Universidade do Minho
Escola de Ciências

Rita Maria Martins Alves

Impact of Contact Lenses on Environment

Outubro 2019

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UMINHO | 2019

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Impact of Contact Lenses on Environment

Tese de Mestrado
Mestrado em Genética Molecular

Trabalho efetuado sob a orientação de
Professora Doutora Ana Paula Fernandes Monteiro Sampaio Carvalho
Professora Doutora Maria Madalena Cunha Faria Lira

Outubro 2019

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

AGRADECIMENTOS

Ao longo da elaboração deste trabalho e deste percurso, existem algumas pessoas às quais é necessário fazer um agradecimento.

Em primeiro lugar gostaria de agradecer às minhas orientadoras, à professora Paula Sampaio e à professora Madalena Lira por me terem permitido abraçar este projeto, pela sua orientação e partilha de conhecimento. Pela sua preocupação e disponibilidade para me ajudar ao longo deste ano.

Aos meus colegas da Micro II pela boa disposição e pelos momentos memoráveis que passamos juntos. Um obrigado à Joana e ao Augusto pela sua alegria contagiante e pela partilha de conhecimento. À Inês pelas suas histórias, e à Marisol pela sua ajuda preciosa e todo o carinho.

À minha lab partner, Rita Rocha um obrigado especial por este ano cheio de feitos e superação. Por ter sido o meu apoio ao longo deste ano, sem ti este ano não teria sido a mesma coisa.

Ao Doutor Pedro Costa e à Doutora Daniela Batista pela paciência, disponibilidade e ajuda prestada na realização deste trabalho.

Ao Professor Doutor Senentxu Lanceros-Mendez por me ter permitido a realização da técnica de AFM e ATR-FTIR.

Aos funcionários do Departamento de Biologia, em especial ao Sr. Luís e à Dona Manuela pela sua simpatia, ajuda e disponibilidade em retirar qualquer dúvida que surgiu ao longo deste percurso.

À Erica e ao João por serem os melhores ouvintes nesta reta final. Por terem levado com o meu stress, e se terem tornado nos melhores motivadores.

Ao meu namorado por ter estado sempre lá nos momentos bons e nos menos bons, e por me ter feito acreditar que era possível quando eu já não acreditava.

À minha família, à minha madrinha, à tia São, ao tio Manel e ao tio Fernando por me ajudarem com o que podiam e por não me deixarem desistir de mim. Às minha primas Joana e Inês por me obrigarem a ser um exemplo para vocês. Por último um obrigado especial ao meu pai por ter permitido que continuasse os meus estudos sem nunca me colocar barreiras, acreditando incondicionalmente em mim e ao meu irmão por todos os dias me mostrar que ser diferente não é obstáculo e me ensinar a apreciar as pequenas vitórias da vida.

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Resumo

As lentes de contato (LC) são mais populares do que nunca e, com o aumento da prevalência da miopia e dos números de pessoas com presbiopia, espera-se que o número de utilizadores aumente. E, por questões de conveniência e de higiene, as lentes de contato descartáveis (LCD) tornam-se a primeira escolha, estando disponíveis para diferentes modalidades de uso. No entanto, estas podem ter um impacto ambiental adverso resultante da necessidade de descartar muitas lentes e as suas embalagens.

Como não existem estudos que mostram se as LC têm impacto ambiental, este estudo tem como objetivo avaliar se os microrganismos conseguem degradar/deteriorar o material das LC. Foram selecionados seis fungos unicelulares (*Pichia orientalis*, *P. fermentans*, *Candida humilis*, *C. intermedia*, *C. tropicalis* e *Wickerhamomyces anomalus*), duas bactérias (*Bacillus megaterium* e *Brucella melitensis*) e três hifomicetes aquáticos (*Articulospora tetracladia*, *Tricladium splendans* e *Varicosporium elodea*) para incubar com os materiais de LC Nesofilcon A (Bausch & Lomb) e Senofilcon A (Johnson & Johnson) por um período desde 40 horas a 8 meses. O impacto da presença do material das LC no crescimento de leveduras, bactérias e hifomicetes aquáticos foi avaliado e os resultados indicaram que a sua presença não afetou significativamente o crescimento. Além disso, ao final do período de incubação com leveduras (40 h), o índice de refração das LC (IR) e o conteúdo em água (CA) não se alterou quando comparados com as lentes novas. A integridade da LC após as incubações com leveduras e bactérias permaneceu intacta, porém, após 8 meses na presença de hifomicetes aquáticos, as LC apresentaram roturas na sua superfície. Para avaliar o impacto da LC no meio ambiente, a LC foi colocada no solo e num extrato do solo e o IR e o CA também foram analisados após 1, 2, 4, 6 e 8 semanas. Os resultados indicaram que os valores de IR e CA não mudaram significativamente ao longo das semanas nas duas condições testadas. As análises aos materiais das LC com Microscopia de Força Atómica (AFM) e com Espectroscopia de Infravermelho com Transformada de Fourier acoplada a um acessório de refletância total atenuada (FTIR-ATR) incubadas por 6 e 8 semanas nestas condições indicam que há um aumento na rugosidade das LC, no entanto, não há alterações na composição polimérica das lentes. Estas LC foram incubadas com bactérias e testadas quanto à sua resistência ao estiramento. Os resultados mostram que a percentagem de alongamento até quebra da LC Senofilcon A piorou em todas as condições, mas o mesmo não foi observado para LC Nesofilcon A.

Concluiu-se que as LC não afetam o crescimento de leveduras, bactérias e hifomicetes aquáticos. Após inseridas durante 8 semanas no solo ou no extrato do solo não parecem degradar-se ou deteriorar-se significativamente. No entanto, a lente Senofilcon A, composta por Silicone-Hidrogel (SiHi), começou a apresentar um sinal de deterioração, o que sugere que esta LC pode contribuir para o aumento dos

microplásticos no meio ambiente. Considerando que o número esperado de utilizadores de LC aumentará, particularmente as LCD diárias, as LC que terminarem nos rios ou nos aterros sanitários devem ser consideradas um problema ambiental.

Palavra-chave: Lentes de Contacto; Nesofilcon A e Senofilcon A; Impacto Ambiental

Abstract

Contact lenses (CL) are more popular than ever and with the increase in the prevalence of myopia and people with presbyopia, it is expected that the number of users will increase. The convenience and hygiene issues make disposable contact lenses (DCL) the first choice as they are available for different wearing modalities. However, they can have a high adverse environmental impact resulting from the need to discard many lenses and their packaging.

Since there aren't studies that show if CL have an environmental impact, this study aims to evaluate if microorganisms can degrade/deteriorate CL materials. Six unicellular fungi were selected (*Pichia orientalis*, *P. fermentans*, *Candida humilis*, *C. intermedia*, *C. tropicalis*, and *Wickerhamomyces anomalus*), two bacteria (*Bacillus megaterium* and *Brucella melitensis*), and three aquatic hyphomycetes (*Articulospora tetracladia*, *Tricladium splendans*, and *Varicosporium elodea*) to incubate with the Nesofilcon A (Bausch&Lomb) and Senofilcon A (Johnson&Johnson) CL for a period from 40 hours to 8 months. The impact of the presence of the CL material on yeast, bacteria and aquatic hyphomycete growth was evaluated and results indicated that their presence didn't significantly affect their growth. Also, at the end of the incubation with yeast (40 h), the CL refractive index (RI) and water content (WC) didn'tt change when compared with the new lens. The CL integrity after the incubations with yeast and bacteria remains intact, however, after 8 months in the presence of aquatic hyphomycetes, the CL presented breaks at their surface. To evaluate the impact of CL on the environment, CL were placed in soil and in a soil extract and RI and WC were analyzed after 1, 2, 4, 6 and 8 weeks. Results indicated that the RI and WC values didn't change significantly over the weeks in both conditions tested. Analyzes of CL materials with Atomic Force Microscopy (AFM) and Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) incubated for 6 and 8 weeks in those conditions indicate that there is an increase in CL roughness, however, there are no changes in the polymeric composition of the CL. These CL and CL incubated with bacteria were tested for their resistance to stretch and the results show that there is an impairment of Senofilcon A in all conditions but the same was not observed for Nesofilcon A.

We concluded that CL do not affect yeast, bacteria and aquatic hyphomycetes growth. After inserted during 8 weeks in soil or soil extract doesn't seem to significantly degrade or deteriorate CL. However, Senofilcon A CL, composed of silicone-Hydrogel (SiHy), began to present a sign of deterioration, which suggests that this monthly DCL may contribute to the enhancement of microplastics in the environment. Considering that the expected number of CL users will increase, particularly the daily DCL, CL ending in the rivers or in the land field should be considered an environmental problem.

Keywords: Contact Lenses; Nesofilcon A and Senofilcon A; Environmental Impact

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Abbreviation and Acronyms List

AFM	Atomic Force Microscopy
ATR	Attenuated Total Reflectance
CL	Contact Lenses
DCL	Disposable Contact Lenses
DMA	N,N-Dimethylacrylamide
DNA	Deoxyribonucleic Acid
DK	Oxygen permeability
FTIR	Fourier transform infrared spectroscopy
HEMA	Hydroxyethyl methacrylate
MA	Methacrylic acid
NVP	*N*-vinylpyrrolidone
OD	Optical density
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PDMS	Polydimethylsiloxane
PHEMA	Poly-hydroxyethyl methacrylate
PV	Polyvinyl
PVC	Polyvinyl chloride
PVP	Polyvinylpyrrolidone
Ra	Average Roughness
RI	Refractive Index
Rpm	Rotations per minute
Rq	Root Mean Squared Roughness
SiHy	Silicone Hydrogel
SCL	Soft Contact Lenses
TAE	Tris-acetate-EDTA
TPU	Thermoplastic polyurethane
TS	Tensile strength
USAN	United States Adopted Name
YPD	Yeast extract peptone dextrose

WC	Water content

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Chapter 1:

Introduction

1. Introduction

Approximately 140 million people worldwide wear contact lenses (CL) to correct refractive ocular errors (Markoulli & Kolanu, 2017; Stapleton *et al.*, 2006). The market of CL, worth over $6 billion at the supplier level, including accessories and solutions. The greatest number of wearers live in North America, 36 million, Asia has 24 million wearers and Europe has 20 million wearers (Holden, Stretton, Evans, & Sweeney, 2003). In 2018, most of soft CL prescribed were monthly (40 %), daily (46 %), with fewer than 15 % of soft lenses prescribed for extended wear (P. Morgan *et al.*, 2019). However, the years leading up to 2020 promise to yield a huge increase in the global CL market, especially the daily disposables, mainly due to the rising number of people with myopia and presbyopia (Holden *et al.*, 2003).

The prevalence of myopia has approximately doubled in the past three decades, and in 2010 it was estimated that myopia affects 108 million people (Holden *et al.*, 2016). Prevalence rates of 70-80 % have been reported amongst populations of schoolchildren and young adults in Asia, and around 20-50 % in America and Europe (Wolffsohn *et al.*, 2016). Holden *et al.* (2016) estimated that myopia will show a significant increase in prevalence globally, affecting nearly 5 billion people by 2050.

Presbyopia was estimated to affect more than 1 billion people globally in 2005 (Holden *et al.*, 2008). The uncorrected presbyopia is currently the most prevalent cause of vision impairment globally and is expected to continue to be, CL are likely to be used increasingly for the correction of these refractive errors (Holden *et al.*, 2003).

1.1 Disposable Contact Lenses

1.1.1 History

In the early days of soft CL development, patients would typically use the same pair of lenses until they became too uncomfortable to wear or were damaged or lost. Regular lens replacement was an obvious solution to some of these problems, although the high unit cost of lenses proved to be a significant discouragement. In the early 1980s, Klas Nilsson of Gothenburg, Sweden, convinced patients to the benefits of replacing lenses on a regular basis and began prescribing lenses in this way (Efron, 2002). Holden *et al.*, (1985) proved that extended CL wear interferes with normal corneal function and that there

are benefits of regular lens replacement. In this way, the concept of regular lens replacement, although relatively expensive for the patient, was born.

The pharmaceutical Johnson & Johnson, which had not previously been involved in CL business, release the Acuvue lens, an inexpensive weekly-replacement extended-wear, which was released in the USA in June 1988, and worldwide shortly thereafter. The success of this lens elevated Johnson & Johnson to a leadership position in the CL market. All other major CL companies followed the suit, and today the majority of soft CL prescribed worldwide (76 %) are designed to be replaced monthly or more frequently (Efron, 2002; P. Morgan *et al.*, 2019).

The introduction of daily disposable lenses (DCL) was in 1994 by Johnson & Johnson who releases the '1-Day Acuvue' daily DCL in the market (Efron, 2002; Efron *et al.*, 2010). Daily DCL offers the advantage that they do not need the 'chemistry set' of cleaning, rinsing, disinfecting and enzyme removing solutions and offers great convenience as they allow the wear of new lens every day. This modality reduces deposit accumulation, enhances comfort, visual quality, and decreases the risk of ocular infection (Efron *et al.*, 2010; Sapkota, Franco & Lira, 2017).

Sapkota *et al.* (2017) concluded that changes in ocular surface physiology and comfort score were similar to daily and monthly wear modalities. This result was due to the fact that the CL practitioners were advised to recommend lenses according to material characteristics rather than wearing modality. However, many clinicians recommend daily disposable lenses as the first choice (Efron *et al.*, 2010).

1.1.2 Soft Contact Lenses Polymers

1.1.2.1 HEMA and PHEMA

Otto Wichterle and Drahoslav Lim designed hydroxyethyl methacrylate (HEMA) at the Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences to be used for a wide range of applications manufacturing (Bennet & Weissman, 2005; Wichterle & Lim, 1960). HEMA (Figure 1) is a monomer, that had a hydrophilic pendant group and the ability to be easily polymerized like the majority of methacrylic derivatives (Montheard, Chatzopoulos, & Chappard, 1992). In 1951, Wichterle adapted the spin-casting technique for lens manufacturing, he claims to have produced 'the first suitable contact lenses' in late 1961(Efron, 2002; Bennet & Weissman, 2005).

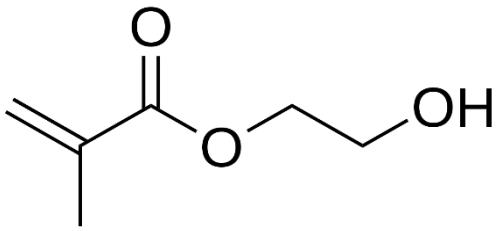

Figure 1- Chemical structure of hydroxyethyl methacrylate (HEMA).

CL manufactured from HEMA were an immediate market success, primarily by their superior comfort and enhanced biocompatibility. The first soft contact lenses (SCL) material, poly-hydroxyethyl methacrylate (PHEMA), was developed by Wichterle in 1961. The patent to develop SCL commercially was subsequently acquired by Bausch & Lomb in the USA, which introduced SCL into the world market in 1972 (Efron, 2002).

Nowadays, the main material component of hydrogel lens is PHEMA, methacrylic acid (MA), *N*-vinylpyrrolidone (NVP), and other monomers that are added to change the iconicity and water content to improve wettability, flexibility and oxygen permeability (Goda & Ishihara, 2006; Stapleton *et al.*, 2006).

MA is commonly introduced into CL materials, such as Johnson & Johnson trademarks, to increase the water content (WC) and therefore to increase oxygen permeability.

1.2.2.2 Silicone Hydrogel

Silicone is the second most abundant element in the Earth's crust and is ubiquitous in the hydrosphere. Silicone (Figure 2) is a general term for organosiloxane polymers containing a backbone of tetrahedral silicon and bridging oxygens and thus is relatively hydrophobic (Hamilton, 2002). The most common silicone polymer, polydimethylsiloxane (PDMS) has a hydrophobic and lipophilic character and is used to enhance oxygen permeability in CL polymers (Nicolson & Vogt, 2001).

Silicone is highly permeable to oxygen and carbon dioxide and therefore provides minimal interference to corneal respiration. However, it is difficult to manufacture and its surface is hydrophobic, and if left unmodified, silicone lenses would be inherently incompatible with the ocular surface (Stapleton *et al.*, 2006).

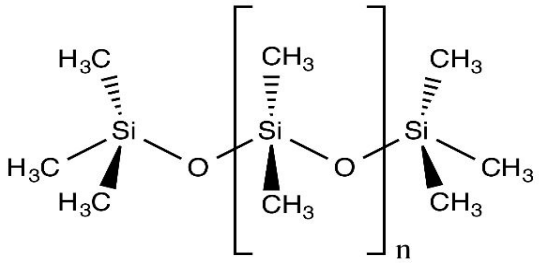

Figure 2- Chemical structure of silicone, containing the backbone of tetrahedral silicon and bridging oxygens.

