0.3857	851.15556	0.00023	0.00017	0.00014		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
80	1	0.3940	867.82779	0.00019	0.00012	0.00006	0.00013	0.00006
		0.6780	1438.29855	0.00015	0.00009	0.00006		
		0.5169	1114.69704	0.00021	0.00014	0.00011		
		0.5105	1101.84138	0.00012	0.00006	0.00004		
		0.5291	1139.20317	0.00014	0.00011	0.00007		
		0.4889	1058.45344	0.00013	0.00008	0.00004		
		0.5219	1124.74053	0.00018	0.00011	0.00009		
		0.5513	1183.79629	0.00012	0.00008	0.00000		
	2	0.4943	1069.30043	0.00016	0.00010	0.00006		
		0.4684	1017.27508	0.00016	0.00010	0.00006		
		0.4752	1030.93423	0.00015	0.00010	0.00006		
		0.6587	1399.53067	0.00012	0.00008	0.00005		
		0.4713	1023.10032	0.00016	0.00011	0.00007		
		0.4819	1044.39254	0.00016	0.00010	0.00007		
		0.5043	1089.38741	0.00015	0.00010	0.00007		
		0.6367	1355.33924	0.00013	0.00008	0.00006		
	3	0.3133	705.72574	0.00024	0.00014	0.00006		
		0.4791	1038.76815	0.00020	0.00012	0.00009		
		0.4970	1074.72392	0.00015	0.00009	0.00004		
		0.4362	952.59493	0.00019	0.00012	0.00007		
		0.5072	1095.21265	0.00015	0.00009	0.00004		
		0.3387	756.74668	0.00024	0.00016	0.00011		
		0.3463	772.01282	0.00029	0.00019	0.00014		
		0.3603	800.13462	0.00034	0.00024	0.00020		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	4	0.4535	987.34546	0.00019	0.00014	0.00009		
		0.3873	854.36954	0.00017	0.00012	0.00006		
		0.4163	912.62181	0.00021	0.00014	0.00010		
		0.3878	855.37388	0.00023	0.00015	0.00011		
		0.3192	717.57706	0.00020	0.00016	0.00006		
		0.3243	727.82139	0.00024	0.00017	0.00011		
		0.3080	695.07960	0.00025	0.00017	0.00011		
		0.3299	739.07012	0.00025	0.00018	0.00014		
120	1	0.5502	1181.58671	0.00015	0.00009	0.00000	0.00010	0.00006
		0.5084	1097.62313	0.00018	0.00012	0.00008		
		0.4859	1052.42736	0.00015	0.00011	0.00008		
		0.4960	1072.71518	0.00020	0.00013	0.00010		
		0.5026	1085.97265	0.00020	0.00012	0.00009		
		0.6119	1305.52347	0.00013	0.00008	0.00005		
		0.4993	1079.34392	0.00014	0.00011	0.00008		
		0.7742	1631.53558	0.00008	0.00006	0.00003		
	2	0.4526	985.53762	0.00017	0.00011	0.00007		
		0.5145	1109.87619	0.00018	0.00011	0.00008		
		0.3678	815.19985	0.00015	0.00008	0.00004		
		0.4761	1032.74207	0.00016	0.00010	0.00007		
		0.5983	1278.20517	0.00013	0.00008	0.00005		
		0.5121	1105.05523	0.00011	0.00007	0.00004		
		0.6635	1409.17247	0.00008	0.00005	0.00003		
		0.4979	1076.53175	0.00015	0.00010	0.00006		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	3	0.3696	818.81552	0.00023	0.00014	0.00008		
		0.3441	767.59367	0.00026	0.00018	0.00012		
		0.5215	1123.93703	0.00017	0.00011	0.00007		
		0.3543	788.08239	0.00026	0.00017	0.00012		
		0.5706	1222.56417	0.00016	0.00008	0.00004		
		0.4311	942.35059	0.00017	0.00011	0.00005		
		0.5222	1125.34312	0.00016	0.00009	0.00005		
		0.6550	1392.09844	0.00004	0.00001	0.00000		
	4	0.4761	1032.74207	0.00018	0.00015	0.00011		
		0.3800	839.70599	0.00023	0.00017	0.00010		
		0.4688	1018.07858	0.00010	0.00006	0.00001		
		0.5050	1090.79349	0.00016	0.00011	0.00007		
		0.3519	783.26155	0.00015	0.00009	0.00003		
		0.8920	1868.16044	0.00005	0.00003	0.00000		
		0.5101	1101.03788	0.00010	0.00006	0.00002		
		-						
140	1	0.5637	1208.70423	0.00013	0.00008	0.00005	0.00011	0.00011
		0.8711	1826.17859	0.00009	0.00006	0.00004		
		0.6784	1439.10204	0.00010	0.00006	0.00003		
		0.5944	1270.37126	0.00011	0.00007	0.00004		
		0.5001	1080.95090	0.00016	0.00010	0.00006		
		0.3311	741.48054	0.00018	0.00011	0.00006		
		0.5456	1172.34671	0.00013	0.00011	0.00008		
		0.5198	1120.52227	0.00015	0.00010	0.00007		

Concentration (mg/L)	Trial	OD600	Biomass Concentration (mg/L)	mg of HSL per mg of Biomass After 60 min	mg of HSL per mg of Biomass After 90 min	mg of HSL per mg of Biomass After 120 min	Average	Standard Deviation
	2	0.5946	1270.77306	0.00013	0.00008	0.00006		
		0.4967	1074.12127	0.00011	0.00007	0.00004		
		0.4142	908.40356	0.00006	0.00003	0.00000		
		1.0525	2190.55677	0.00004	0.00002	0.00000		
		0.8161	1715.70007	0.00012	0.00008	0.00006		
		0.5793	1240.03988	0.00014	0.00009	0.00006		
		0.5089	1098.62740	0.00019	0.00012	0.00009		
		0.4867	1054.03429	0.00011	0.00007	0.00005		
	3	0.4387	957.61667	0.00013	0.00007	0.00002		
		0.3832	846.13382	0.00000	0.00009	0.00002		
		0.8689	1821.75943	0.00007	0.00005	0.00002		
		0.5618	1204.88767	0.00013	0.00008	0.00003		
		0.4556	991.56371	0.00014	0.00007	0.00002		
		0.6013	1284.23131	0.00013	0.00007	0.00003		
		0.3471	773.61975	0.00022	0.00017	0.00011		
		0.3778	835.28684	0.00025	0.00017	0.00011		
	4	0.3994	878.67477	0.00028	0.00016	0.00010		
		0.3868	853.36514	0.00011	0.00006	0.00000		
		0.6244	1330.63232	0.00000	0.00041	0.00008		
		0.4308	941.74794	0.00012	0.00018	0.00011		
		0.4520	984.33238	0.00040	0.00037	0.00004		
		0.4188	917.64355	0.00028	0.00000	0.00000		
		0.4022	884.29917	0.00038	0.00023	0.00068		
		0.4972	1075.12566	0.00003	0.00034	0.00010		

12.3. ADHERED BIOMASS ANALYSIS

Table 30, Table 31, Table 32 and Table 33present the Cell Confluence data obtained from *Cellavista* image analysis, for the different trials, with the correspondent average and standard deviation, for each *Pseudomonas aeruginosa* strain, nanoparticles and respective concentrations.

