TALLINN UNIVERSITY OF TECHNOLOGY
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Department of sustainable technology

SILVER NANOPARTICLES APPLICABILITY IN STRAW BALE CONSTRUCTION AS ANTIBACTERIAL AND ANTIFUNGAL AGENT

HÕBENANOOSAKESTE ANTIBAKTERIAALSETE NING SEENEVASTASTE OMADUSTE RAKENDATAVUS PÕHUEHITUSES

Master thesis
Environmental engineering focusing on material recycling

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Hereby I declare that this master thesis is my original investigation and achievement. All used materials of other authors, important statements from literature or somewhere else originated data in this thesis are referred.

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ABSTRAKT

Käesoleva rakendusuurimusliku teadustöö autor on Küünal, S., pealkiri on „Hõbenanoosakeste antibakteriaalsete ning seenevastaste omaduste rakendatavus põhuehituses“ ning sellega taotletakse tehnikateaduste magistrikraadi. Magistritöö on kirjutatud Tartus, 2014, koosneb kahest köitest, 69 leheküljest, 3 tabelist, 25 joonisest, 148 viitest ning on koostatud inglise keeles. Käesolev magistritöö on ühes köites.

Käesoleva magistritöö eesmärgiks oli uurida hõbenanoosakeste antibakteriaalset ning seenevastast mõju põhust valmistatud ökoloogilistel ehitusplokkidel. Selleks viidi läbi järgnevad etapid:

1. Koostati kirjanduse ülevaade ökoehituse vajadusest ning ökoloogiliste ehitusmaterjalidega seotud probleemidest. Teostati põhjalik uurimustöö hõbenanoosakeste omaduste ning rakenduste kohta.
2. Viidi läbi hõbenanoosakeste süntees.
3. Sünteesitud hõbenanoosakesed identifitseeriti ning karakteriseeriti nende parameetrid.
4. Eelkatse tarvis kasvatati laboritingimustes põhust pärinevate mikroorganismide kolooniad.
5. Valmistati erineva kontsentratsiooniga hõbenanoosakeste lahused kasutades eelnevalt teada saadud parameetreid.
6. Eelkatse käigus katsetati erinevaid kontsentratsioone kahekümne erineva mikroorganismi peal.
7. Eelkatsest tulenev efektiivseim hõbenanoosakeste lahuse kontsentratsioon valiti välja katsetuseks põhust katsekehadel.
8. Analüüsiti saadud tulemusi ning arutleti meetodi tulususe ning ohutuse üle.

Magistritöö esimene pool koosneb kirjanduse ülevaatest, milles sisalduvad teemad ökoehituse vajadustest, võimalikest probleemidest ökoloogiliste ehitusmaterjalidega, nanorevolutsioonist, hõbenanoosakeste rakendustest erinevates valdkondades ning sellega

kaasnevatest võimalikest ohtudest. Lisaks keskenduti hõbenanoosakeste loodussõbralikematele sünteesi meetoditele ning utiliseerimisvõimalustele.

Tulenevalt töö eesmärgist oli tarvis sünteesida kitsa suurusjaotusega hõbenanoosakesed. Sünteesitud hõbenanoosakesi analüüsiti koostöös Oslo Ülikooliga kasutades TGA, XRD meetodeid ning kõrge resolutsiooniga transmission-elektronmikroskoopi. Leiti, et sünteesi tulemiks olid kerakujulised hõbenanoosakesed keskmise läbimõõduga kolm nanomeetrit. Sellest tulenevalt võis ette valmistada erinevate hõbenanoosakeste kontsentratsiooniga lahuseid, kasutades erinevaid lahusteid (destilleeritud vesi, etanool, metanool).

Esmane katse hõlmas erinevate kontsentratsioonide katsetamist juba varem põhust välja külvatud mikro-organismidel. Mõjutatud said pigem bakterid kui seened, kuna tavaolukorras tagab seenorganismide kitiinist rakukest suurema vastupidavuse välispidistele mõjutustele. Eelkatse tulemused näitasid suurimat efektiivsust 1 $\mu M/dm^3$ kontsentratsiooniga lahusel, sellest tingituna valiti vastav kontsentratsioon antibakteriaalse ja seenevastase toime testimiseks põhuplokkidel. Testimiseks valmistati rukkikõrtest kolm katsekeha. Esimene leotati kraanivees, teine immutati liitri puhta etanooliga ning kolmas liitri etanooli 1 $\mu M/dm^3$ hõbenanoosakeste lahusega. Alkohol lasti ära auruda ning katsekehad asetati välitingimustesse, hoitud otsesest päiksevalgusest ning sademete eest kaitstuna.