The first two spherical-design silicone hydrogel (SiHy) lenses were introduced into the market in 1998, Focus Night & Day (Ciba Vision) and PureVision (Bausch & Lomb). The introduction of these lenses is considered one of the most significant advances in the CL field, since the development of HEMA (Efron, 2002). This material combines the comfort of the traditional hydrogel lenses with the high oxygen and carbon dioxide permeability of silicone used in the elastomeric CL and the siloxane materials used in rigid gas permeable CL (JoséManuel González-Méijome, Lo, Almeida, Parafita, & Refojo, 2005). Within a decade of these products entering the market, all major CL manufacturers had introduced new SiHy lenses (Efron, 2002). Currently, SiHy is the most widely prescribe material (54 %) (Morgan *et al.*, 2016).

1.2 Contact Lenses properties

1.2.1 Refractive Index and Water content

There are many properties important for manufacture and design CL, both at the physiological and visual levels. The Refractive index (RI) of CL is an important parameter not only from the optical but also from the physiological perspective since it is a measurable parameter that reflects changes in the water content (WC) of the polymer. The WC of CL can be affected by temperature, pH and solution tonicity, and can be measured immediately following removal from its storage solution. The amount of water in a hydrated CL may be expressed in terms of weight or volume and is calculated according to the following formula (Equation 1):

$$WC = \frac{wet - dry\ weight}{wet\ weight} \times 100 \qquad \text{Equation 1}$$

RI for any substance is defined as the ratio of the velocity of light of a given wavelength in the vacuum in comparison to its velocity in the substance. RI as the ratio of velocity and the parameter is mainly used for calculation purposes. (Efron, 1980; Efron & Brennan, 1987). The WC may be related to protein and lipid deposits, and the adhesion of bacteria and formation of biofilms in the CL surface (Elder, Tapleton, Evans, & Dart, 1995; Henriques *et al.*, 2005; Teichroeb *et al.*, 2008). It is possible to estimate the WC of a CL by measuring its refractive index because there is a negative relationship between these quantities (J. González-Méijome *et al.*, 2006; Insua Pereira & Lira, 2017)

1.2.2 Atomic Force Microscopy

A smooth surface is essential for the optical quality and the biocompatibility of CL and the ocular surface (JoséManuel González-Méijome et al., 2005). The mechanical properties of a polymer composite such as tensile strength, stiffness, elastic modulus, fracture toughness, and mode of failure depend on the properties of the filler, properties of the matrix, to a large extent, the polymer-filler interfacial interaction (Dvir, Jopp, & Gottlieb, 2006).

The atomic force microscope (AFM) is a type of microscopy that doesn't use lenses, photons, or electrons, which explores directly the sample surface by means of mechanical scanning, which allowed a possibility for microstructural and mechanical analysis of biological specimens. This microscopy is based on the concept of near-field (Braga & Ricci, 2004), in **Figure 3** a schematic diagram of an AFM is shown. The heart of the instrument is the tip mounted at the end of a small cantilever, typically made of silicon or silicon nitride. The tip is responsible for the closest contact with sample and cantilever and when the tip is brought into the proximity of a sample surface, forces between the tip and the sample lead to a deflection of the cantilever according to Hooke's law (Baguet, Sommer, & Duc, 1993; Braga & Ricci, 2004; Lira, Santos, Azeredo, & Oliveira, 2007).

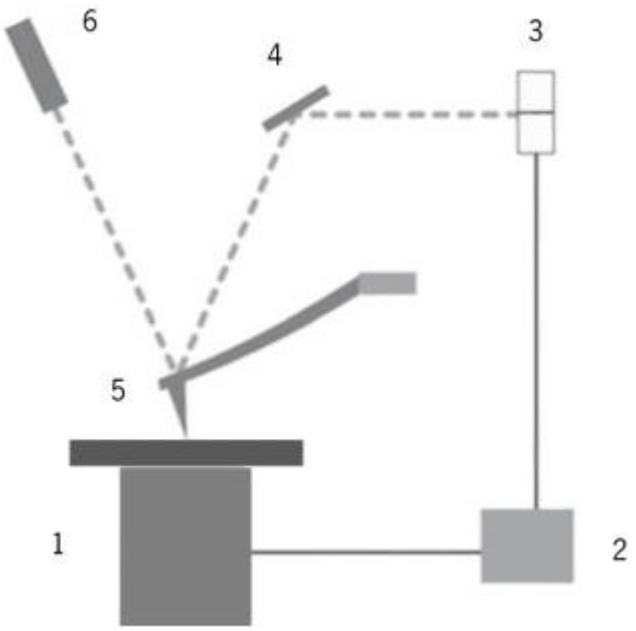

Figure 3- Scheme of atomic force microscopy.1- Scanner with sample; 2- Eletronics;3- Photodetector; 4- Mirror; 5-Cantilever; 6- Laser (adapeted from Braga & Ricci, 2004).

AFM allows the analysis of surface topography and roughness, and it can be quantified at extremely high lateral and vertical resolutions and by means of a non-destructively methodology (Lira *et al.*, 2007). This technique showed to be a powerful tool for studying the surface properties of hydrophilic CL material in aqueous environments (Lira *et al.*, 2007; Méndez-Vilas, Bruque, & González–Martín, 2007).

The roughness parameters of a real surface are evaluated from the deviations of the two-dimensional measured profile (z values measured by means of the AFM technique on the surface) with respect to an ideal surface (D Antonio, Lasalvia, Perna, & Capozzi, 2012). An important parameter to characterize the irregularities is the Roughness Average (Ra) that represents the mean deviation of the amplitude z relative to the center line and the Root Mean Square Roughness (Rq) (Baguet *et al.*, 1993).

1.2.3 Attenuated Total Reflectance-Fourier Transform Infrared

Fourier Transform Infrared (FTIR) is an old powerful physic-chemical technique for identifying types of chemical bonds in a molecule. One of the strengths of this technique is its ability to obtain spectra from a very wide range of different compounds (C. Santos, Fraga, Kozakiewicz, & Lima, 2010).

Currently, FTIR instruments are in digital format, which makes them faster and more sensitive than the older ones. The basis of FTIR is the absorption of the infrared light by several molecules in a sample,

which can be solid, liquid or gas. This technique had the advantage that the sample preparation is simple, needs no reagent, and data acquisition is faster than other physic-chemical technique (Mansur, Sadahira, Souza, & Mansur, 2008; C. Santos *et al.*, 2010).

FTIR spectroscopy is recognized as a valuable tool for the analysis of the secondary structure of polypeptides, proteins, and to characterize the presence of specific chemical groups in the material (Kong & Yu, 2007; Mansur et al., 2008). FTIR spectra of pure compounds are generally so unique that they look like molecular "fingerprints". Organic compounds have very rich detailed spectra, while, inorganic compounds are usually much simpler. So, the peak positions in an infrared spectrum correlate with molecular structure, and these spectra can be used to identify the molecules in an unknown sample by comparison with a library of known compounds (Smith, 2011). When a chemical bond absorbs the infrared light, it vibrates in varying ways depending on its own nature. The emitted infrared light may be absorbed by the sample molecules (C. Santos *et al.*, 2010).

Absorbance and percentage of transmittance are mathematically related to each other and using FTIR software it can be converted from one to the other. Absorbance is linearly proportional to concentration, so absorbance units are used for quantitative analysis, while the transmittance spectrum is used for qualitative analysis since the peaks are not linearly proportional to the concentration (Smith, 2011).

FTIR and other techniques such as gel permeation chromatography, and differential scanning calorimetry (DSC) can be used to evaluate polymer degradation (C. A. Santos et al., 1999).

1.2.4 Mechanical properties

Mechanical properties are extremely important in the quality control and design of soft CL materials since they should make the interaction between CL and the ocular surface less traumatic as they could (Efron, 1980; Oliveira & González-Méijome, 2005). Stress in the CL materials provoked by repeated application, removal or eye movement can cause irreversible deformation or fracture, resulting in loss of optical performance, user discomfort or even complete disintegration. Therefore, an important characteristic of CL materials is its ability to maintain its physical dimensions or return to its original shape after being exposed to external forces (Tranoudis & Efron, 2004).

Efron (1980) reported that to investigate the tensile properties of a CL a standard shape must be cut, and then the tensile stress can start and be analyzed. The material will immediately elongate, and the extent of this elongation is termed as tensile strain.

The tensile stress can be applied in CL until breakage occurs and at this stage is referred to as the tensile strength (TS) of the material. If the strain is gradually increased the material will continue to elongate until a point is reached where there will be an increase in strain without a further increase in stress, this is known as the yield point. The tensile stress at this point is a useful indicator of material strength since it indicates the degree of stress that material can withstand without being deformed permanently and indicates the strength of a material (Efron, 1980; Tranoudis & Efron, 2004).

Since the stress and the strain are obtained by dividing for constant factors, the load-elongation curve has the same shape as the engineering stress-strain curve. In conventional engineering tension tests, and engineering curve stress-strain curve (Figure 4) is constructed from the load-elongation measurements made on the specimen.

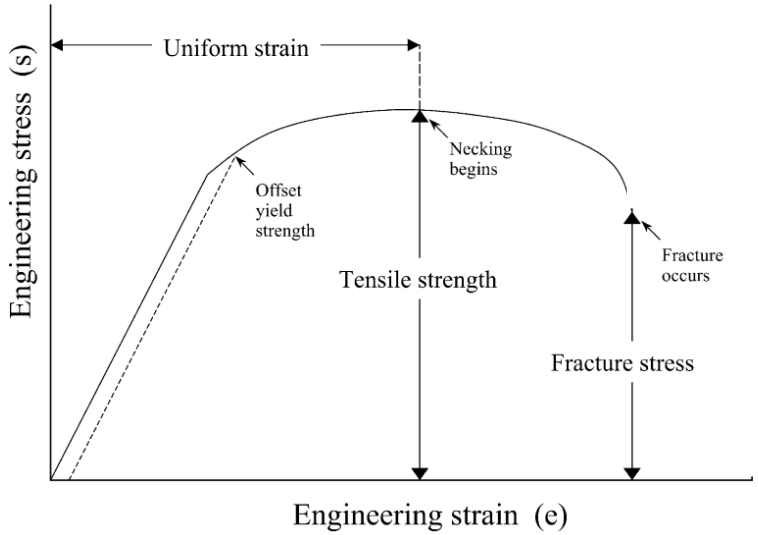

Figure 4- An engineering curve stress-strain curve (adapted from (Tranoudis & Efron, 2004).

1.3 Degradation and recycling of lens constituents

1.3.1 Environmental impact

Daily disposable lenses can have a large adverse environmental impact, resulting from the need to discard the lenses and their packages every day. While the cardboard boxes and the plastic blister packs that contain the lenses could be recycled, the lens ends up in the trash, contaminating the environment (Efron *et al.*, 2010). Many of the DCL are typically made of SiHy, and hardly degradable. In addition, their very small size makes them nearly impossible for most recycling facility's machinery to process, meaning that DCL polymers end up in landfills. Pereira carried out a questionnaire (n=128) about the Portuguese habits of discarding the CL, and 60.2% of respondents eliminate their CL in the organic waste, 26.5% in the toilet and 13.3% in the recycling waste (unpublished data).

S. L. Morgan, Morgan, and Efron, (2003) carried out a study to measure the masses of different constituent materials created as waste when disposable CL are used. They conclude that the environmental impact of waste generated using CL products by the end consumer is insignificant compared with the amount of domestic waste generated in everyday life. However, there are no studies showing if CL have or not significant environmental impacts.

1.3.1.1 Degradation of Silicone polymers

Polymer degradation has been classified as photo-oxidative degradation, thermal degradation, ozone-induced degradation, mechanochemical degradation, catalytic degradation, and biodegradation. The degradation of most synthetic polymers in nature is a very slow process that includes environmental factors, followed by the action of wild microorganisms. The major degradation will either be photodegradation, thermal degradation, or biological degradation (Efron, 2002).

PDMS is the most commonly used silicone polymer due to its excellent chemical and thermal stability. Its physical and dielectric properties remain constant over a wide range of temperatures (Hamilton, 2002). In soil, PDMS polymer could be hydrolyzed to small, water-soluble siloxanols with the ultimate product being the monomeric dimethylsilanediol (DMSD)(Corning, 1998).

DMSD is the main vehicle of silicone contamination in the environment and is also the most likely silicone derivate to be bioactive (Hamilton, 2002). In addition, is highly susceptible to condensation polymerization reactions, resulting in two key intermediates in the production of siloxane polymers, cyclic dimethylsiloxanes and linear polydisiloxanols and it can be microbially degraded to CO_2 and inorganic

silicate, and it also volatilize from soil at about 1-7% per week, this loss mechanism suggests that DMSD will not persist in the soil environment (Corning, 1998).

1.3.2 Degradation by Microorganisms

1.3.2.1 Biodegradation

Biodegradation has been recently defined by the IUPAC (2012) as the degradation of a polymeric item due to cell-mediated phenomena (Vert *et al.*, 2012).

Microorganisms can damage the structure and function of synthetic polymers (Cappitelli & Sorlini, 2008). Microbial degradation of polymers depends on their molecular compositions, molecular weights and the presence of specific microorganisms on surfaces of the materials. Generally, a high molecular weight polymer takes more time and is harder biodegradable compared to polymers with low weight (Gu, Ford, & Mitchell, 2011). Additionally, an increase in polymers molecular weight leads to a decrease in solubility, which make them unfavorable for attack by microorganism. In contrast, monomers, dimers, and oligomers of a polymer's repeating units are much easily degraded and mineralized. Some polymers can be almost completely utilized as a source of carbon and energy while others are only partially degraded (Gu, 2003).

There are different ways in which microorganisms can interfere with the structure and function of synthetic polymers. It could be by the presence of microorganisms which results from the accumulated biomass represented by biofilms, enzymatic attack, physical penetration, and disruption, increased leaching of additives and monomers that are used as nutrients, production of metabolites, and water accumulation and excretion of pigments (Cappitelli & Sorlini, 2008). The first mechanism for biodegradation of high molecular weight polymer is the oxidation or hydrolysis by enzymes to create functional groups that improve its hydrophilicity (Shah *et al.*, 2008). Hydrolysis of polymer backbone will produce low molecular weight by-products. Although for hydrolysis to occur, the polymer must contain hydrolytically unstable bonds which should be reasonably hydrophilic for water access. Biodegradable polymers are mainly esters and ester-derivative (Park *et al.*, 1993).

The breakdown of large polymers to carbon dioxide requires several different organisms, with one breaking down the polymer into its constituent monomers, other using the monomers and excreting simpler waste compounds as by-products and others able to use the excreted wastes. The degradation process is called depolymerization, when the end products are CO_2, H_2O, or CH_4, (Gu, 2003; Gu *et al.*, 2011).

A commonly recognized rule is that the closer the similarity of a polymeric structure to a natural molecule, the easier it is to be degraded and mineralized. During degradation, exoenzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, like oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer bacterial membranes, and then to be utilized as carbon and energy sources that can end as a mineralization process.

1.3.2.2 Biodeterioration

Deterioration is connected to a loss of performances and thus to the function, whereas degradation relates to a loss of properties, a polymer deterioration is more general than polymer degradation (Vert *et al.*, 2012).

Hueck (2001) defined biodeterioration as any unwanted change in the properties of a material caused by the vital activities of organisms. The material process of biodeterioration can be divided into 3 types. When the material is damaged mechanically it means the organism disrupts or distorts the material by growth or movement. When material suffers a chemical attack, the organisms use the material as a food source (assimilation) or by excretion products or vital phenomena other than nutrition (dissimilation). Biodeterioration is not necessarily caused by any 'conscious' process of the organism (Allsopp *et al.*, 2004; Hueck, 2001). Biodeterioration of polyurethane polymers occurs through the enzymatic action of hydrolases, such as ureases, proteases, and esterases. Most of the synthetic polymers are resistant to biodeterioration because of their chemical nature (Cappitelli & Sorlini, 2008; Gu, 2003).