Table 30 – Cell Confluence data retrieved from *Cellavista* image analysis, with respective average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AgNP

Concentration (mg/L)	Trial Cell Confluence Values (%)		Average Cell Confluence	Standard Deviation	CC/ Final Biomass (mg/mL)		CC/ Final Biomass Average	Standard Deviation
	1	2			1	2		
0	7.43	72.77	61.60	28.88	3.26	31.98	27.07	12.69
	45.39	92.90			19.95	40.83		
	47.82	83.20			21.02	36.57		
	29.54	88.64			12.98	38.96		
	58.28	92.38			25.61	40.60		
	23.30	93.69			10.24	41.18		
	21.68	95.13			9.53	41.81		
	52.56	80.84			23.10	35.53		
10	64.17	38.16	55.05	28.11	30.47	18.12	26.14	13.35
	39.58	71.88			18.79	34.13		
	36.43	87.74			17.30	41.67		
	14.29	81.19			6.78	38.55		
	13.46	87.73			6.39	41.66		
	25.00	90.76			11.87	43.10		
	11.88	80.05			5.64	38.01		
	61.34	77.13			29.13	36.62		

Concentration (mg/L)	Trial Cell Confluence Values (%)		Average Cell Confluence	Standard Deviation	CC/ Final Biomass (mg/mL)		CC/ Final Biomass Average	Standard Deviation
	1	2			1	2		
20	38.86	75.78	53.62	29.11	20.23	39.45	27.91	15.15
	52.15	78.94			27.15	41.09		
	8.77	75.21			4.56	39.15		
	3.59	47.48			1.87	24.72		
	21.92	80.35			11.41	41.82		
	64.69	83.60			33.67	43.52		
	30.11	90.79			15.67	47.26		
	17.40	88.31			9.06	45.97		
30	66.36	93.67	54.28	26.83	40.04	56.51	32.75	16.19
	31.55	75.61			19.03	45.61		
	27.01	73.70			16.30	44.46		
	18.41	66.60			11.11	40.18		
	22.43	76.93			13.53	46.41		
	49.80	84.95			30.05	51.25		
	15.40	74.66			9.29	45.04		
	16.34	75.13			9.86	45.33		
40	57.86	97.81	42.79	23.69	40.83	69.02	30.20	16.72
	9.20	42.88			6.49	30.26		
	19.01	57.88			13.41	40.84		
	23.84	71.12			16.82	50.19		
	29.48	39.42			20.80	27.82		
	14.72	29.20			10.39	20.60		
	53.45	64.29			37.72	45.37		
	16.73	57.75			11.81	40.75		

Concentration (mg/L)	Trial Cell Confluence Values (%)		Average Cell Confluence	Standard Deviation	CC/ Final Biomass (mg/mL)		CC/ Final Biomass Average	Standard Deviation
	1	2			1	2		
50	65.22	92.25	36.32	28.67	52.27	73.93	29.11	22.98
	11.48	81.65			9.20	65.44		
	16.35	88.66			13.11	71.06		
	17.54	34.95			14.05	28.01		
	12.74	52.55			10.21	42.12		
	14.16	27.89			11.34	22.35		
	12.57	23.08			10.07	18.50		
	15.18	14.93			12.17	11.96		

Table 31 - Cell Confluence data retrieved from *Cellavista* image analysis, with respective average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AuNP

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/ Final Biomass (mg/mL)				CC/ Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
0	95.21	11.64	15.43	87.52	68.44	26.09	41.84	5.12	6.78	38.46	30.08	11.47
	92.50	73.23	72.44	80.62			40.65	32.18	31.84	35.43		
	86.68	73.11	82.55	85.55			38.09	32.13	36.28	37.60		
	68.60	20.35	60.36	96.80			30.15	8.94	26.53	42.54		
	82.44	65.19	78.99	97.86			36.23	28.65	34.72	43.01		
	78.04	16.53	41.21	84.29			34.30	7.27	18.11	37.04		
	72.05	15.21	59.13	88.40			31.66	6.68	25.98	38.85		
	95.29	51.00	69.98	91.77			41.88	22.41	30.76	40.33		

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/ Final Biomass (mg/mL)				CC/ Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
40	26.21	64.85	43.13	98.34	56.51	24.58	18.05	47.41	17.43	45.61	38.90	16.92
	16.84	68.87	25.32	66.25			11.59	32.79	48.62	66.86		
	44.40	47.63	70.63	97.12			30.57	25.97	27.82	61.15		
	37.08	37.72	40.41	88.81			25.53	28.43	13.31	56.57		
	58.55	41.30	19.33	82.17			40.31	48.24	25.85	52.59		
	52.85	70.07	37.55	76.39			36.39	11.70	34.37	60.43		
	31.13	16.99	49.92	87.78			21.43	58.14	33.22	66.04		
	82.06	84.45	48.25	95.92			56.49	29.70	67.70	45.61		
50	45.17	77.10	30.67	99.51	59.42	24.08	32.48	55.43	22.05	71.55	42.72	17.31
	62.98	62.23	34.75	90.27			45.29	44.74	24.99	64.91		
	47.69	48.31	30.21	85.35			34.29	34.74	21.72	61.37		
	67.10	47.37	21.44	75.44			48.24	34.06	15.42	54.25		
	35.69	36.01	20.60	80.92			25.66	25.89	14.81	58.18		
	37.16	87.05	46.56	94.80			26.72	62.59	33.48	68.16		
	28.08	50.57	54.37	91.74			20.19	36.36	39.09	65.96		
	54.21	83.38	75.10	99.45			38.98	59.95	54.00	71.50		
80	25.33	87.55	59.78	98.55	53.58	28.13	19.27	66.61	45.48	74.97	40.76	21.40
	11.47	42.89	21.80	86.84			8.73	32.63	16.59	66.06		
	37.04	84.14	44.32	57.01			28.18	64.01	33.72	43.37		
	67.45	52.53	31.66	82.83			51.31	39.96	24.09	63.02		
	16.84	66.67	27.29	41.14			12.81	50.72	20.76	31.30		
	36.61	87.31	11.96	90.40			27.86	66.42	9.10	68.78		
	38.30	74.30	11.99	85.47			29.14	56.52	9.13	65.02		
	34.50	90.14	19.99	90.34			26.24	68.58	15.21	68.73		

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/ Final Biomass (mg/mL)				CC/ Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
120	40.19	84.51	23.25	96.61	50.75	30.02	31.21	65.63	18.06	75.02	39.41	23.31
	27.41	50.49	31.55	86.99			21.29	39.21	24.50	67.56		
	38.17	78.64	5.26	91.89			29.64	61.07	4.08	71.36		
	55.01	57.66	10.37	75.24			42.72	44.77	8.05	58.43		
	10.79	81.49	14.88	69.90			8.38	63.28	11.56	54.28		
	7.41	55.81	15.45	91.30			5.75	43.34	12.00	70.90		
	22.59	66.05	30.44	72.85			17.54	51.29	23.64	56.57		
	25.04	85.20	25.01	96.41			19.44	66.17	19.42	74.87		
140	41.23	27.34	45.04	86.48	52.79	23.44	34.12	22.63	37.28	71.58	43.69	19.40
	28.83	52.61	42.90	79.25			23.86	43.54	35.51	65.59		
	54.85	57.04	38.87	97.79			45.40	47.21	32.17	80.93		
	22.54	59.22	47.64	88.56			18.65	49.01	39.43	73.30		
	36.81	30.70	57.92	93.79			30.47	25.40	47.94	77.62		
	42.19	44.56	56.73	83.59			34.92	36.88	46.96	69.18		
	30.04	83.91	51.32	51.32			24.87	69.45	42.48	42.47		
	9.22	83.05	10.00	53.78			7.63	68.73	8.28	44.51		

Table 32 - Cell Confluence data retrieved from *Cellavista* image analysis, with respective average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AgNP

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/Final Biomass (mg/mL)				CC/Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
0	1.50	14.61	25.36	43.78	26.19	15.25	0.67	6.53	11.33	19.55	11.70	6.81
	22.24	25.92	14.27	37.89			9.93	11.58	6.38	16.92		
	15.51	36.77	19.94	52.02			6.93	16.42	8.90	23.23		
	3.49	7.05	28.46	45.75			1.56	3.15	12.71	20.43		
	33.14	56.45	23.68	24.29			14.80	25.21	10.58	10.85		
	4.92	9.86	32.55	19.72			2.20	4.40	14.54	8.81		
	4.16	10.35	29.78	53.39			1.86	4.62	13.30	23.84		
	33.20	28.07	30.22	49.79			14.83	12.54	13.50	22.24		
10	25.31	35.25	12.73	20.94	33.71	14.12	12.87	17.93	6.47	10.65	17.15	7.19
	12.03	53.45	43.20	32.18			6.12	27.19	21.98	16.37		
	19.82	54.31	54.38	51.64			10.08	27.63	27.67	26.27		
	8.92	18.91	26.22	38.67			4.54	9.62	13.34	19.67		
	16.05	52.03	27.99	27.27			8.17	26.47	14.24	13.87		
	26.59	68.02	29.81	29.79			13.53	34.61	15.17	15.15		
	27.45	42.21	40.19	31.51			13.96	21.47	20.45	16.03		
	43.90	30.01	30.08	48.01			22.34	15.27	15.30	24.43		

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/Final Biomass (mg/mL)				CC/Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
20	55.26	45.12	36.55	45.22	37.72	15.12	31.44	25.67	20.80	25.73	21.46	8.61
	26.69	63.71	48.46	59.23			15.19	36.25	27.57	33.70		
	30.47	60.08	42.52	45.36			17.34	34.18	24.19	25.81		
	10.99	24.16	23.90	27.39			6.25	13.75	13.60	15.58		
	35.41	35.33	24.36	32.71			20.15	20.10	13.86	18.61		
	23.58	54.25	32.39	46.40			13.42	30.87	18.43	26.40		
	23.91	7.20	45.05	56.57			13.60	4.09	25.63	32.19		
	16.87	18.90	57.88	50.99			9.60	10.75	32.93	29.01		
30	29.55	25.51	40.24	14.85	40.04	18.01	18.27	15.77	24.88	10.13	24.76	11.14
	38.13	46.01	36.29	16.39			23.58	28.45	22.44	25.48		
	18.85	59.81	27.65	41.20			11.65	36.98	17.10	37.62		
	27.42	48.15	38.97	60.84			16.95	29.77	24.10	30.04		
	31.97	75.67	22.42	48.58			19.77	46.79	13.86	41.70		
	17.48	53.52	47.36	67.43			10.81	33.09	29.28	32.41		
	22.97	64.50	47.24	52.41			14.20	39.88	29.21	10.82		
	31.69	25.33	85.40	17.50			19.60	15.66	52.81	10.13		
40	22.96	39.26	26.75	7.75	32.82	19.90	16.61	28.40	19.35	5.60	23.74	14.39
	28.12	44.49	21.76	36.63			20.34	32.19	15.74	26.50		
	10.91	52.52	35.00	13.59			7.89	38.00	25.32	9.83		
	7.66	52.61	14.85	31.42			5.54	38.06	10.74	22.73		
	18.89	71.08	37.95	38.73			13.66	51.42	27.45	28.02		
	20.96	68.84	9.23	9.57			15.17	49.80	6.68	6.92		
	7.72	57.21	22.57	17.74			5.59	41.39	16.33	12.84		
	40.57	43.38	81.22	58.31			29.35	31.38	58.76	42.18		

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/Final Biomass (mg/mL)				CC/Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
50	20.26	12.74	40.73	27.87	31.11	12.90	17.14	24.39	48.84	11.66	26.32	10.91
	39.21	44.19	22.92	29.41			33.16	10.77	34.45	23.57		
	30.47	36.09	38.09	34.33			25.77	37.38	19.39	24.88		
	22.36	25.48	23.42	40.53			18.91	30.52	32.22	29.04		
	22.64	33.12	29.13	33.20			19.15	21.55	19.81	34.28		
	36.98	32.37	29.07	31.20			31.28	28.01	24.64	28.08		
	62.56	60.65	8.23	16.43			52.92	27.38	24.59	26.39		
	28.84	57.74	13.79	11.57			24.39	51.30	6.96	13.89		

Table 33 - Cell Confluence data retrieved from *Cellavista* image analysis, with respective average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AuNP

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/Final Biomass (mg/mL)				CC/Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
0	61.32	36.51	52.37	89.10	68.35	19.71	39.41	23.47	33.66	57.27	43.93	12.67
	40.25	80.65	60.35	93.47			25.87	51.83	38.79	60.08		
	59.54	87.32	63.21	99.23			38.27	56.12	40.63	63.78		
	35.13	55.89	67.75	98.25			22.58	35.92	43.55	63.15		
	52.93	78.39	47.72	97.26			34.02	50.38	30.67	62.51		
	52.31	56.84	59.64	88.75			33.62	36.54	38.33	57.04		
	49.89	58.28	64.84	97.90			32.07	37.46	41.67	62.92		
	73.97	91.38	44.90	91.98			47.54	58.73	28.86	59.12		