1.3.3 Microorganisms involved in synthetic polymers degradation

Polymeric materials, such as polyethylene, polystyrene, polyvinyl chloride (PVC) and polyesters are resistant to microbial attack, but the addition of various other materials makes them susceptible to microbial attack. Synthetic and natural polymers can be potential substrates for heterotrophic microorganisms including bacteria and fungi (Gu, 2003; Shah *et al.*, 2008).

Under aerobic conditions, *Mucor rouxii* species proved to be efficient in the degradation of PVC, while *Flavobacterium* sp. and *Pseudomonas* sp. can mineralize polyethylene glycols completely (Gu, 2003; Kawai & Schink, 1987; Singh & Pant, 2016). Additionally, polyester segments of polyurethane can be degraded relatively easy by microorganisms, while polyether segments of polyurethane are more resistant to microbial attack (Gewert, Plassmann, & Macleod, 2015). Normally filamentous fungi were the agents

causing deterioration of PVC, polyurethane, nylon, and acrylics, although some bacteria, yeasts, algae, and lichens can grow on synthetic polymers (Cappitelli & Sorlini, 2008).

It is important to notice that biodeterioration and degradation of polymer substrate can rarely reach 100 % and the reason is that a small portion of the polymer will be incorporated into microbial biomass, hummus, and the other portion go into natural products (Gu, 2003).

1.3.4 Aquatic Hyphomycetes- an ecological relevant group

Microplastics can be produced from degradation and /or deterioration of polymers. Most of the microplastics in oceans are believed to originate from a larger item, through mechanical action and degradation, driven by UV-radiation-induced photooxidation, releasing low-molecular-weight polymer fragments such as monomers and oligomers, and forming fragments of increasingly smaller size. Rivers are susceptible to synthetic polymers and microplastics and can carry them to marine environments. The influence of microplastics on freshwater biota remain largely unstudied, although for marine ecosystems there is a concern due to its small size and to the fact that they are an optimal prey for many animals in the marine food chain (Galloway, Cole, & Lewis, 2017; McCormick *et al.*, 2016). There are many evidences that microplastics are entering the marine food chain, by filter feeders living in the water column and bottom sediments. The microplastics have already been found in the guts of invertebrates, fish and other larger animals (Galloway *et al.*, 2017; Karlsson *et al.*, 2017; Van Cauwenberghe & Janssen, 2014).

Microplastics may also affect lower trophic by presenting a novel habitat for colonization by microbial biofilms in aquatic ecosystems. Biofilms are composed of bacteria, archaea, and microbial eukaryotes attached to surfaces and embedded in an extracellular matrix of polymeric substances. Biofilm microbes are essential for heterotrophic organic matter processing in aquatic systems and provide an energy input to the food chain (Galloway *et al.*, 2017; Gewert *et al.*, 2015; McCormick *et al.*, 2016). Nelms *et al.* (2018) present evidence that microplastics can be transferred across trophic levels, from fish to a marine mammal top predator.

Aquatic fungi, more specifically aquatic hyphomycetes, are an ecological relevant group of freshwater fungi that play a key role as intermediaries trophic level between plant detritus and invertebrates in either clean or metal-polluted streams, and as such, they are excellent candidates for bioindicators (Pascoal, Pinho, Cássio, & Gomes, 2003). They are the predominant microorganisms that colonize leaves in streams, and their activity is affected by several environmental variables (Chauvet & Suberkropp, 1998).

There are evidences that aquatic hyphomycetes are responsible for dominating the microbial leaf breakdown, and they produce a variety of extracellular enzymes capable of degrading complex polysaccharides of plant cell walls, including cellulose, lignin, and hemicellulose.

Seena, Graça, Bartels, and Cornut (2019) have demonstrated that the nanosized polystyrene impaired the ecological functions of aquatic hyphomycetes, and aquatic fungal species differ in their tolerance to nanosized polystyrene. Although, it is unclear if, in a multispecies natural system, the more tolerant hyphomycetes can substitute the least tolerant species, this study highlighted the importance of high fungal diversity in freshwater streams to them be more tolerant to plastic pollution.

1.3.5 The different use of Hydrogels and CL polymers

Hydrogels are water-swollen polymeric materials that maintain a distinct three-dimensional structure even after being deformed for a very long time. The CL is one of the most representative application products of hydrogels (Goda & Ishihara, 2006; Kopeček, 2007).

The term hydrogel implies that the material is already swollen in water. In addition, they are usually made of hydrophobic polymer molecules that are crosslinked by chemical bonds or other cohesion forces such as ionic interaction, hydrophobic interaction or hydrogen bond (Qiu & Park, 2001). A wide and diverse range of polymer compositions has been used to fabricate hydrogels which can be divided into natural polymer hydrogels, synthetic polymer hydrogels, as well as the combinations of the two classes (Hoffman, 2012). To design a hydrogel for SCL, the chosen polymers must satisfy several requirements such as chemical and thermal stability, be optically transparent and show a higher tensile and tear strength (Goda & Ishihara, 2006). Polymers of natural origin have the advantage of being biocompatible and biodegradable but may suffer from weak mechanical strength, high batch-to-batch variability, and immunogenicity which make them less attractive than synthetic polymers. Synthetic polymers allow for better control of the hydrogel's preparation, are more reproducible and versatile but the biocompatibility and biodegradability must be evaluated (Kamaly, Yameen, Wu, & Farokhzad, 2016; Kopeček, 2007).

Hydrogels have shown good biocompatibility and resistance, so they can be applicated in different fields such as tissue engineering, synthetic extracellular matrix, implantable devices, food packing, materials controlling the activity of enzymes or cell attachment. Also, they are used extensively in the development of the smart drug delivery for molecules that are sensitive to, or incompatible with organic solvents (Kopeček, 2007; Roy, Saha, Kitano, & Saha, 2012). The capacity of different drugs to diffuse into polymers has been used in different types of biotechnologies (Kopeček, 2007; Qiu & Park, 2001).

PHEMA had a great interest in the pharmaceutical area due to this its biocompatibility, high permeability for water, mechanical properties and softness with the surrounding tissues. According to these characteristics, HEMA had been used to entrapped (or immobilized) many different compounds such as salicylic acid or liposomes to develop drug delivery devices (Mahattanadul, Sunintaboon, Sirithip, & Tuchinda, 2016; Mokry, Karbanova, Lukas, Paleckova, & Dvorankova, 2000).

As CL are hydrogels and the majority have PHEMA in its constitution so, many researches have attempted to use them for ophthalmic drug delivery (Gulsen & Chauhan, 2005). PHEMA and HEMA are hardly degradable *in vivo,* which in the case of CL is necessary, but it is also difficult to degrade in a natural environment (Mokry *et al.*, 2000).

Chapter 2:

Objectives

2. Objectives

The increase of people with refractive errors leads to an increase of people using contact lenses, being the most significant rise in the disposable CL, such as daily and monthly. The need to discard these lenses can cause an environmental impact due to its degradation/deterioration into small particles which increases the number of microplastics in Nature. Previous studies already implicated microorganisms, yeasts, and bacteria, in the degradation and or deterioration of polymeric surfaces.

Therefore, we specifically aimed to:

I. Evaluate if the presence of contact lenses affects the growth of yeasts, bacteria and aquatic hyphomycetes and if they have the capacity of biodegraded contact lenses.

II. Evaluate if contact lenses are degraded or deteriorated in the soil, by ATR-FTIR, AFM, mechanical studies of the CL after eight weeks of incubation.

The work presented was develop as a collaborative project between the Centre of Molecular and Environmental Biology (CBMA) and the Centre of Physics, of the University of Minho.

Chapter 3:

Material and Methods

3. Material and Methods

3.1 Contact Lenses

To perform this study 2 different CL were selected (Table 1). These CL were chosen according to the wearing modality and their composition in order to have one traditional or conventional CL and composed of SiHy, the most widely prescribed material nowadays.

Table 1- Contact lenses used in this study and their characteristics.

Wear Modality	USAN	Manufacturer	DK	Water Content	Centre thickness (mm) for -3.00D	Polymer composition	Type
Daily	Nesofilcon A	Bausch & Lomb	42	78 %	0.10	PHEMA; PVP; NVP	Conventional
Monthly	Senofilcon A	Johnson & Johnson	103	38 %	0.07	HEMA; PDMS; DMA+PVP	SiHy

USAN: United States Adopted Name

DK: Oxygen permeability (units: x 10^{-11} cm^2/s ml O_2/ml.mm Hg).

HEMA: Hydroxyethyl methacrylate;

PVP: Polyvinylpyrrolidone

NVP: N-Vinylpyrrolidone

PDMS: Polydimethylsiloxane

DMA: N, N-Dimethylacrylamide;

3.2 Yeast strains, growth conditions, and media

In order to evaluate the impact that yeasts could have in CL and the impact that CL could have in yeasts grow, six unicellular fungi were selected from the Yeast collection of the Department of Biology (Table 2).

Table 2- Yeast species used in this study and the respective code.

Code	Yeast Species
TB200	*Pichia orientalis*
TB105	*Pichia fermentans*
TB13	*Candida humilis*
TB474	*Candida intermedia*
TB140	*Candida tropicalis*
TB73	*Wickerhamomyces anomalus*

Yeast cell cultures were prepared on YPD (Yeast extract peptone dextrose) medium 1 % (w/v) yeast extract, 2 % (w/v) peptone, 2 % (w/v) glucose, for solid medium (YPDA) 2 % agar (w/ v) was added.

For the incubation of CL with the yeast, the cultures of *C. humilis*, *C. intermedia*, *P. orientalis,* and *P. fermentans* were diluted with 20 ml fresh medium to OD_{600} = 0.1 and incubated with 1, 3, 5 and 10 lenses at 25 °C, and 120 rpm. The OD_{600} was measured throughout 2 to 3 days of incubation. After the incubations, the CL were collected and placed in deionized water until RI and WC analysis. Incubation of the yeast cells in the same conditions with no CL was used as control.

3.2.2 Soil and Soil extract

The protocol followed to prepare soil extract was adapted from Roy *et al.* (2012), 200 mL de soil was mixed and stirred with 500 mL distilled water. After the solid part settled down the supernatant (soil extract) and the settled solid phase were collected. A new CL was incubated in 20 mL of soil extract separately and in the middle of 30 mL of soil. The CL were then incubated at 22 °C for 8 weeks. After 1, 2, 4, 6 and 8 weeks of incubation, the CL were removed, photographed and kept in deionized water until RI and WC analysis.

The biofilm in the surface of the CL incubated in soil and in soil extract was collected, after 6 to 8 weeks of incubation, and a smear in YPDA was prepared and incubated at 30 °C for 2 days. From the YPDA plates, the morphologically different microorganisms were isolated.

3.3 Gram staining and PCR

Initially, to characterize the bacterial isolates a Gram staining protocol was done and then a PCR (Polymerase chain reaction) and the identification of the strains at the species level was performed by DNA (Deoxyribonucleic Acid) sequencing of the isolates. For Gram staining, the bacterial isolates, a Gram-positive bacteria (*Bacillus subtilis*) and a Gram-negative bacteria (*Escherichia coli*) were stained with a drop of crystal violet (60 s), Lugol's solution (60 s), 99 % ethanol (10 s) and safranin solution 0.25 % (30 s). Between each dye, a wash with water was made. In an optical microscope (Leitz Laborlux K), bacteria were observed and photographed.

For species identification, the genomic DNA of the isolates was extracted by manual protocol, adapted from the Wizard Genomic DNA extraction kit (Promega) and quantified in a NanoDropTM ND1000 spectrophotometer. For PCR amplificated, the following primers were used: 16S 8F (5'-AGAGTTTGATCCTGGCTCAG-3') as forward and 1492R (5'-GGYTACCTTGTTACGACTT-3') as the reverse primer. The composition of PCR reaction was: 2 µL of bacterial DNA, 2.5 µL PCR buffer, 2 µL MgCl2 (25 mM), 1 µL 27F primer (10 µM), 1 µL 1492R primer (10 µM), 0.5 µL dNTPs (0.2 mM), 0.3 µL Taq 5U and 12.7 µL of ultra-pure H_2O, in a total volume of 25 µL. The PCR amplification was performed according to the following conditions: initial denaturing at 96 °C for 3 min, followed by 30 cycles of denaturing at 96 °C for 30 s, annealing at 50 °C for 30 min, and extension at 72 °C for 1.5 min.

PCR amplification was confirmed by visualizing the PCR fragments on 1.2% (w/v) agarose gel in 1x Tris-acetate-EDTA (TAE) buffer (45 min, 100 V and 400 mA). The amplified PCR products were purified using the NZYGelpure Kit (NZYTech®), and quantified in a NanoDropTM ND1000 spectrophotometer, and sequenced with each primer (8F, 1492R). For the identification of the species, the obtained sequence was alignment via MEGABLAST in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.2.3 Bacteria strains

Two of the identified bacteria were selected to grow individually in the presence of the CL to see if they were able to deteriorate the lenses. Bacteria cell cultures were prepared on liquid LB medium 1 % (w/v) tryptone, 0.5 % (w/v) yeast extract, 0,5 % (w/v) NaCl in a ratio flask/medium volume 5/1 and the incubation was performed at 30 °C at 200 rpm. To prepare cells for the experiment, the culture was diluted in 20 ml fresh medium to OD_{600} = 0.1 and incubated in the presence of 3 lenses, at 22 °C with no shaking. Throughout the 2 months of incubation, the OD_{600} was measured for 1 month to monitoring the

health of the cultures, and after the incubation, the CL were placed in dH_2O until the tensile strength test. Incubation of the bacteria in the same conditions with no CL was used as control.

3.2.4 Aquatic Hyphomycetes

To evaluate if CL affects the growth of aquatic fungi, more specifically aquatic hyphomycetes, three different species from the collection of the Biology Department of the University of Minho were selected (Table 3).

Table 3- The aquatic fungi species used, code and location of isolation.

Species	Code	Isolation local	
Articulospora tetracladia	At61	Leaves	Este River
Tricladium splendens	Ts54		
Varicosporium elodea	Ve142	Foam	S. João do Campo

To prepare the aquatic fungi for the experiment, the 3 fungi were incubated in 1 % Malt Extract Agar (MEA) at 16 °C for 28 days. To evaluate if the presence of the CL affects the growth of fungi the lenses were aseptically cut (Figure 5) to allow them to be flattened in the middle of a 1 % MEA plate.

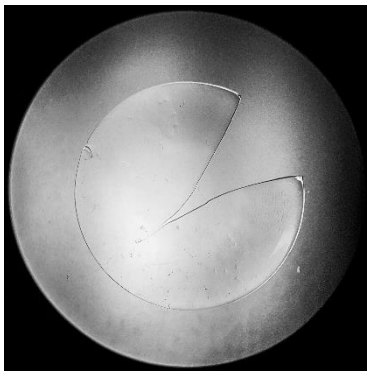

Figure 5- Representation of the contact lenses cut.

Then agar plugs with a 6 mm diameter collected from the edge of 28-days-old colonies of the fungus were placed on the top of the CL. As a control, a similar agar plug was placed in the middle of a plate of 1 % MEA without CL. For each fungus, 3 different conditions were tested: no lens, a Nesofilcon A lens, and a Senofilcon A lens (Figure 6). Throughout the 28 days of the experiment, plates were placed in 16 °C in darkness and the mycelial diameter was measured every 2 days.

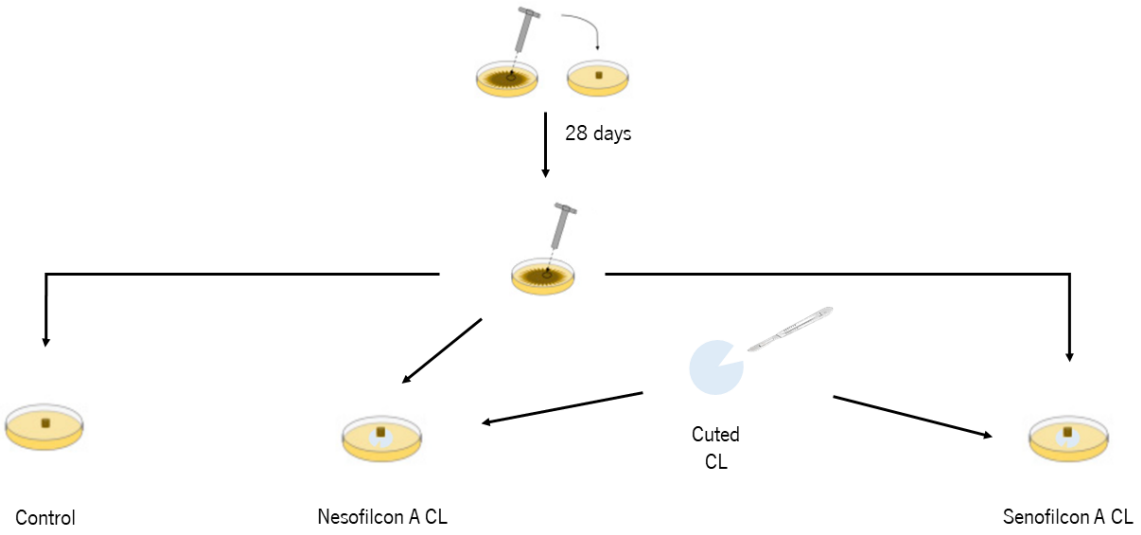

Figure 6- Illustration of aquatic hyphomycetes experiment.

3.4 Contact Lenses properties analysis

3.4.1 Refractive Index and Water Content

The analysis of refractive index (RI) and water content (WC) were performed after the incubations to verify the changes in these parameters. The instrument used for the analyses were the digital automated refractometer CLR 12-70 (Index Instruments, Cambridge, United Kingdom). The equipment measures RI by back reflection at 589 nm and provides a direct reading of the measure. The WC was obtained directly from the refractometer, according to Equation 2:

$$WC\ (\%) = \frac{n_1 - n_2}{n_1 - n_s} \qquad \text{Equation 2}$$

This equation relates WC with the RI of the CL (n2), the dehydrated polymer (n1), and the solution (ns) in which the lenses were in.

Before the measurements the excess of water on the surface of the CL were gently removed with a filter paper, and to increase reliability, three measurements per lens were performed and the mean value was considered for the analyses.

3.4.2 Attenuated Total Reflectance-Fourier Transform Infrared and atomic force microscopy

To perform the FTIR-ATR (Fourier transform-Attenuated Total Reflectance) and AFM (Atomic Force Microscopy) assays all CL were dried at room temperature. The surface of a new Nesofilcon A and Senofilcon A CL and the CL that remain 6 and 8 weeks in soil and in soil extract were characterized by atomic force microscopy (AFM), using the equipment AFM Nano-Observer CSI. The measurements were made by scanning an area of 20 µm x 20 µm, and the obtained images were analyzed with Gwyddion software to determine statistical parameters of roughness. A correction of polynomial type (2 degrees) was applied to all the original images, to allow the sample to become flat and the surface free of curvature. Once the data were corrected, the roughness parameters Ra e Rq were determined.

To evaluate the existence of absorption peaks in various areas of the spectrum and identify the presence of different functional groups, the same CL were characterized by infrared spectrometry with FTIR-ATR, using a Jasco FT / IR-6100 spectrometer in a range of 600 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3.4.3 Tensile properties

Tensile properties assays were performed to investigate if yeasts, bacteria and time in the soil or in soil extract can change the strength of the CL. The protocol followed for this assay was adapted from Tranoudis and Efron (2004), and the CL was cut to the size of 10 mm x 14 mm as shown in Figure 7. The tensile properties were measured with a Shimadzu Autograph Test Machine, Model AG-500, using a 4-8 mm gauge length. The crosshead speed was 2 mm mm^{-1} min^{-1} and the test was performed until CL breaks. It was tested 3 lenses of 7 different conditions for both CL in the study: new CL; after 6 weeks in soil extract; after 6 weeks in the soil; 8 weeks in soil extract; 8 weeks in the soil; 2 months incubate with *B. megaterium*; 2 months with *B. melitensis*.

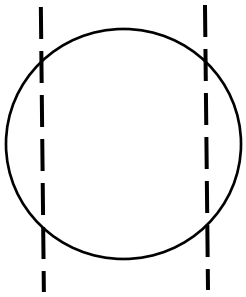

Figure 7- Two parallel representative cuts from a single contact lens.

The engineering stress, s, was calculated by dividing the load, P (N), by the original area of the cross-section of the specimen, A_0, according to Equation 3, and the percentage of elongation was calculated according to the Equation 4.

$$S = \frac{P}{A_0} \qquad \text{Equation 3}$$

$$Percentage\ elongation = \left(\frac{L}{L_0}\right) \times 100 \qquad \text{Equation 4}$$

When L is the length between gauge marks and L_0 is the original gauge length. TS in megapascals (MPa) was calculated dividing the engineering stress by the original cross-section area according to Equation 5.

$$TS = \frac{S}{A_0} \qquad \text{Equation 5}$$

3.5 Statistical Analysis

All experiments were done in triplicate, and the results are presented as the mean of the 3 experiments ± standard deviation (SD). To perform statistical analysis, it was used GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA). For comparison of more than two means it was used the One-way analysis of variance (ANOVA), and Tukey's test to multiple comparisons. Whenever the differences were considered statistically significant, they were represented by asterisks: * means $p < 0.05$, ** means $p < 0.01$, and *** $p < 0.001$ when compared to the control.

Chapter 4:

Results

4. Results

4.1 Effects of CL on microorganism's growth

In order to highlight if CL affects the yeast growth, six unicellular fungi (Table 2), were incubated with the Nesofilcon A and Senofilcon A CL.

The incubation of *P. orientalis*, *P. fermentans*, *C. humilis*, and *C. intermedia* were made using 3 lenses of each CL in the study. In Figure 8, it is possible to observe that the growth of these 4 unicellular fungi was not affected by the presence of 3 daily CL (3D) or 3 monthly CL (3M).

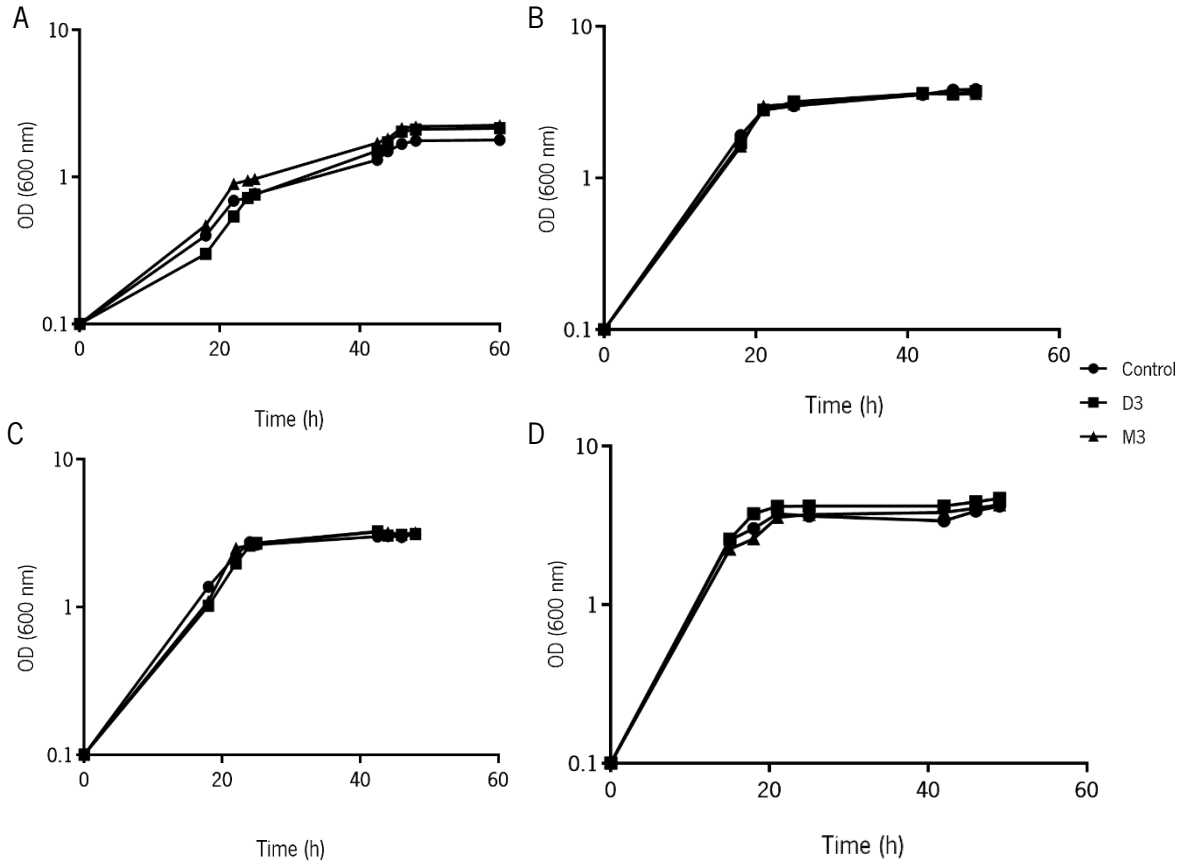

Figure 8- Growth curve of *P. orientalis* (A), *P. fermentans* (B), *C. humilis* (C), and *C. intermedia* (D) at 25 °C, in the presence of Nesofilcon A CL and Senofilcon A CL. The grow of yeasts was made in the presence of three Nesofilcon A CL (D3), three Senofilcon A CL (M3), and no lens (control).

However, to evaluate growth with a higher number of lenses, incubations were performed with the yeast strains that were not tested yet. In Figure 9A, it is possible to observe that there is no difference in *C. tropicalis* growth with no lens or after the incubation for 40 h with one (D1), five (D5), or ten daily disposable (D10) CL. The same was observed for the growth of this yeast species in the presence of the monthly CL (M1, M5, M10) (Figure 9B).

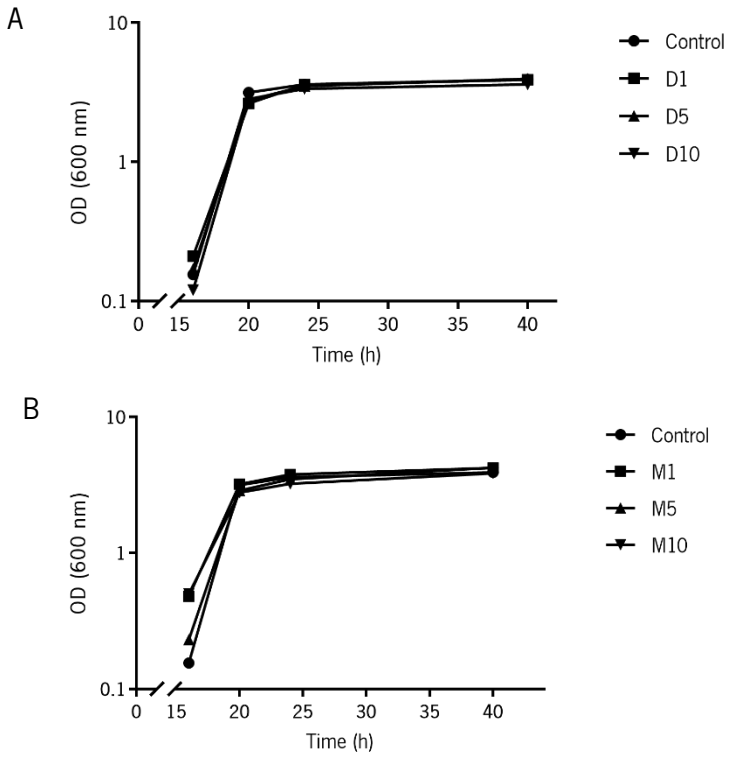

Figure 9- Growth curve of *C. tropicalis* at 25 °C for 40 h, in the presence of daily CL (A) and monthly CL (B). The grow of yeast was made in the presence of one Nesofilcon A CL (D1), five (D5), and ten (D10), one Senofilcon A CL (M1), five (M5), and ten (M10).

The growth of *W. anomalus* was performed at same conditions as for *C. tropicalis* for 2 days, and in Figure 10 it is possible to observe that *W. anomalus* had more difficulty to grow in the presence of 10 Nesofilcon A lenses (D10) (Figure 10A), and 10 Senofilcon A lens (M10) (Figure 10B). Regardless of the slower onset of growth when incubated with 10 lenses compared to the control or to the other conditions (M1 and M5), at the end of the incubation period (40h) *W. anomalus* recovered and reached the same cellular density as the other conditions. It was also possible to observe that initially, the cellular density with 10 lenses is higher for daily CL then for monthly CL.

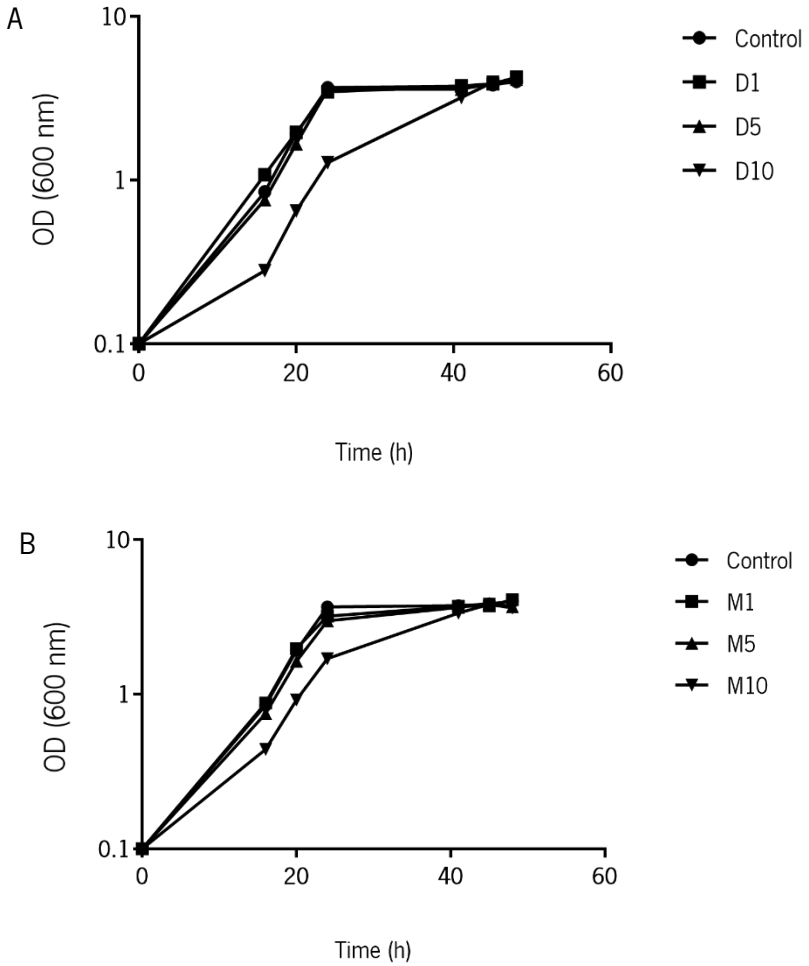

Figure 10- Growth curve of *W. anomalus* at 25 °C for 40 h, in the presence of daily CL (A) and monthly CL (B). The grow of yeast was made in the presence of one Nesofilcon A CL (D1), five (D5), and ten (D10), one Senofilcon A CL (M1), five (M5), and ten (M10), and no lenses (control).

These results show that these six unicellular fungi can grow in the presence of Nesofilcon A and Senofilcon A, and surprisingly they can grow at the same rate in the presence of both CL and with no lens. However, the initial growth of *W. anomalus* seems to be affected when there are higher amounts if lenses in the medium. Apparently, this yeast seems to need more time to adjust.

4.2 Soil

To evaluate if CL are degraded or deteriorated in the soil or in soil extracted, the two different CL were observed for 8 weeks of incubation. In Figure 11, it is possible to see that after 6 weeks in soil and in soil extract Senofilcon A CL shows more microorganisms' attachment than both CL incubated for less time and Nesofilcon A incubated for the same time. After 8 weeks both CL seem to have similarly more predisposition for microorganisms to adhere.