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/Final Biomass (mg/mL)				CC/Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
40	23.67	71.64	25.14	69.53	67.59	20.98	19.67	59.55	20.90	57.80	56.18	17.44
	26.51	76.53	85.25	83.16			22.03	63.62	70.86	69.12		
	44.38	81.24	86.67	90.08			36.89	67.53	72.04	74.88		
	43.72	83.06	67.17	84.31			36.34	69.04	55.84	70.08		
	36.61	83.17	85.33	81.48			30.43	69.13	70.93	67.73		
	41.07	85.20	69.78	82.25			34.14	70.82	58.00	68.37		
	51.87	33.22	81.91	91.09			43.11	27.61	68.09	75.72		
	74.82	75.24	72.41	75.36			62.20	62.54	60.19	62.64		
50	17.06	69.68	47.05	67.33	59.95	20.74	15.12	61.76	41.70	59.67	53.13	18.38
	26.80	81.91	59.35	82.77			23.76	72.60	52.60	73.36		
	28.86	73.18	79.16	83.06			25.58	64.86	70.16	73.62		
	34.34	45.04	76.14	72.54			30.43	39.92	67.48	64.29		
	37.83	80.65	63.59	78.26			33.53	71.48	56.36	69.37		
	28.69	80.07	79.14	84.60			25.43	70.97	70.15	74.98		
	30.79	48.01	61.77	78.16			27.29	42.55	54.74	69.28		
	39.05	40.92	80.04	62.55			34.61	36.27	70.94	55.44		
80	21.27	45.92	53.11	60.05	60.70	19.05	21.45	46.29	53.54	60.54	61.19	19.21
	38.83	52.04	72.23	81.06			39.15	52.47	72.82	81.72		
	44.82	52.06	91.40	77.25			45.19	52.48	92.14	77.88		
	29.69	71.03	72.46	78.64			29.93	71.60	73.05	79.28		
	24.94	69.75	69.86	45.44			25.14	70.31	70.43	45.81		
	20.30	69.82	74.91	77.48			20.46	70.39	75.52	78.10		
	43.15	70.04	53.86	84.57			43.50	70.61	54.30	85.25		
	76.83	72.98	73.39	73.13			77.45	73.57	73.98	73.72		

Concentration (mg/L)	Trial Cell Confluence Values (%)				Average Cell Confluence	Standard Deviation	CC/Final Biomass (mg/mL)				CC/Final Biomass Average	Standard Deviation
	1	2	3	4			1	2	3	4		
120	28.87	60.12	49.88	83.57	69.64	14.50	26.14	54.44	45.17	75.68	63.06	13.13
	41.72	64.04	90.10	82.57			37.78	57.99	81.59	74.77		
	63.52	67.90	67.68	62.36			57.52	61.49	61.29	56.47		
	55.78	73.65	88.43	69.38			50.51	66.69	80.08	62.83		
	66.39	85.44	85.44	74.46			60.12	77.37	77.37	67.43		
	65.39	41.50	83.27	66.82			59.21	37.58	75.41	60.51		
	74.63	84.19	80.11	77.09			67.58	76.24	72.55	69.81		
	82.68	79.34	58.83	73.32			74.87	71.85	53.27	66.39		
140	44.03	29.23	56.88	84.40	69.16	14.01	38.09	25.28	49.20	73.01	59.83	12.12
	81.54	57.84	62.42	72.04			70.53	50.03	53.99	62.31		
	68.60	65.93	61.09	73.71			59.34	57.03	52.84	63.76		
	66.99	75.26	51.11	74.59			57.95	65.10	44.21	64.53		
	73.69	80.75	55.98	87.72			63.74	69.85	48.43	75.88		
	69.44	91.57	71.56	70.14			60.07	79.21	61.90	60.67		
	77.88	92.48	77.47	60.47			67.37	79.99	67.01	52.31		
	82.94	62.87	84.44	48.24			71.74	54.38	73.05	41.73		

Table 34, Table 35, Table 36 and

Table 37present the optical density values, at 590 nm, obtained for the different Crystal Violet trials, with the correspondent average and standard deviation, for each *Pseudomonas aeruginosa* strain, nanoparticles and respective concentrations.

Table 34 – OD data collected, for the different assays, from the CV assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AgNP.

Concentration (mg/L)	Trial CV OD Values	Average CV OD Values	Standard Deviation	CV/Final Biomass (mg/mL)	CV/Final Biomass Average	Standard Deviation
0	3.4264	3.4461	0.3467	2.0277	1.5437	0.1553
	3.4286			1.7587		
	3.9669			1.9839		
	3.1345			1.5398		
	3.7399			1.6942		
	OVER			-		
	3.5860			1.3723		
	2.8407			0.9564		
10	3.6709	3.1023	0.3743	1.7886	1.4731	0.1777
	2.8505			1.3378		
	3.0445			1.4095		
	2.7942			1.2816		
	3.2877			1.4418		
	3.5047			1.5065		
	2.4494			1.0339		
	3.2164			1.3015		

Concentration (mg/L)	Trial CV OD Values	Average CV OD Values	Standard Deviation	CV/Final Biomass (mg/mL)	CV/Final Biomass Average	Standard Deviation
20	3.6438	3.3155	0.4498	1.8013	1.7258	0.2342
	2.7299			1.3295		
	3.1698			1.5424		
	3.5837			1.6872		
	3.7658			1.7540		
	2.8106			1.3056		
	2.8630			1.2612		
	3.9578			1.7115		
30	2.9277	2.6045	0.7695	1.5088	1.5713	0.4642
	1.4081			0.7129		
	2.1069			1.0572		
	3.6871			1.8164		
	2.8747			1.4152		
	1.9443			0.9191		
	2.2216			1.0189		
	3.6654			1.5995		
40	1.8713	2.1618	0.2894	1.0230	1.5256	0.2042
	2.0954			1.1312		
	2.2338			1.1522		
	1.8687			0.9598		
	1.9204			0.9720		
	2.1213			1.0693		
	2.7658			1.3751		
	2.4177			1.1156		

Concentration (mg/L)	Trial CV OD Values	Average CV OD Values	Standard Deviation	CV/Final Biomass (mg/mL)	CV/Final Biomass Average	Standard Deviation
50	2.1934	2.8144	0.5733	1.2149	2.2556	0.4595
	2.1324			1.1767		
	2.3383			1.2483		
	2.5335			1.3342		
	2.9284			1.5015		
	3.7468			1.9012		
	3.1244			1.5757		
	3.5177			1.7715		

*Outlier rejected with Dixon's Q test.
**Over the limit of the measure capability of Bioscreen C.

Table 35 – OD data collected, for the different assays, from the CV assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AuNP.