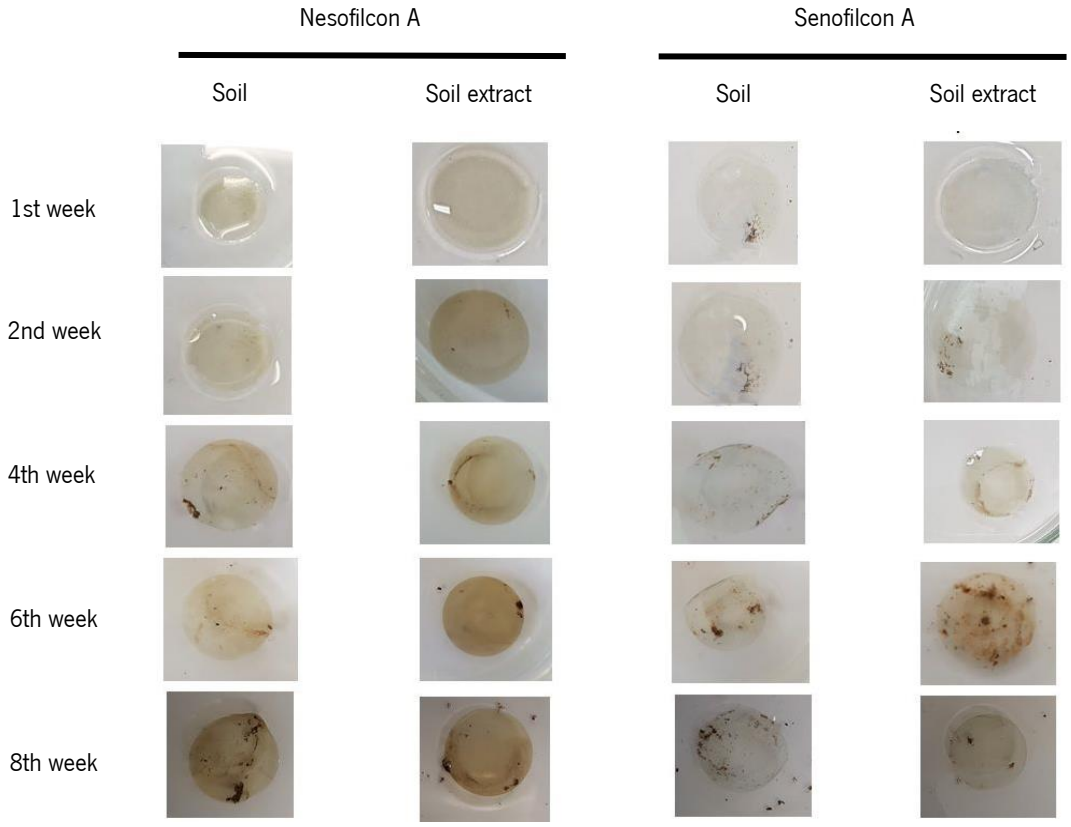

Figure 11- Nesofilcon A and Senofilcon A after 1, 2, 4, 6 and 8 weeks of incubation in soil and in soil extract.

In order to identify what was attached to the lenses after 8 weeks of incubation, a smear from the CL surface biofilms was performed in YPD Agar plates (Figure 12). It was possible to observe that there is a higher variety of microorganisms growing in both mediums and both CL.

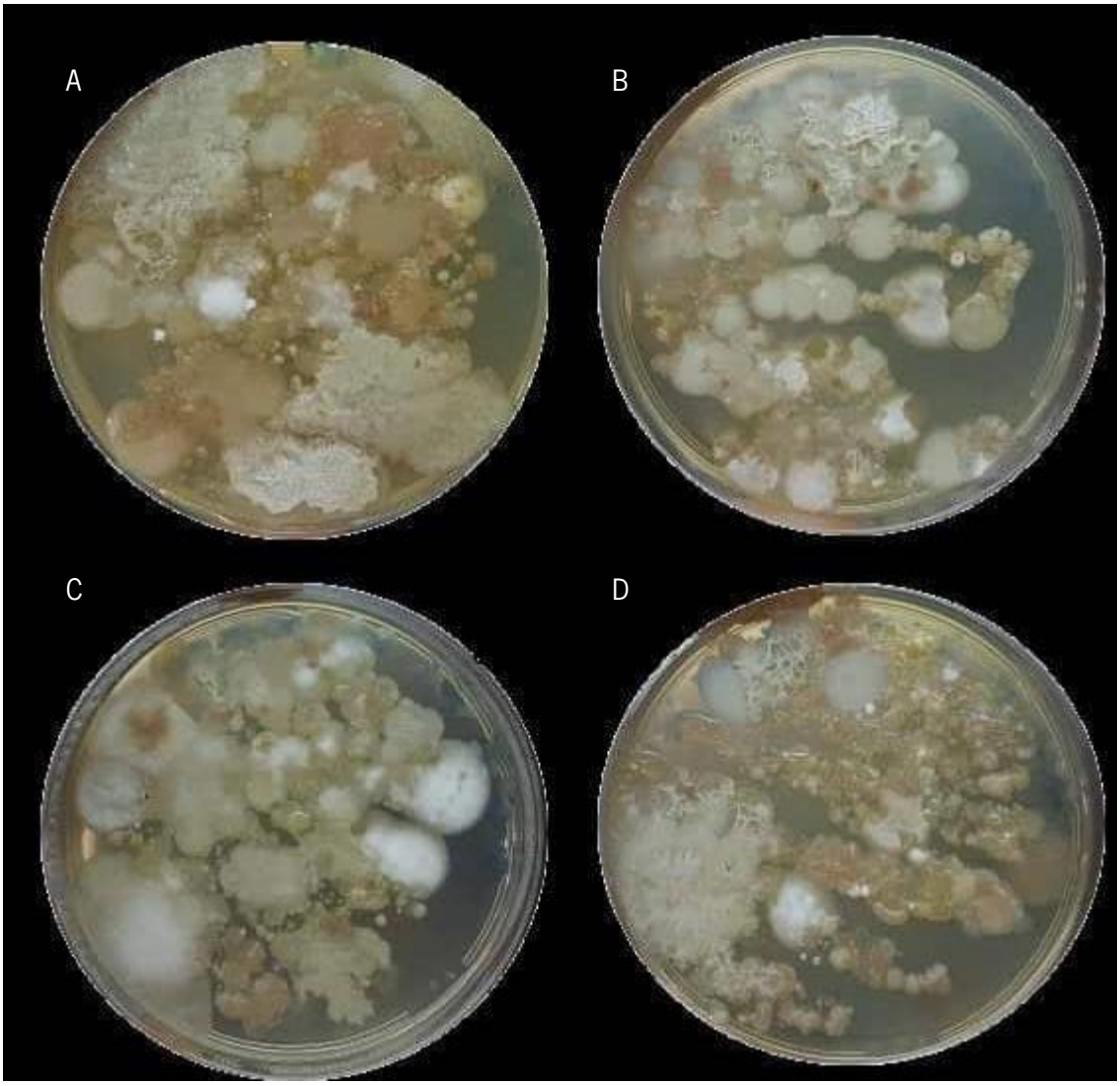

Figure 12- The growth in YPDA plates of microorganisms attached to contact lenses after incubation in Soil and in Soil Extract. The smear of Nesofilcon A for 8 weeks in soil (A), in soil extract (B), and the smear of Senofilcon A after 8 weeks in soil (C), and soil extract (D).

4.2.1 Gram staining

From the previous YPDA plates (Figure 11), it was possible to isolate and regrow four bacterial strains. To identify these bacterial strains to the species level, gram staining (Figure 13) and sequencing DNA were performed.

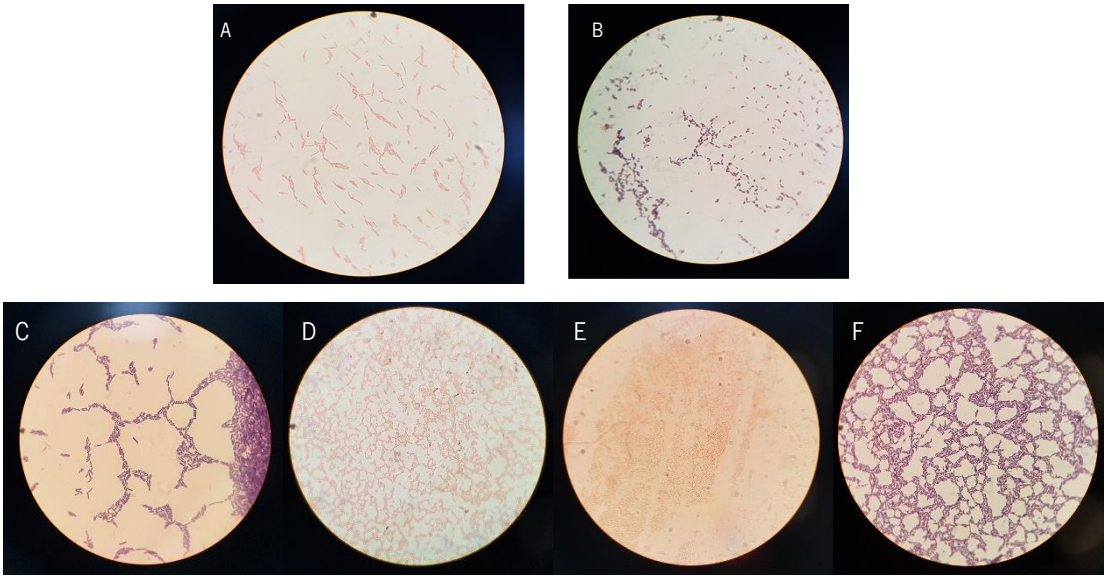

Figure 13- Gram staining of *E. coli* (A), *B. subtilis* (B), *Bacillus megaterium* (C), *Brucella melitensis* (D), *Achromobacter xylosoxidans* (E), and *Bacillus subtilis* (F).

According to gram staining, 2 gram-positive, and 2-gram negative bacteria were isolated which were then identified, by sequencing of the 16S region, as *Bacillus megaterium* (C), *Brucella melitensis* (D), *Achromobacter xylosoxidans* (E), and *Bacillus subtilis* (F).

4.1.2 Bacteria

In order to evaluate if the isolated bacteria could deteriorate the CL, *B. megaterium* and *B. melitensis* were incubated with 3 lenses of each CL in the study. In Figure 14, it is possible to observe that the growth of these two bacteria was not affected by the presence of 3 daily CL (3D) or 3 monthly CL (3M), throughout the 2 months of incubation, as expected.

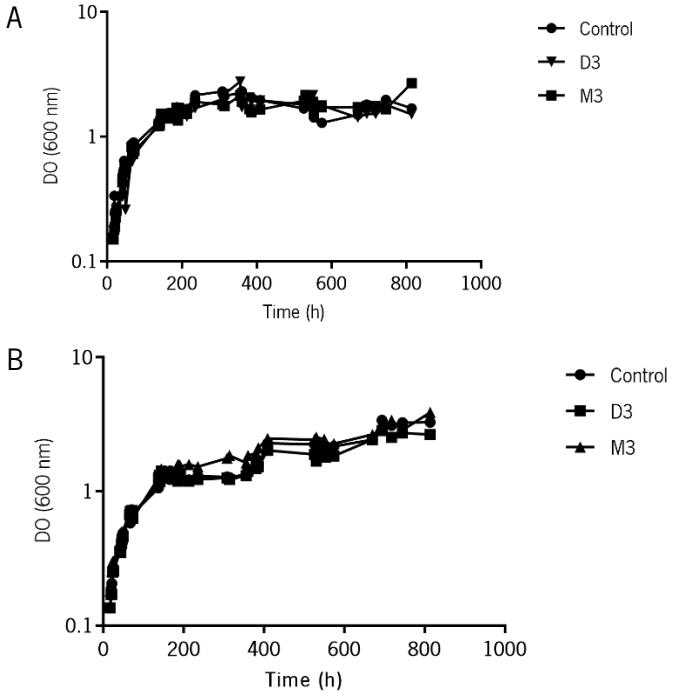

Figure 14- Growth curve of *B. megaterium* (A), and *B. melitensis*, (B) in the presence of three Nesofilcon A (D3), three Senofilcon A (M3), and no lenses (Control).

4.1.3 Aquatic Hyphomycetes

To evaluate if CL affects the growth of three aquatic fungi *A. tetracladia* (Figure 15), *T. splendans* (Figure 16) and *V. elodea* (Figure 17), fungal plugs were placed for 28 days to grow on top of the Nesofilcon A and Senofilcon A CL in MEA plates. In Figure 15, Figure 16 and Figure 17 it is possible to see that the mycelial diameter (cm) of the aquatic fungi did not change in the presence of either CL) when compared to the control with no lens.

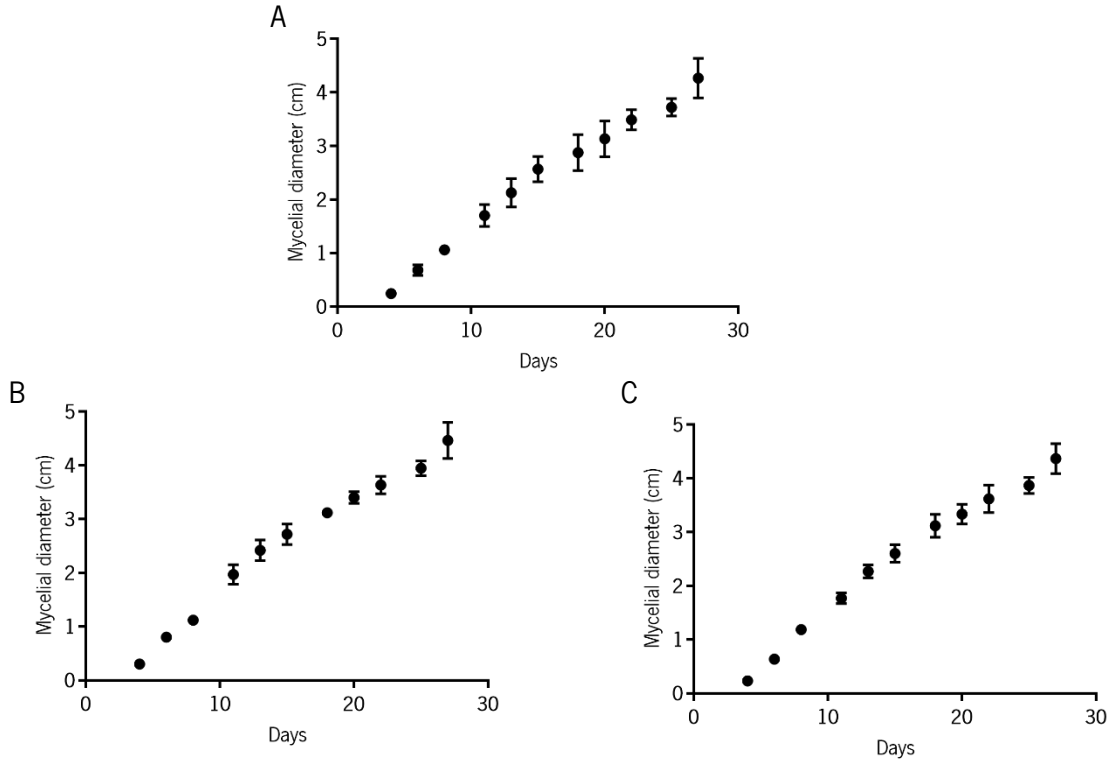

Figure 15- Mycelial diameter (cm) during 30 days of *A. tetracladia* with no CL (A), with Nesofilcon A (B), and with Senofilcon A (C).

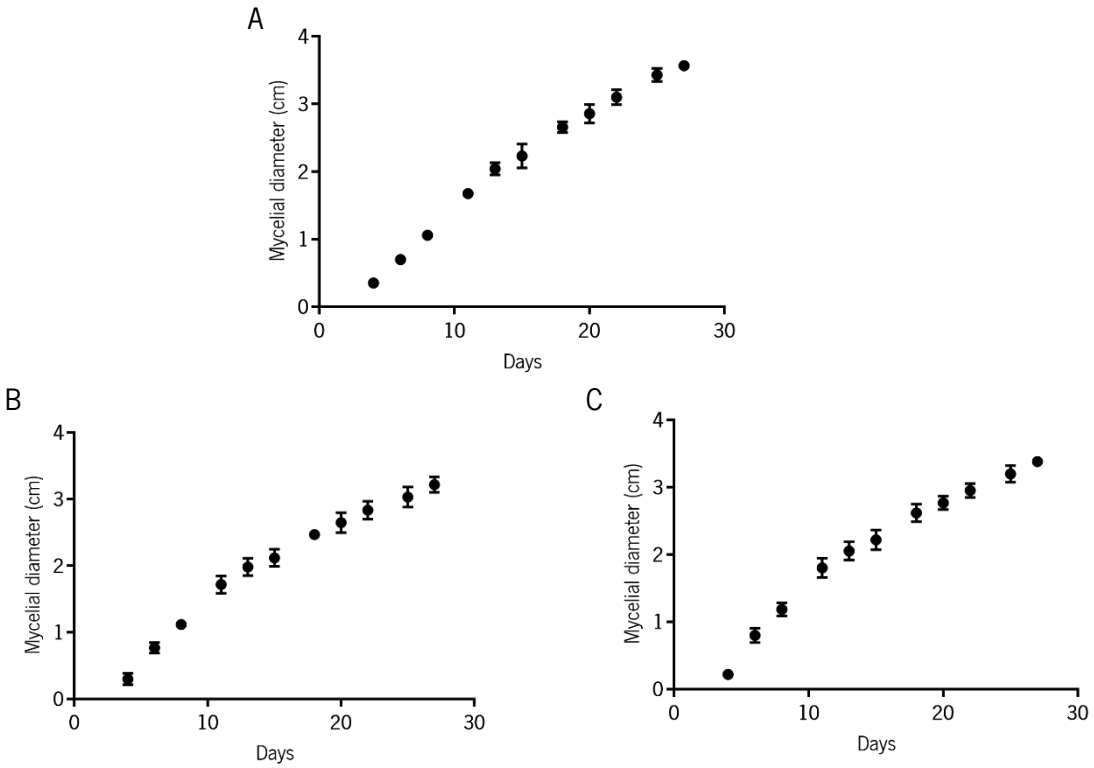

Figure 16- Mycelial diameter (cm) during 30 days of *T. splendans* with no lens (A), with Nesofilcon A (B), and with Senofilcon A (C).