Concentration (mg/L)	Trial OD Values		Average CV OD Values	Standard Deviation	CV/Final Biomass		CV/Final Biomass Average	Standard Deviation
	1	2			1	2		
0	3.1445	3.6244	3.4762	0.2665	1.3820	1.5929	1.4797	0.2122
	3.1160	3.7832			1.3694	1.6627		
	3.8006	3.3938			1.6703	1.4915		
	OVER**	3.4363			-	1.5102		
	3.2939	3.4339			1.4476	1.5092		
	3.5550	3.4189			1.5624	1.5026		
	1.8381*	3.0022			0.8078	1.3194		
	3.8649	3.7991			1.6986	1.6697		

Concentration (mg/L)	Trial OD Values		Average CV OD Values	Standard Deviation	CV/Final Biomass		CV/Final Biomass Average	Standard Deviation
	1	2			1	2		
40	3.4090	3.7294	3.3209	0.2642	2.3470	2.5676	2.2863	0.1819
	3.4258	3.2642			2.3585	2.2473		
	OVER**	2.9946			-	2.0617		
	3.3357	3.4987			2.2965	2.4087		
	3.6742	3.1398			2.5295	2.1616		
	OVER	3.2423			-	2.2322		
	2.8354	2.9143			1.9521	2.0064		
	3.4426	3.5865			2.3701	2.4692		
50	2.8123	3.8738	3.1245	0.5269	2.0221	2.7853	2.2466	0.3788
	OVER**	2.0534			-	1.4764		
	3.6232	2.5845			2.6052	1.8583		
	3.4911	2.7215			2.5102	1.9568		
	3.9021	2.6679			2.8057	1.9183		
	2.8781	2.8764			2.0694	2.0682		
	3.5722	3.2677			2.5685	2.3495		
	OVER**	3.4189			-	2.4583		
80	3.0915	2.2124	2.3995	0.3319	2.3519	1.6831	1.8255	0.2525
	2.4863	2.1855			1.8915	1.6627		
	2.2083	1.9744			1.6800	1.5021		
	2.9730	2.5851			2.2618	1.9667		
	2.1389	2.4235			1.6272	1.8437		
	1.9096	2.5285			1.4528	1.9236		
	2.6005	2.3761			1.9784	1.8077		
	2.0004	2.6986			1.5219	2.0530		

Concentration (mg/L)	Trial OD Values		Average CV OD Values	Standard Deviation	CV/Final Biomass		CV/Final Biomass Average	Standard Deviation
	1	2			1	2		
120	2.7227	1.5379	1.8686	0.3243	2.1144	1.1943	1.4511	0.2518
	1.9886	1.5421			1.5443	1.1975		
	1.8753	1.7466			1.4563	1.3563		
	1.9869	1.7710			1.5430	1.3753		
	1.5732	1.6347			1.2217	1.2695		
	1.8353	1.4890			1.4252	1.1563		
	1.7369	2.0490			1.3488	1.5912		
	1.9580	2.4510			1.5205	1.9034		
140	1.5408	0.8703	1.3475	0.2707	1.2752	0.7203	1.1152	0.2240
	1.4924	1.1705			1.2352	0.9687		
	1.2525	1.0536			1.0366	0.8720		
	1.3491	1.4160			1.1166	1.1719		
	1.4950	0.9223			1.2373	0.7633		
	1.6271	1.3722			1.3466	1.1357		
	1.5316	1.0524			1.2676	0.8710		
	1.9237	1.4907			1.5921	1.2338		

*Outlier rejected with Dixon's Q test.
**Over the limit of the measure capability of Bioscreen C.

Table 36 – OD data collected, for the different assays, from the CV assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AgNP.

Concentration (mg/L)	Trial OD Values		Average CV OD Values	Standard Deviation	CV/Final Biomass		CV/Final Biomass Average	Standard Deviation
	1	2			1	2		
0	1.1956	3.6053	2.0956	1.0882	0.5340	1.6102	0.9360	0.4860
	1.2837	2.3264			0.5733	1.0390		
	1.9191	1.7006			0.8571	0.7595		
	1.0887	2.1516			0.4862	0.9610		
	0.9211	3.3329			0.4114	1.4886		
	1.0387	3.4569			0.4639	1.5440		
	0.9357	3.5508			0.4179	1.5859		
	1.0529	3.9696			0.4703	1.7729		
10	2.9127	1.9865	1.8534	0.7829	1.3009	0.8872	0.8278	0.3497
	1.1861	1.3701			0.5297	0.6119		
	0.9746	1.5188			0.4353	0.6783		
	1.3076	3.1188			0.5840	1.3929		
	1.0502	2.3383			0.4691	1.0444		
	1.3359	2.4016			0.5967	1.0726		
	1.1121	2.2793			0.4967	1.0180		
	1.2696	3.4920			0.5670	1.5596		
20	1.8365	1.8205	1.4227	0.5382	0.8202	0.8131	0.6354	0.2404
	1.0699	1.4555			0.4778	0.6501		
	1.0462	1.7904			0.4673	0.7996		
	0.6403	2.0522			0.2860	0.9166		
	0.7564	1.0751			0.3378	0.4802		
	1.1087	1.9331			0.4952	0.8634		
	0.7463	2.0136			0.3333	0.8993		
	1.0025	2.4158			0.4477	1.0790		

Concentration (mg/L)	Trial OD Values		Average CV OD Values	Standard Deviation	CV/Final Biomass		CV/Final Biomass Average	Standard Deviation
	1	2			1	2		
30	1.4248	1.3338	1.3569	0.5604	0.6364	0.5957	0.6060	0.2503
	0.9884	1.1982			0.4414	0.5352		
	0.9518	1.3663			0.4251	0.6102		
	1.2519	1.2068			0.5591	0.5390		
	0.8946	1.3356			0.3996	0.5965		
	0.7671	2.7859			0.3426	1.2443		
	1.0166	1.2521			0.4540	0.5592		
	1.1990	2.7369			0.5355	1.2224		
40	1.8206	1.0200	1.2152	0.4562	0.8131	0.4556	0.5428	0.2037
	0.6880	0.9929			0.3073	0.4435		
	0.9216	1.5129			0.4116	0.6757		
	1.4334	1.7972			0.6402	0.8027		
	0.9379	1.5534			0.4189	0.6938		
	0.8050	1.9814			0.3595	0.8850		
	0.5774	1.4502			0.2579	0.6477		
	0.4695	1.4824			0.2097	0.6621		
50	1.0855	0.7457	0.9350	0.3402	0.4848	0.3331	0.4176	0.151950447
	1.4051	1.1646			0.6276	0.5201		
	0.8405	1.5331			0.3754	0.6847		
	0.5615	1.0325			0.2508	0.4611		
	0.3853	1.1815			0.1721	0.5277		
	0.6108	1.2477			0.2728	0.5573		
	0.4269	0.7260			0.1907	0.3243		
	1.0788	2.2196*			0.4818	-		

*Outlier rejected with Dixon's Q test.

Table 37 – OD data collected, for the different assays, from the CV assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AuNP.

Concentration (mg/L)	**Trial OD Values 1**	**Trial OD Values 2**	**Average CV OD Values**	**Standard Deviation**	**CV/Final Biomass 1**	**CV/Final Biomass 2**	**CV/Final Biomass Average**	**Standard Deviation**
0	OVER	3.9019	5.6655	1.7711	-	2.5079	2.1725	0.2455
	3.5640	3.0248			2.2907	1.9441		
	3.4688	3.1687			2.2295	2.0366		
	2.8576	3.6447			1.8367	2.3425		
	3.1764	2.8455			2.0416	1.8289		
	3.8300	2.3951*			2.4616	-		
	3.9520	2.8347			2.5401	1.8219		
	3.6200	3.4331			2.3267	2.2065		
40	3.0288	3.8278	4.2492	1.1467	1.9467	2.4602	1.5960	0.4908
	1.6690	2.3840			1.0727	1.5323		
	1.3585	3.2727			0.8731	2.1035		
	1.6850	2.5743			1.0830	1.6546		
	1.4979	3.4832			0.9627	2.2387		
	2.0259	3.2772			1.3021	2.1063		
	2.0207	2.2806			1.2988	1.4658		
	2.0098	3.3356			1.2918	2.1439		
50	2.3923	3.2702	4.5664	1.4929	1.5376	2.1018	1.3716	0.4528
	1.8554	3.3526			1.1925	2.1548		
	0.9159	2.1732			0.5887	1.3968		
	1.8169	1.8569			1.1678	1.1935		
	1.7548	2.1349			1.1279	1.3722		
	1.0890	2.1082			0.6999	1.3550		
	1.4064	2.4950			0.9039	1.6036		
	2.1803	3.3426			1.4013	2.1484		

Concentration (mg/L)	Trial OD Values		Average CV OD Values	Standard Deviation	CV/Final Biomass		CV/Final Biomass Average	Standard Deviation
	1	2			1	2		
80	2.4007	1.2847	4.5664	1.2083	1.5430	0.8257	0.9739	0.3111
	1.4259	2.5287			0.9165	1.6253		
	1.0829	1.9018			0.6960	1.2223		
	1.3056	1.7582			0.8391	1.1300		
	1.0222	0.8041			0.6570	0.5168		
	1.0929	0.9334			0.7024	0.5999		
	1.6239	1.5859			1.0437	1.0193		
	1.5949	1.8993			1.0251	1.2207		
120	1.8122	1.9616	3.1300	1.3631	1.1648	1.2608	1.0178	0.3476
	0.8535	1.9930			0.5486	1.2810		
	1.1980	2.1608			0.7700	1.3888		
	0.6871	2.1834			0.4416	1.4033		
	0.8825	1.6744			0.5672	1.0762		
	0.5746	2.1577			0.3693	1.3868		
	1.6408	1.9736			1.0546	1.2685		
	1.6371	1.9479			1.0522	1.2520		
140	1.6354	1.3351	2.6442	0.9194	1.0511	0.8581	0.8594	0.1787
	0.9053	1.3069			0.5819	0.8400		
	0.9392	1.2293			0.6036	0.7901		
	1.1204	1.7719			0.7201	1.1388		
	1.3894	1.0687			0.8930	0.6869		
	1.3235	1.6709			0.8506	1.0739		
	1.0496	1.7091			0.6746	1.0985		
	1.6015	2.1149*			1.0293	-		

*Outlier rejected with Dixon's Q test.
**Over the limit of the measure capability of Bioscreen C.

Table 38, Table 39, Table 40 and Table 41 present the optical density values obtained for the different MTT trials, with the correspondent average and standard deviation, for each *Pseudomonas aeruginosa* strain, nanoparticles and respective concentrations.

Table 38 - Data collected, for the different assays, from the MTT assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AgNP.

Cell Confluence (%)	Concentration (mg/L)	Trial MTT OD Values	MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence	MTT OD/Cell Confluence Average	Standard Deviation
72.768	0	1.3677	1.3308	0.5758	1.8795	1.5468	0.7011
92.901		2.0943			2.2543		
83.204		2.4156			2.9032		
88.639		0.6531			0.7368		
92.382		1.0785			1.1674		
93.693		0.9597			1.0243		
95.132		0.8651			0.9094		
80.845		1.2120			1.4992		
38.159	10	0.8487	1.3203	0.4102	2.2241	1.7612	0.4890
71.878		1.6217			2.2562		
87.744		2.1703			2.4734		
81.193		1.4633			1.8022		
87.729		1.1445			1.3046		
90.764		1.0373			1.1428		
80.054		1.3758			1.7186		
77.128		0.9008			1.1679		

Cell Confluence (%)	Concentration (mg/L)	Trial MTT OD Values	MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence	MTT OD/Cell Confluence Average	Standard Deviation
75.783	20	0.6710	0.7623	0.1513	0.8854	1.0147	0.2813
78.940		0.7644			0.9683		
75.208		0.5966			0.7933		
47.483		0.7561			1.5924		
80.348		1.0690			1.3305		
83.603		0.5605			0.6704		
90.790		0.8269			0.9108		
88.307		0.8535			0.9665		
93.665	30	0.4467	0.7852	0.2439	0.4769	1.0229	0.3261
75.605		0.8163			1.0797		
73.697		0.6583			0.8932		
66.603		0.5644			0.8474		
76.932		1.1883			1.5446		
84.948		0.8754			1.0305		
74.659		0.6296			0.8433		
75.134		1.1025			1.4674		
97.807	40	0.9516	0.7036	0.2069	0.9729	1.3343	0.5089
42.875		1.0302			2.4028		
57.876		0.6829			1.1799		
71.117		0.4860			0.6834		
39.416		0.6468			1.6410		
29.196		0.4578			1.5680		
64.289		0.8632			1.3427		
57.749		0.5104			0.8838		

Cell Confluence (%)	Concentration (mg/L)	Trial MTT OD Values	MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence	MTT OD/Cell Confluence Average	Standard Deviation
92.249	50	1.4290*	0.6209	0.2098	-	2.2372	1.6863
81.653		0.3273			0.4008		
88.664		0.3294			0.3715		
34.949		0.7742			2.2152		
52.551		0.5079			0.9665		
27.892		0.7864			2.8195		
23.078		0.8350			3.6182		
14.926		0.7864			5.2688		

*Outlier rejected with Dixon's Q test.

Table 39 - Data collected, for the different assays, from the MTT assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3777 with AuNP.