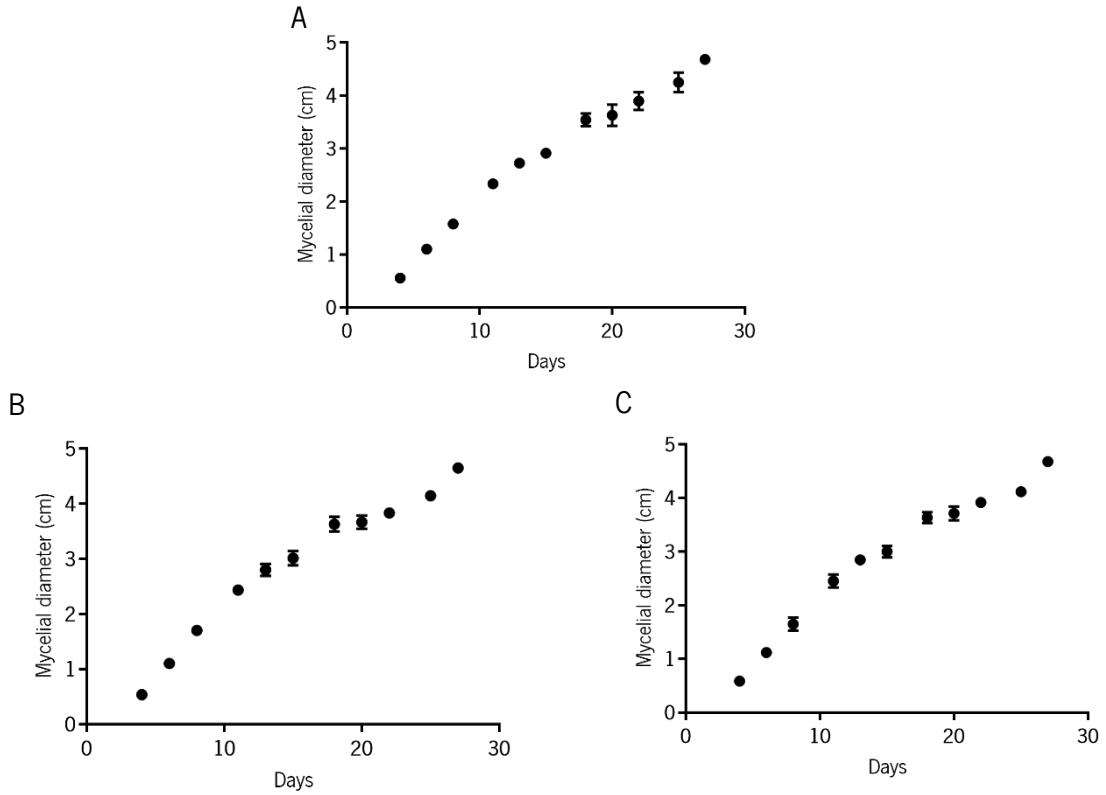

Figure 17- Mycelial diameter (cm) during 30 days of *V. elodea* with no lens (A), with Nesofilcon A (B), and with Senofilcon A (D).

After the 28 days, the plates were kept in dark for another 8 months after which the CL were observed (Figure 18). It is possible to see that all CL are broken, probably due to the mechanical forces of the hyphae. The daily CL (Nesofilcon A) are more broken after incubation with *A. tetracladia* (A) and *T. splendans* (C) than with *V. elodea* (E). However, the monthly CL (Senofilcon A) seems to be more broken when it was incubated with *A. tetracladia* (B), and *V. elodea* (F) than with *T. splendans* (D).

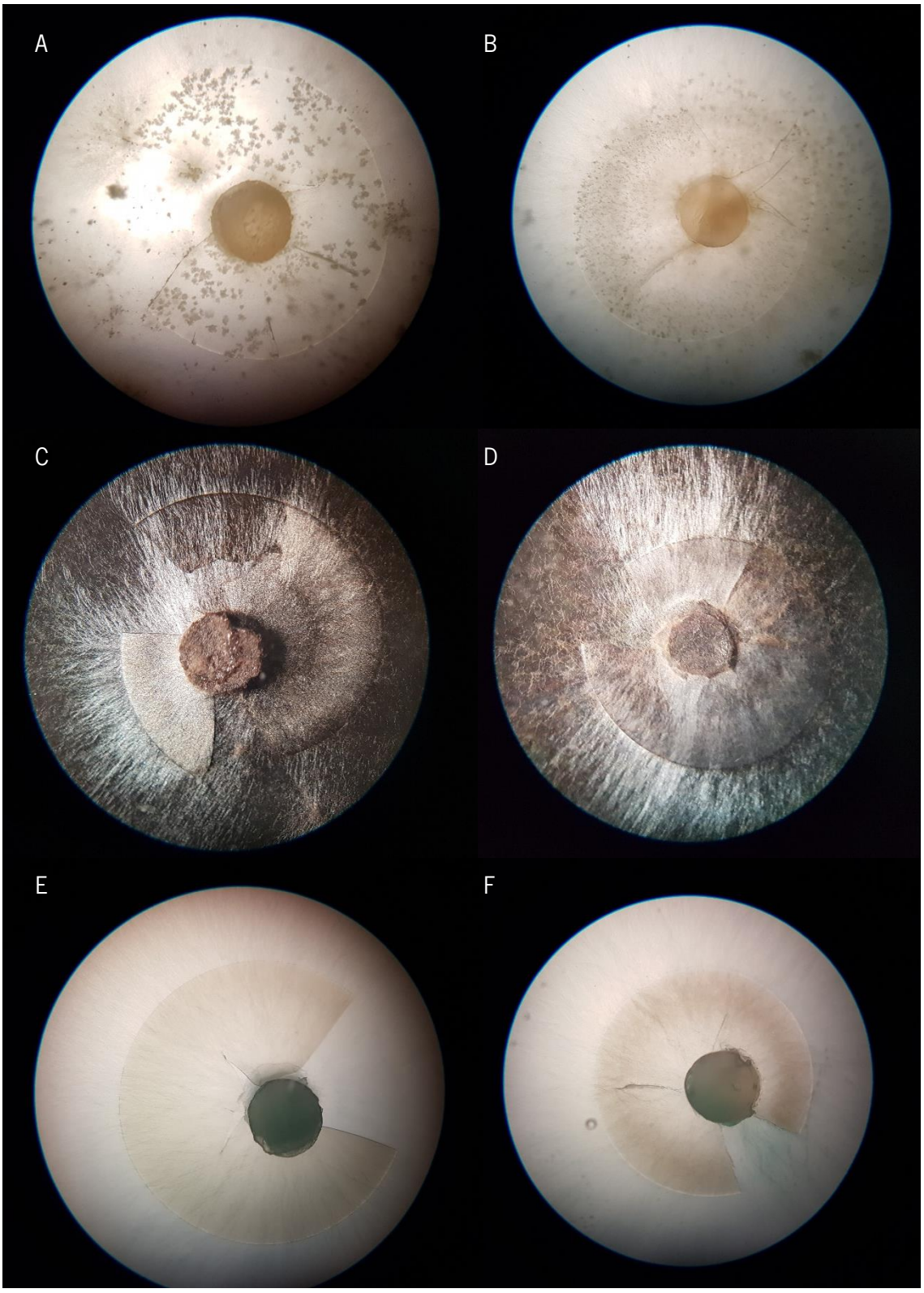

Figure 18- Contact lenses after incubation with the 3 aquatic hyphomycetes for 8 months. Nesofilcon A with *A. tetracladia* (A), *T. splendans* (C), and *V. elodea* (E). Senofilcon A with *A. tetracladia* (B), *T. splendans* (D), and *V. elodea* (F).

4.3 Contact Lenses properties

4.3.1 Refractive index and water content

After the incubation with the 6 different fungi (*Pichia orientalis*, *Pichia fermentans*, *Candida humilis*, *Candida intermedia*, *Candida tropicalis*, and *Wickerhamomyces anomalus*) the water content (WC) and refractive index of Nesofilcon A and Senofilcon were measured. In Figure 19, the incubation with these 6 different fungi did not change the WC of both CL when compared with a new CL. The WC values obtained for Nesofilcon A range between 77.1 % to 77.8 %, and for Senofilcon A range between 37.1 % to 39.1 % which are very similar to the nominal value provided by the manufacturer (Table 1). It can also be observed that the WC of Senofilcon A is more variable compared to the results for Nesofilcon A, however, this variability was not statistically significant.

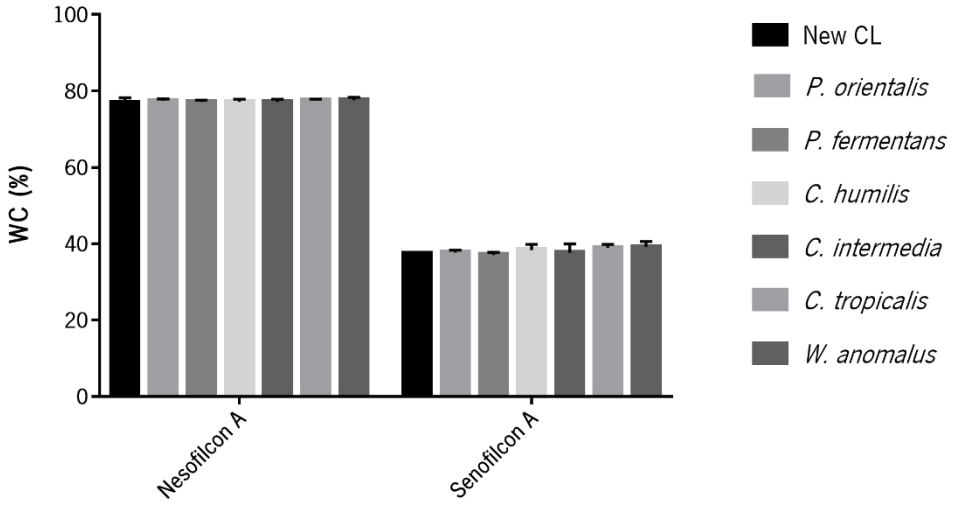

Figure 19 - The water content of new Nesofilcon A CL, new Senofilcon A CL, Nesocofilcon A and Senofilcon A incubated for 4 days with *P. orientalis*, *P. fermentans*, *C.humilis*, *C. intermedia*, *C. tropicalis* and *W. anomalus*.

In Figure 20, the RI after the incubation with 6 unicellular fungi do not change for both CL when compared to the respective new CL. The RI values obtained for Nesofilcon A range between 1.374 to 1.375, and for Senofilcon A range between 1.419 to 1.431 which are very similar to the nominal value provided by the manufacturer (Table 1).

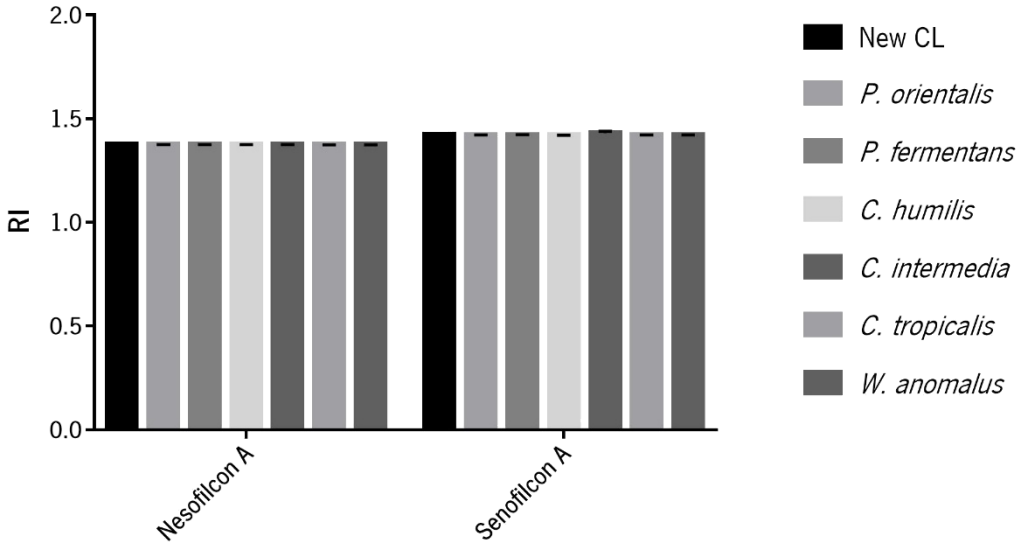

Figure 20- The refractive index of new Nesofilcon A CL, new Senofilcon A CL, Nesocofilcon A, and Senofilcon A incubated for 4 days with *P. orientalis*, *P. fermentans*, *C.humilis*, *C. intermedia*, *C. tropicalis* and *W. anomalus*.

The WC of Nesofilcon A and Senofilcon A CL incubated with soil, in soil and soil extract, for eight weeks was also evaluated. In Figure 21, It is possible to observe that WC does not change significantly over the 8 weeks compared to new CL and that the WC of Senofilcon A in both mediums is more variable compared to the results observed for Nesofilcon A, however, this variability was not statistically significant.

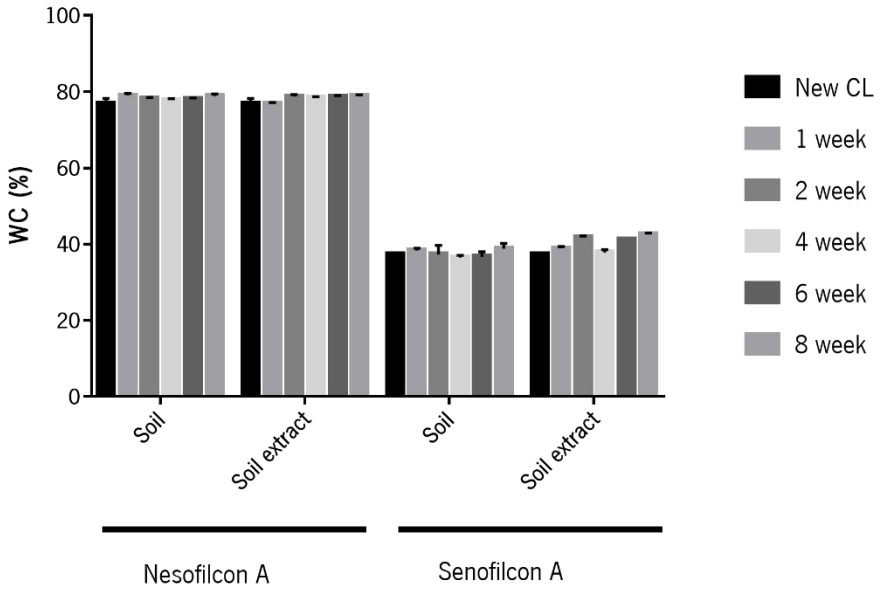

Figure 21- The water content of new Nesofilcon A CL, new Senofilcon A CL and the Nesofilcon A and Senofilcon A incubated 1, 2, 4, 6 and 8 weeks in soil and in soil extract.

The same results were observed for the RI, as it was not observed changes for both CL during the 8 weeks of incubation compared to new CL (Figure 22).