Cell Confluence (%)		Concentration (mg/L)	Trial MTT OD Values		MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence		MTT OD/Cell Confluence Average	Standard Deviation
1	2		1	2			1	2		
15.4263	87.5215	0	0.9587	0.7726	0.7703	0.1337	6.2147	0.8828	1.3620	1.3153
72.4401	80.6209		1.0221	0.6991			1.4110	0.8671		
82.5468	85.5489		0.8198	0.6895			0.9931	0.8060		
60.3629	96.8013		0.9340	0.6201			1.5473	0.6406		
78.9928	97.8632		0.9114	0.6698			1.1538	0.6844		
41.2079	84.2889		0.9162	0.6208			2.2234	0.7365		
59.1255	88.4027		0.6982	0.6511			1.1809	0.7365		
69.9803	91.7691		0.7433	0.5975			1.0622	0.6510		

Cell Confluence (%)		Concentration (mg/L)	Trial MTT OD Values		MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence		MTT OD/Cell Confluence Average	Standard Deviation
1	2		1	2						
43.1332	98.3400	40	0.8283	1.0467	0.8063	0.1551	1.9203	1.0644	1.5299	0.8076
25.3198	66.2466		0.8001	0.8193			3.1600	1.2367		
70.6252	97.1179		0.6723	1.1392			0.9519	1.1730		
40.4110	88.8141		0.9272	0.9447			2.2944	1.0637		
19.3264	82.1741		0.6885	0.6528			3.5625	0.7944		
37.5547	76.3883		0.6911	0.9613			1.8403	1.2584		
49.9220	87.7787		0.6919	0.6700			1.3860	0.7633		
48.2463	95.9186		0.5663	0.8013			1.1738	0.8354		
30.6715	99.5099	50	0.8749	0.9594	0.8021	0.1260	2.8525	0.9641	1.5945	0.8556
34.7542	90.2714		0.9809	0.9730			2.8224	1.0779		
30.2111	85.3532		0.6334	0.7327			2.0966	0.8584		
21.4445	75.4443		0.5388	0.9155			2.5125	1.2135		
20.5979	80.9174		0.7205	0.8605			3.4979	1.0634		
46.5624	94.7990		0.7606	0.7503			1.6335	0.7915		
54.3692	91.7422		0.8424	0.8130			1.5494	0.8862		
75.1005	99.4462		0.6317	0.8459			0.8411	0.8506		
59.7771	98.5472	80	0.7023	1.0570	0.7529	0.2157	1.1749	1.0726	1.9842	1.5176
21.8029	86.8387		0.6151	1.2094			2.8212	1.3927		
44.3166	57.0102		0.6395	0.8493			1.4430	1.4897		
31.6600	82.8328		0.6260	0.9530			1.9773	1.1505		
27.2936	41.1427		0.5343	0.8507			1.9576	2.0677		
11.9586	90.4042		0.8566	0.7156			7.1630	0.7916		
11.9950	85.4685		0.4316	0.8363			3.5982	0.9785		
19.9941	90.3362		0.3530	0.8163			1.7655	0.9036		

Cell Confluence (%)		Concentration (mg/L)	Trial MTT OD Values		MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence		MTT OD/Cell Confluence Average	Standard Deviation
1	2		1	2						
23.2511	96.6093	120	0.3625	0.7393	0.6209	0.2300	1.5591	0.7652	1.9894	1.9489
31.5457	86.9934		0.6979	0.9960			2.2123	1.1449		
5.2595	91.8867		0.4569	0.7148			8.6871	0.7779		
10.3716	75.2361		0.3923	0.7916			3.7824	1.0522		
14.8825	69.8999		0.5161	0.8776			3.4678	1.2555		
15.4550	91.2973		0.3024	0.7564			1.9566	0.8285		
30.4379	72.8526		0.5029	0.8589			1.6522	1.1790		
25.0107	96.4082		0.1704	0.7987			0.6813	0.8285		
45.0412	86.4850	140	0.2514	0.7680	0.4691	0.3022	0.5582	0.8880	0.7594	0.4143
42.9005	79.2456		0.4280	0.9357			0.9977	1.1808		
38.8744	97.7889		0.2139	0.6351			0.5502	0.6495		
47.6399	88.5624		0.1309	0.7444			0.2748	0.8405		
57.9199	93.7857		0.1027	0.6790			0.1773	0.7240		
56.7350	83.5865		0.1322	0.6933			0.2330	0.8294		
51.3216	51.3154		0.1044	0.8374			0.2034	1.6319		
10.0040	53.7822		0.1023	0.7468			1.0226	1.3886		

Table 40 - Data collected, for the different assays, from the MTT assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AgNP.

Cell Confluence (%) 1	Cell Confluence (%) 2	Concentration (mg/L)	Trial MTT OD Values 1	Trial MTT OD Values 2	MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence 1	MTT OD/Cell Confluence 2	MTT OD/Cell Confluence Average	Standard Deviation
25.3598	43.7811	0	0.5986	0.5829	0.5367	0.0931	2.3604	1.3314	1.8394	0.6892
14.2741	37.8877		0.4933	0.5030			3.4559	1.3276		
19.9365	52.0182		0.5025	0.3694			2.5205	0.7101		
28.4558	45.7467		0.5126	0.4058			1.8014	0.8871		
23.6785	24.2898		0.3828	0.5163			1.6167	2.1256		
32.5487	19.7223		0.5855	0.5218			1.7988	2.6457		
29.7763	53.3852		0.6189	0.6623			2.0785	1.2406		
30.2227	49.7899		0.6579	0.6741			2.1768	1.3539		
12.7258	20.9380	10	1.1842	0.7384	0.7758	0.1952	9.3055	3.5266	2.6967	1.8453
43.2049	32.1790		0.5743	0.7562			1.3292	2.3500		
54.3830	51.6358		0.8313	0.6434			1.5286	1.2460		
26.2249	38.6676		0.8147	0.4359			3.1066	1.1273		
27.9867	27.2675		0.5479	0.9426			1.9577	3.4569		
29.8090	29.7865		0.7508	0.6539			2.5187	2.1953		
40.1944	31.5121		0.8165	0.8403			2.0314	2.6666		
30.0824	48.0128		0.7093	1.1728			2.3579	2.4427		
36.5531	45.2189	20	0.8938	1.3285	0.8267	0.2590	2.4452	2.9379	2.0774	0.8077
48.4570	59.2327		1.2260	0.9412			2.5301	1.5890		
42.5224	45.3649		1.0111	0.4635			2.3778	1.0217		
23.8998	27.3853		0.9726	0.5678			4.0695	2.0734		
24.3642	32.7065		0.6054	0.3971			2.4848	1.2141		
32.3891	46.3998		1.0089	0.5963			3.1149	1.2851		
45.0530	56.5732		0.6827	1.0119			1.5153	1.7887		
57.8781	50.9901		0.8189	0.7017			1.4149	1.3761		

Cell Confluence (%)		Concentration (mg/L)	Trial MTT OD Values		MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence		MTT OD/Cell Confluence Average	Standard Deviation
1	2		1	2			1	2		
40.2423	14.8499	30	0.8166	0.6923	0.7070	0.2199	2.0292	4.6620	1.9171	1.0786
36.2923	16.3872		0.8396	0.6145			2.3134	3.7499		
27.6542	41.2010		0.8216	0.6473			2.9710	1.5711		
38.9722	60.8429		0.5587	0.8599			1.4336	1.4133		
22.4215	48.5831		0.4278	0.8458			1.9080	1.7409		
47.3594	67.4333		0.5376	0.6333			1.1351	0.9391		
47.2433	52.4076		0.4229	0.7366			0.8952	1.4055		
85.4041	17.4997		0.5029	1.3540*			0.5888	-		
26.7497	7.7472	40	0.8919	0.5369	0.5487	0.2327	3.3342	6.9302	2.7860	2.0711
21.7553	36.6317		0.4780	0.8592			2.1972	2.3455		
34.9952	13.5947		0.4319	0.4257			1.2342	3.1314		
14.8470	31.4213		0.3383	0.7313			2.2786	2.3274		
37.9477	38.7274		0.2889	0.6387			0.7613	1.6492		
9.2321	9.5692		0.3455	0.7952			3.7424	8.3100		
22.5740	17.7416		0.2445	0.5914			1.0831	3.3334		
81.2160	58.3107		0.2271	0.9550			0.2796	1.6378		
40.7324	27.8708	50	0.6536	0.2760	0.3768	0.1300	1.6046	0.9903	1.5631	0.7415
22.9207	29.4149		0.4934	0.3563			2.1526	1.2113		
38.0941	34.3287		0.3574	0.4335			0.9382	1.2628		
23.4244	40.5283		0.2821	0.5434			1.2043	1.3408		
29.1330	33.2026		0.2679	0.4441			0.9196	1.3375		
29.0675	31.2013		0.2718	0.4345			0.9351	1.3926		
8.2330	16.4272		0.1738	0.4592			2.1110	2.7954		
13.7909	11.5706		0.1573	0.4250			1.1406	3.6731		

*Outlier rejected with Dixon's Q test.

Table 41 - Data collected, for the different assays, from the MTT assay, with the correspondent average and standard deviation, for *Pseudomonas aeruginosa* 3081 with AuNP.

Cell Confluence (%)		**Concentration (mg/L)**	**Trial MTT OD Values**		**MTT OD Values Average**	**Standard Deviation**	**MTT OD/Cell Confluence**		**MTT OD/Cell Confluence Average**	**Standard Deviation**
1	**2**		**1**	**2**			**1**	**2**		
52.3659	89.0983	0	0.5050	0.5822	0.5267	0.0931	0.9644	0.6534	0.6757	0.1773
60.3524	93.4704		0.2380	0.6324			0.3944	0.6766		
63.2127	99.2309		0.3150	0.6529			0.4983	0.6580		
67.7543	98.2467		0.3044	0.6563			0.4493	0.6680		
47.7242	97.2601		0.3810	0.7826			0.7983	0.8046		
59.6390	88.7514		0.2882	0.7827			0.4832	0.8819		
64.8402	97.8977		0.3711	0.8363			0.5723	0.8543		
44.9005	91.9801		0.2272	0.8720			0.5060	0.9480		
25.1436	69.5340	40	0.2380	0.6634	0.6693	0.1952	0.9466	0.9541	0.8652	0.3933
85.2464	83.1570		0.4130	0.8135			0.4845	0.9783		
86.6684	90.0830		0.5125	0.9463			0.5913	1.0505		
67.1748	84.3085		0.3342	0.9473			0.4975	1.1236		
85.3339	81.4771		0.3547	0.9544			0.4157	1.1714		
69.7787	82.2530		0.3675	1.0290			0.5267	1.2510		
81.9116	91.0932		0.3745	1.2268			0.4572	1.3468		
72.4111	75.3577		0.2339	1.3002			0.3230	1.7254		
47.0463	67.3264	50	0.1439	0.5050	0.4960	0.2590	0.3059	0.7501	0.6792	0.4147
59.3476	82.7679		0.2925	0.5618			0.4929	0.6788		
79.1606	83.0636		0.2367	0.5972			0.2990	0.7190		
76.1352	72.5401		0.2478	0.6411			0.3255	0.8838		
63.5917	78.2637		0.1904	0.7753			0.2994	0.9906		
79.1442	84.5997		0.2613	0.9336			0.3302	1.1036		
61.7660	78.1623		0.2463	0.9748			0.3988	1.2471		
80.0420	62.5477		0.2293	1.0986			0.2865	1.7564		

Cell Confluence (%)		Concentration (mg/L)	Trial MTT OD Values		MTT OD Values Average	Standard Deviation	MTT OD/Cell Confluence		MTT OD/Cell Confluence Average	Standard Deviation
1	2		1	2			1	2		
53.1147	60.0512	80	0.1262	0.4303	0.3912	0.2199	0.2376	0.7166	0.5641	0.3873
72.2311	81.0641		0.1997	0.4922			0.2765	0.6072		
91.4002	77.2534		0.1468	0.5168			0.1606	0.6690		
72.4638	78.6427		0.1918	0.5309			0.2647	0.6751		
69.8644	45.4414		0.1479	0.6907			0.2117	1.5200		
74.9149	77.4761		0.1509	0.6935			0.2014	0.8951		
53.8613	84.5703		0.1715	0.7962			0.3184	0.9415		
73.3865	73.1285		0.1571	0.8166			0.2141	1.1167		
49.8788	83.5728	120	0.1531	0.2909	0.3326	0.2327	0.3069	0.3481	0.4570	0.2838
90.1043	82.5728		0.1342	0.3939			0.1489	0.4770		
67.6786	62.3639		0.1967	0.4228			0.2906	0.6780		
88.4308	69.3842		0.2204	0.4352			0.2492	0.6272		
85.4397	74.4581		0.2162	0.4780			0.2530	0.6420		
83.2747	66.8193		0.1372	0.6187			0.1648	0.9259		
80.1142	77.0890		0.1136	0.6351			0.1418	0.8239		
58.8278	73.3185		0.1190	0.7567			0.2023	1.0321		
56.8776	84.4027	140	0.1584	0.3601	0.3125	0.1300	0.2785	0.4266	0.4664	0.2529
62.4155	72.0356		0.1946	0.4346			0.3118	0.6033		
61.0887	73.7132		0.1665	0.4411			0.2726	0.5984		
51.1091	74.5949		0.1228	0.4544			0.2403	0.6092		
55.9836	87.7153		0.1499	0.4629			0.2678	0.5277		
71.5578	70.1384		0.1985	0.4743			0.2774	0.6762		
77.4695	60.4711		0.1796	0.5063			0.2318	0.8373		
84.4436	48.2397		0.1577	0.5388			0.1868	1.1169		