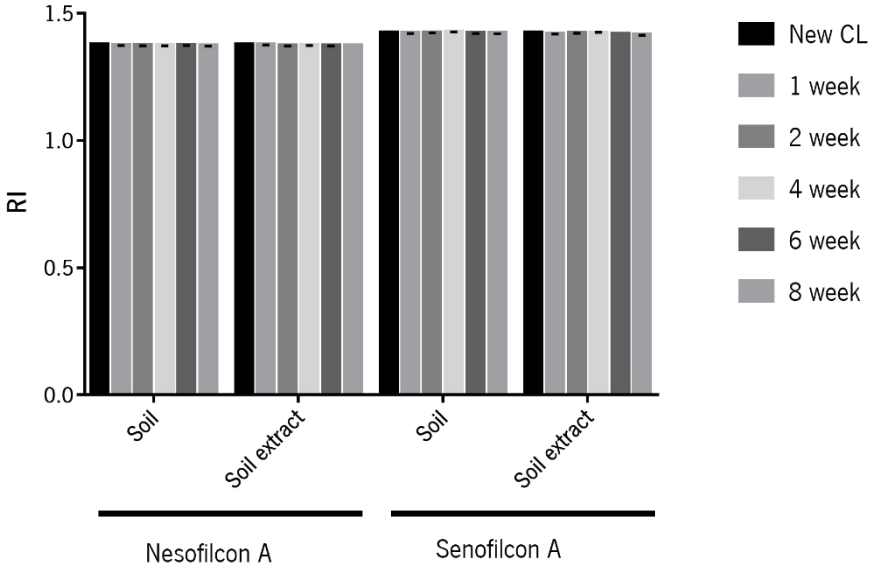

Figure 22- The refractive index of new Nesofilcon A CL, new Senofilcon A CL and the Nesofilcon A and Senofilcon A incubated 1, 2, 4, 6 and 8 weeks in soil and in soil extract.

According to these results, yeasts and incubation in soil or soil extract do not interfere in the two parameters evaluated (WC and RI). This means maybe the incubation time evaluated was not enough to change these two properties for both CL.

4.3.2 Atomic Force Microscopy

Due to the previous results (Figure 11) that suggest that after 6 weeks the surface of the lenses appears to be altered by microorganisms, the CL surface was analyzed after 6 weeks and 8 weeks of incubation in soil extract and soil. A comparison between the 3D topographic image of a new Nesofilcon A and the CL after been placed for 6 and 8 weeks in soil extract or soil obtained by AFM is presented in Figure 23. It is observed that the surface roughness increased considerably in a homogeneous way during the 8 weeks of the experiment in soil, although the same was not observed for soil extract once the roughness is higher in the CL that was incubated 6 weeks compared to the CL incubated 8 weeks (Table 4).

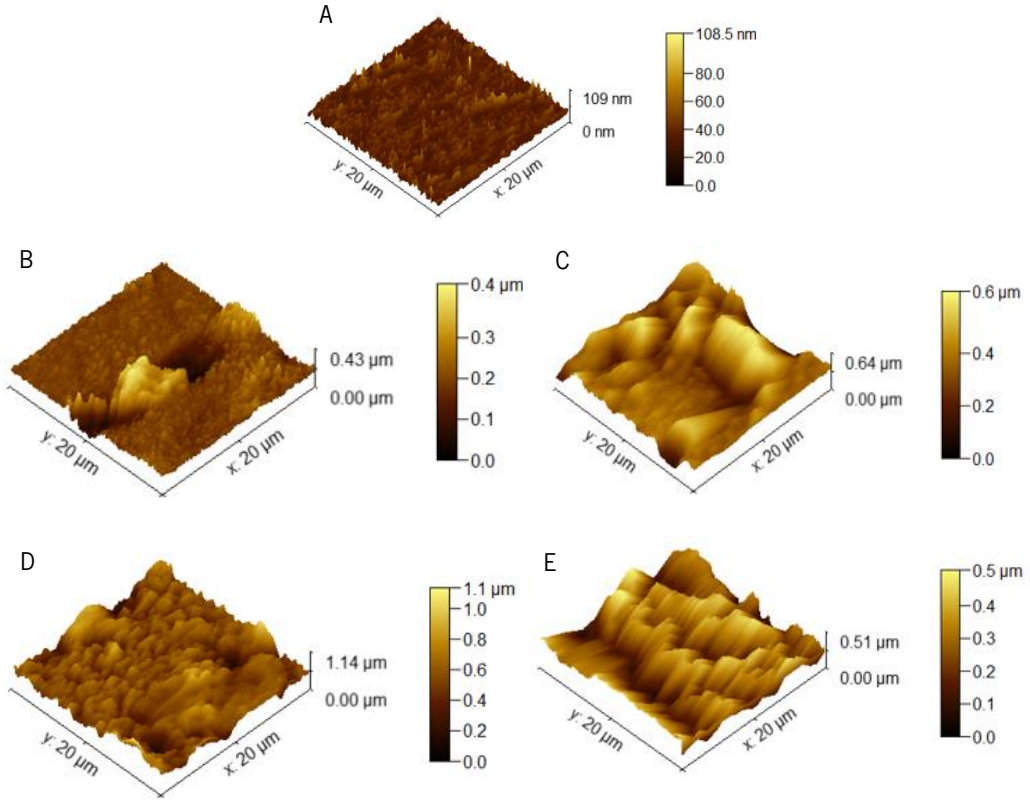

Figure 23- Three-dimensional images generated by the AFM analysis of a 40 µm² area of (A) a new Nesofilcon A, a Nesofilcon A incubated 6 weeks in soil (B), 8 weeks in soil (C), and a Nesofilcon A incubated 6 weeks in soil extract (D), and 8 weeks in soil extract (E).

Similarly, three-dimensional images of the surface of a new Senofilcon A and Senofilcon A incubated for 6 and 8 weeks in soil or soil extract was also obtained and can be observed in (Figure 24).

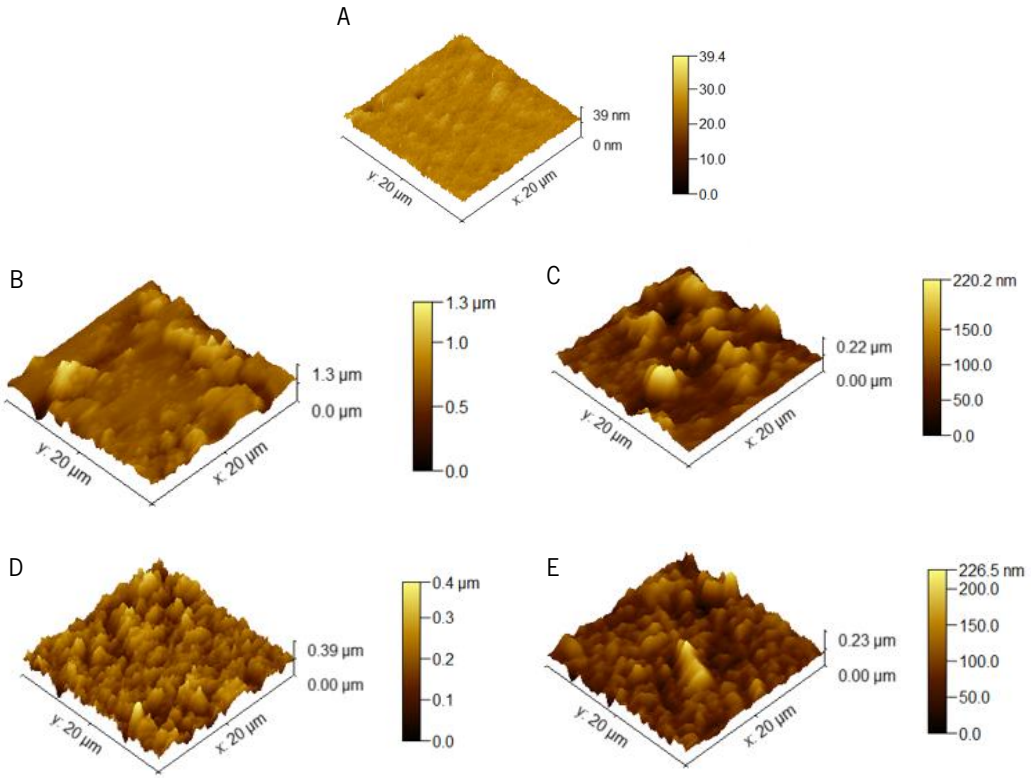

Figure 24- Three-dimensional images generated by the AFM analysis of a 40 µm² area of (A) a new Senofilcon A, a Senofilcon A incubated 6 weeks in soil (B), 8 weeks in soil (C), and Senofilcon A incubated 6 weeks in soil extract (D) and 8 weeks soil extract (E).

The roughness parameters evaluated, Ra and Rq, which show an increase in both CL after the in incubation in soil and soil extract when compared to the new ones are presented in Table 4. This increase is higher when the Nesofilcon A CL was incubated in soil extract rather than in soil, contrary the roughness parameters are higher when the Senofilcon A CL was incubated in soil. Although the roughness of Nesofilcon A CL incubated 8 weeks in the soil is higher than 6 weeks in the same medium, all other CL in both mediums shows a higher value of rugosity after 6 weeks of incubation than after 8 weeks. It is also possible to observe that the increase in rugosity is higher for Nesofilcon A CL compared to Senofilcon A CL.

Table 4- Average Roughness (Ra) and root mean squared (Rq) of new Nesofilcon A and Senofilcon A CL, Nesofilcon A and Senofilcon A incubated 6 and 8 weeks in soil and in soil extract.

			Ra (nm)	Rq (nm)
Nesofilcon A	New		4.481 ± 0.800	5.919 ± 1.304
	Soil Extract	6 weeks	132.10 ± 43.25	118.7 ± 43.58
		8 weeks	67.85 ± 16.11	82.88 ± 17.51
	Soil	6 weeks	30.19 ± 29.48	48.80 ± 40.90
		8 weeks	70.73 ± 22.16	93.38 ± 24.01
Senofilcon A	New		1.295 ± 0.343	1.775 ± 0.885
	Soil Extract	6 weeks	36.53 ± 8.66	46.44 ± 11.18
		8 weeks	18.68 ± 5.81	25.00 ± 7.98
	Soil	6 weeks	76.43 ± 32.28	109.40 ± 52.30
		8 weeks	21.10 ± 9.16	28.49 ± 11.63

4.3.3 Attenuated Total Reflectance-Fourier Transform Infrared

The analysis of FTIR-ATR analysis will let us comprehend if there was a biodeterioration of CL polymers by seeing the existence of absorption peaks in different spectrum zones and identify the presence of different functional groups. The FTIR-ATR results of daily CL (Nesofilcon A) in soil extract (Figure 25) or in soil (Figure 26) are compared to the new CL. A considerable variation of the transmittance spectrum in the area of 1600 cm^{-1}, can be observed in both Figures, corresponding to aromatic groups or ester groups. Changes in absorption peaks at 1300 cm^{-1}, 1400 cm^{-1}, and in the area of 3300 cm^{-1} can also be seen, corresponding to -OH groups. The CL incubated 8 weeks in soil extract show less intensity peaks at the three wavelengths mentioned previously compared to the CL incubated 6 weeks.

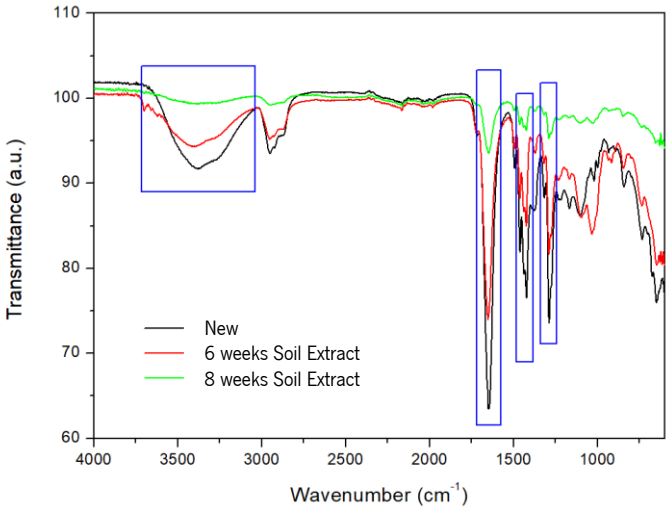

Figure 25- FTIR of a new Nesofilcon A CL (black line) and a Nesofilcon A CL incubated 6 weeks (red line) and 8 weeks (green line) in soil extract.

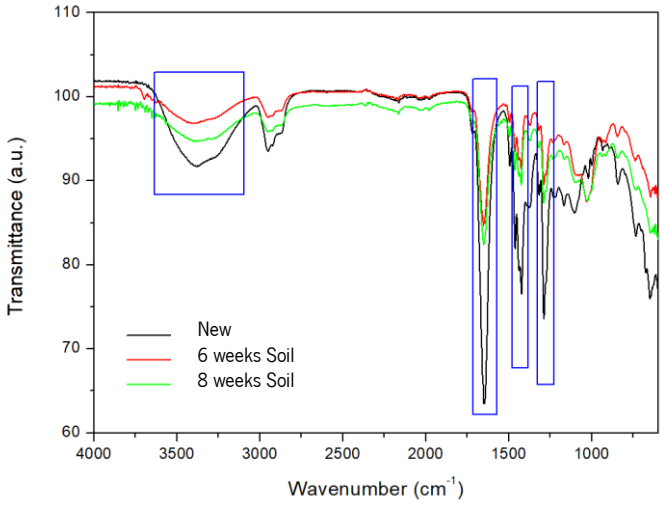

Figure 26- FTIR of a new Nesofilcon A CL (black line) and a Nesofilcon A CL incubated 6 weeks (red line) and 8 weeks (green line) in soil.

The FTIR results of monthly CL (Senofilcon A) in soil extract (Figure 27) and in soil (Figure 28) were compared to the new Senofilcon A. In these two spectrums the CL placed 6 or 8 weeks in both mediums show a peak around 1011 cm^{-1} with less intensity compared to a new CL, this peak may be associated with saccharide groups.

Comparing the spectrums of daily CL (Figure 25, Figure 26) with monthly CL (Figure 27, Figure 28) we can conclude that absorption peaks vary more in daily that in monthly CL, nevertheless there are no changes in the positions of the different peaks observed.

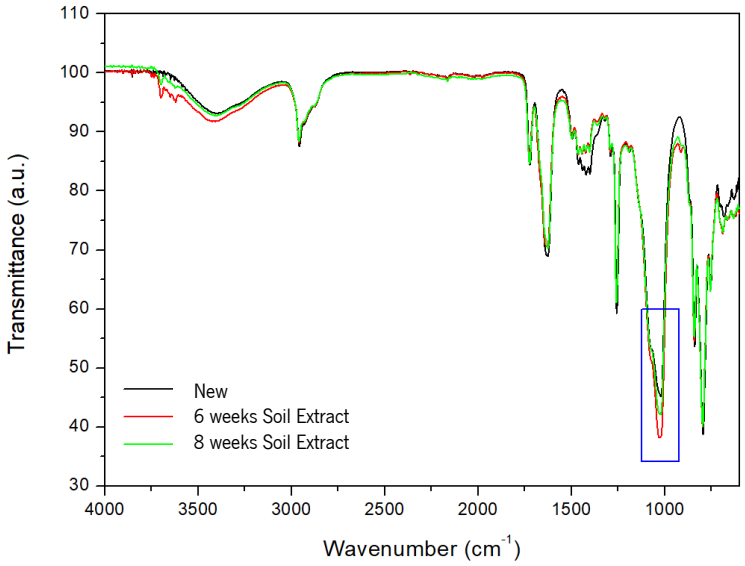

Figure 27- FTIR of a new Senofilcon A CL (black line) and a Senofilcon A CL incubated 6 weeks (red line) and 8 weeks (green line) in soil extract.

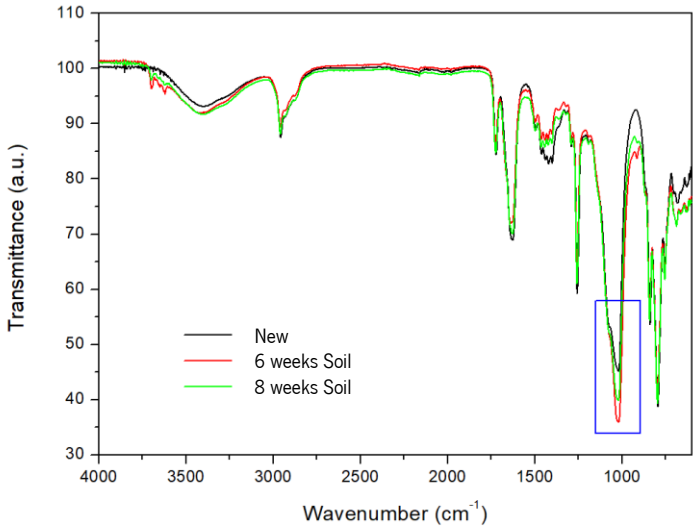

Figure 28- FTIR of a new Senofilcon A CL (black line) and a Senofilcon A CL incubated 6 weeks (red line) and 8 weeks (green line) in soil.

4.3.4 Tensile Properties

According to previous results (Figure 11), the CL seems to become more vulnerable to microorganism's attack after 6 weeks in soil extract or in soil. Since tensile properties are an important parameter in the degradability of CL an elongation test was done in the samples. In Figure 29, it is represented the percentage of elongation at break for a new daily CL, and for the ones that were incubated in soil and in soil extract for 6 and 8 weeks. The CL that was incubated for 8 weeks in soil extract has a less percentage of elongation at break compared to a new CL. Although, the same is not observed for the CL that was incubated for 6 weeks in soil extract. It seems that CL placed in the soil had less elongation at break compared to the ones placed in soil extract, however, these differences were not statistically significant.

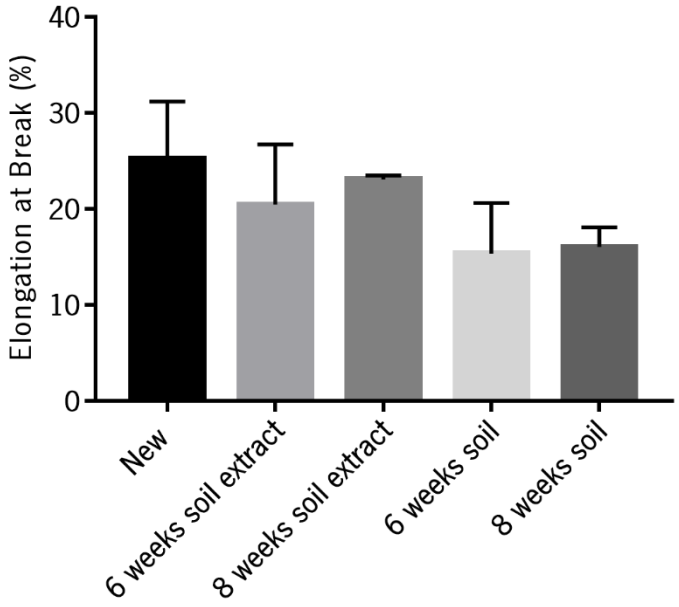

Figure 29- Elongation at break (%) of new Nesofilcon A CL, and Nesofilcon A CL incubated 6 and 8 weeks in soil extract and in soil. Mean ± SD values are from three independent experiments.

Monthly CL incubated during 6 and 8 weeks in both mediums show a less percentage of elongation at break compared to the new CL (Figure 30). It is possible to observe that in soil extract medium, the elongation at break of CL decreases over the weeks of the experiment, while in the soil the elongation at break is higher after 8 weeks rather than 6 weeks. The differences with 6 weeks and 8 weeks of incubation

in soil and soil extract with a new CL are statistically significant ($p<0.05$, Tukey). Statistical differences between 8 weeks in soil extract and a new CL ($p<0.01$, Tukey) were also observed.

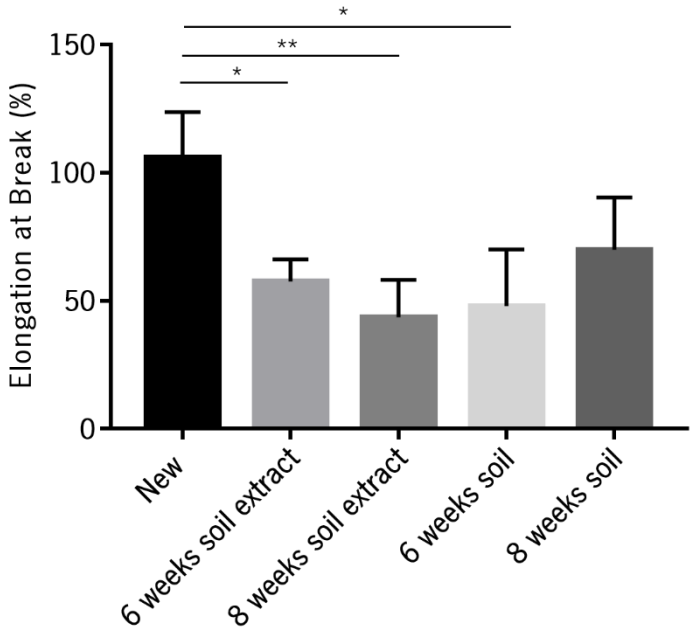

Figure 30- Elongation at break (%) of new Senoofilcon A CL, and Nesofilcon A CL incubated 6 and 8 weeks in soil extract and in soil. Mean ± SD values are from three independent experiments (* means $p < 0.05$, ** means $p < 0.01$ and *** means $p < 0.001$).

The percentage of elongation at break of CL incubated with the two bacteria isolated in this study for 2 months was also compared to the new CL of Nesofilcon A (Figure 31) and of Senofilcon A (Figure 32).

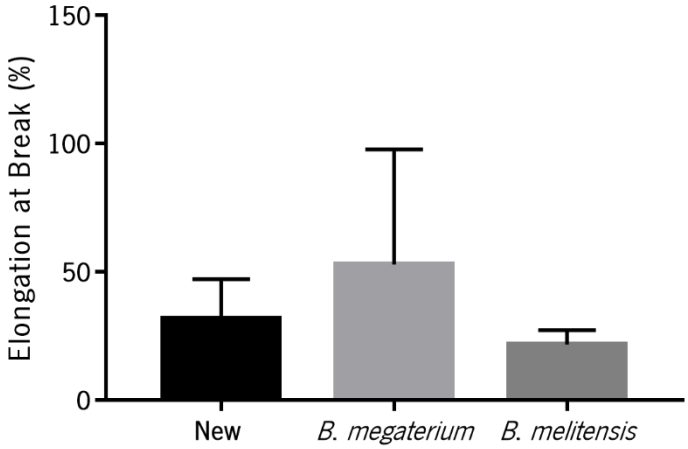

Figure 31- Elongation at break (%) of new Nesofilcon A CL, Nesofilcon A CL incubated 8 weeks with *Bacillus megaterium*, and *Brucella melitensis*. Mean±SD values are from three independent lenses.

As it can be observed in Figure 31, after the incubation with *Bacillus megaterium* the strength of CL is higher when compared with the strength of the new Nesofilcon A. The same was not observed for *Brucella melitensis*, as the strength of lenses after the incubation is lower than the new CL, albeit the difference is not statistically significant for both cases. The contrary to what is observed for daily CL (Figure 31), the strength of monthly CL after the incubation with *B. megaterium* and *B. melitensis* is lower when compared to a new CL (Figure 32), and these differences are statistically significant ($p<0.01$, Tukey).

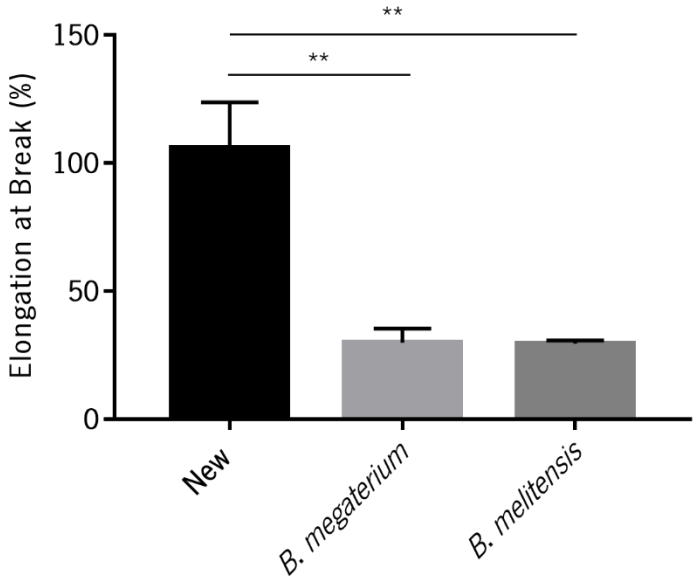

Figure 32- Elongation at break (%) of new Senofilcon A CL, Senofilcon A CL incubated 8 weeks with *Bacillus megaterium*, and *Brucella melitensis*. Mean ± SD values are from three independent lenses (* means $p < 0.05$, ** means $p < 0.01$ and *** means $p < 0.001$).

Chapter 5:

Discussion

5. Discussion

Daily disposable lenses can have a large adverse environmental impact, resulting from the need to discard the lenses and their packages every day. While the cardboard boxes and the plastic blister packs that contain the lenses could be recycled, the lens ends up in the trash, contaminating the environment. Degradation/deterioration of the CL polymers into small particles increases the number of microplastics in the environment.

In this study, it was evaluated the impact of two different CL in the growth of different microorganisms and if they have the capacity of biodegraded these polymers. The results show that all unicellular fungi were able to grow in the presence of Nesofilcon A and Senofilcon A CL with the same growth rate as the control except for *W. anomalus*, which in the presence of a higher amount of polymer had more difficult to adapt.

Regardless that this yeast can grow in anaerobic and aerobic conditions, under anaerobic conditions show low growth rates and biomass yields (Bagheri, Bauer, & Setati, 2015; Walker, 2011). The ten lenses in the test tube may be restricting the oxygen availability to the yeast cells, which contributed to the slow growth rate in these conditions. Furthermore, the slower growth rate is even more notorious in the presence of the 10 Nesofilcon A CL than in the presence of 10 Senofilcon A CL, which can be related to the differences in oxygen permeability of the materials. Senofilcon A is a SiHy (Table 1), which has a high level of oxygen permeability compared with PHEMA (Stapleton *et al.*, 2006), which is the main polymer of Nesofilcon A.

The results with the aquatic hyphomycetes show that CL does not affect the growth of the 3 aquatic hyphomycetes used in this study. The different polymer composition of the CL does not interfere with their growth since they can grow attached to CL, and after a long period of time, the aquatic hyphomycetes had the capacity to break the CL (Figure 18). These results suggest that if these 2 types of lenses end up in the rivers, these 3 aquatic hyphomycetes can attach to the CL and break (deteriorate) them. Nevertheless, our study does not evaluate if these aquatic hyphomycetes prefer to attach to CL or leaves, so it is not possible to infer the real impact of CL in ecosystems rivers. Despite that our results lead to an environmental question about the capacity of fungi to break the CL turning them into microplastics or nanosized particles. This study shows that these fungi can break the CL but do not show their capacity of using CL as carbon source and, although there are no studies about CL impact in rivers ecosystems, it has already been shown that nanosized polystyrene plastics impaired the function of aquatic hyphomycetes (Seena *et al.*, 2019). Since these fungi have an important role in the ecosystem, their

impairment will have consequences in the superior trophic levels (Nelms *et al.*, 2018). Although more studies are needed to assess the impact of CL in the aquatic ecosystem, it seems that if CL, as the nanosized polystyrene plastics, ends up into a river's course, they could have a negative impact.

It was possible to observe that microorganisms need a considerable time to physically degrade the CL and alter their properties. Thus, after the incubations with yeast, soil or soil extract the results of CL'WC and RI did not suffer significant alterations. Senofilcon A is a silicone hydrogel, and it is recognized that silicone hydrogels lenses present lower dehydration rates compared with conventional hydrogels. Nevertheless, Nesofilcon A also presents a high resistance to dehydration (Insua Pereira & Lira, 2017). Since these two materials are resistant to dehydration or have the ability to rehydrate, this could explain why the WC of the Senofilcon A and Nesofilcon A do not change significantly within the time frame of the study. Because WC and RI are related (Equation 2), it is possible to assume that if WC does not change with incubations the RI will not change. It seemed that we need considerably more time to physically degrade the CL. Once the incubation with yeasts, soil and soil extract do not interfere with these two parameters of CL.

Despite the unmodified WC and RI, the AFM results show that after 6 and 8 weeks in soil and soil extract the CL seems to have alterations on their surface. The analyses of the CL surface prove that there is an increase of CL roughness compared to the new CL. Teichroeb *et al.* (2008) observed that Senofilcon A has the lower rugosity of eight different CL, despite having a strong structure. In our results is possible to see that the Senofilcon A lenses show lower values of roughness when compared to Nesofilcon A. The increase of CL roughness after the incubation in both mediums is probably related to microorganisms and organic deposits attachment, which can be confirmed by observation of a great variety of microorganisms attached to CL, particularly bacteria (Figure 12). The microbial contamination and protein deposits in the lens surface have been studied for clinical purposes (Henriques *et al.*, 2005; Teichroeb *et al.*, 2008), and showed that CL provides a suitable substratum for bacterial adherence and biofilm formation (Elder et al., 1995). Protein deposits depend upon the constituent monomers, which is the surface structure of the material and the WC of the CL (Teichroeb *et al.*, 2008). Since the higher the WC the higher the affinity to organic deposits, it was expected that Nesofilcon A would show a higher increase of roughness than Senofilcon A, which was exactly what was observed (Table 4). Regardless, the AFM results showing differences between new and incubated CL, the peaks of FTIR-ATR spectrums do not change between new and incubated CL, which means that the polymeric composition of the CL does not change significantly. Thus, with AFM and FTIR results, it is possible to conclude that the increase of

roughness in AFM results is due to the organic deposits on the CL surface and not for degradation of the constituent polymer of the CL matrix.

Nesofilcon A has in its constitution the polymer PHEMA (Table 1), which has already shown its hard degradability (Mokry *et al.*, 2000), albeit it also has the PVP, which in combination with a biopolymer (20:80) has been shown to be biodegradable (Roy *et al.* 2012). Although Senofilcon A has in its constitution the polymer HEMA, it also has the PDMS (Table 1), which had already shown degradability in soil (Corning, 1998). In our results, it is possible to see that Senofilcon A incubated in soil, soil extract or with bacteria decrease significantly its capacity of elongation, contrary to Nesofilcon A. Their different mechanical behavior is correlated with their different constitution, and the incubation in both mediums (soil and soil extract) and with bacteria impaired the Senofilcon A and not Nesofilcon A. The results of elongation at break could be elucidative of the environmental effect of CL since it appears that the actual most widely prescribed material (SiHy) is easier to degrade than the most conventional material (PHEMA). Taken together, our results reveal that microorganisms can attach to CL and their growth is not significantly impaired, however, none of the incubations were able to significantly damage the Nesofilcon A CL, and the only property that was impaired in Senofilcon A was the tensile property. So, we can conclude that the incubations in both mediums (soil and soil extract) and with different microorganisms individually, probably need a much longer time of incubation to significantly affect CL properties. However, we could see that, although these CL are hardly degradable in soil, the SiHy CL (the monthly disposable lens) is more prone to degradation/deterioration than the PHEMA CL (daily disposable lens). Given that the prescription of daily disposable CL showed an increase of 16 %, while monthly soft CL a decrease of 2 % from 2015 to 2018 (P. B. Morgan *et al.*, 2016; P. Morgan *et al.*, 2019), the impact on the environment of the daily CL must be a concern they can contribute to the enhancement of microplastics in the environment.

Chapter 6:

Concluding Remarks and Future Perspectives

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6.1 Concluding Remarks

Considering that the expected number of CL users will increase in coming years particularly the daily DCL, our results shows that the presence of CL do not affect the grow of yeasts, bacteria and aquatic hyphomycetes. Although, after 8 months of incubation with aquatic hyphomycetes they have the capacity to turn CL into microplastics. The soil and soil extract experiment highlight for the more resistance of Nesofilcon A to be deteriorate rather than Senofilcon A

In conclusion our results show that if CL ending in the rivers or in the land field should be considered an environmental problem.

6.2 Future Perspectives

A future investigation regarding the impact of CL on the environment and the development of a new valuable product to recycle contact lenses include:

I. Analyze if aquatic hyphomycete colonizes CL in the same way that colonizes leaves and evaluate if CL has an impact in superior trophic levels in rivers, such as shredder;
II. Evaluate what happens to CL in the sludge of wastewater treatment plants;
III. Collect used and out of date CL to create a polymer with added value

Chapter 7:

References

7. References

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