Mikroorganismidel lasti areneda 14 päeva ning seejärel tehti otsekülv Petri tasside söötmetele. Pärast nelja päeva arengut 20^oC temperatuuri all inkubaatoris, identifitseeriti mikro-organismide kolooniad kõigil kolmel juhul ning võrreldi tulemusi.

Katse tulemused näitasid hõbenanoosakeste 100%-list tõhusust seenorganismide vastu. Siiski ei suutnud hõbenanoosakeste 1 $\mu M/dm^3$ etanooli lahus mõjuda põhus koloniseerivatele bakteritele. Antud lahuse kehva antibakteriaalset toimet võib seletada sellega, et stressiolukorras, mida looduslik keskkond suure konkurentsi tõttu on, aktiveerivad bakterid kaitsva limakihi. Tekitatud limakihist ei suutnud järelikult antud kontsentratsioonis hõbenanoosakesed end läbi suruda. Eelkatse käigus Petri tassidele isoleeritud bakteritel ei olnud konkureerivaid mikroorganismide kolooniaid, seetõttu kaitsemehhanisme ei rakendatud ja bakterid olid hõbenanoosakeste vastu haavatavamad.

Ühe versioonina võib põhukatsest ilmnenud hõbenanoosakeste suurt tõhusust seente vastu selgitada faktiga, et seentel ei ole võimet end limakapsliga kaitsta. Katsetassis jäi toime nõrgemaks, kuna seentel ei olnud liikidevahelisest konkurentsist tulenevat stressi ning kaitsevõime ei olnud ilmselt häiritud. Samas agressiivses keskkonnas toidu üle konkureerides sai ilmselt hõbenanoosakeste etanooli lahus otsustavaks teguriks, mis lõpuks seente kolooniad hävitas.

Tulenevalt katsest võib väita, et töö käigus sünteesitud hõbenanoosakestel on tõhusad seenevastased omadused ning need sobivad rakendamiseks põhuplokkides. Turul olevate mitmete hallitusvastaste vahenditega võrreldes on hõbenanoosakeste ühe mikromoolise lahuse tootmine tulus. Kõrvalproduktina toodetakse sünteesi käigus ammoniaaki, mis väärtusliku gaasina leiab enamikes laborites kasutust ja muudab antud sünteesi veelgi kasumlikumaks. Võimalikku ohtlikku keskkonnamõju saab vältida oma aja ära elanud hoone korrektse käitlemisega, kui lammutustööde käigus eraldatud põhk tuhastatakse ning hõbenanoosakesed tuhast eraldatakse ning taaskasutatakse. Koostöös Tartu Ülikooli Biomeedikumiga leiti esimeste katsetuste põhjal ka kinnitust antud töö käigus sünteesitud hõbenanoosakeste ohutusele, kui testiti 500 nM/dm^3 kontsentratiooniga lahust inimese embüronaalsetel neerurakkudel.

Antud magistritööst võib kokkvõtteks järeldada, et hõbenanoosakeste ühe mikromooline lahus on sobilik seenevastaseks kasutuseks põhuplokkides. Siiski on tarvis edasisi uuringuid, et leida sobiv hõbenanoosakeste kontsentratsioon, mis mõjuks ka põhuplokkides koloniseerivatele bakteritele. Samuti tuleks võtta kordusproove katsete käigus uuritud põhuplokkidelt, et hinnata hõbenanoosakeste pikaajalist mõju. Kindlasti peaks jätkuma paralleelselt toksilisuse kontrollimine erinevatel kontsentratsioonidel.

Märksõnad: hõbenanoosakesed, sool-geel-meetod, ökoloogilised ehitusmaterjalid, põhuehitus, antibakteriaalsed omadused, seenevastased omadused.

TABLE OF CONTENTS

ABSTRAKT 3

TABLE OF CONTENTS 6

ACRONYMS AND ABBREVIATIONS 8

INTRODUCTION 9

1. LITERATURE OVERVIEW 11

1.1 Sustainable construction 11

1.2 Overview about nanoparticles 14

1.3 Properties and applications of silver nanoparticles 17

1.3.1 Optical properties and applications 18

1.3.2 Electrical properties and applications 18

1.3.3 Antibacterial properties and applications 19

1.4 Toxicity and environmental concern 22

1.5 Silver nanoparticles syntheses 25

1.5.1 Conventional bottom-up syntheses 26

1.5.2 Green synthesis 27

1.6 Utilization of nanoparticles 28

2. THE OBJECTIVES 31

3. MATERIALS AND METHODOLOGY 32

3.1 Research strategy and inclusion criteria 32

3.2 Silver nanoparticle synthesis 32

3.2.1 Materials 32

3.2.2 Methods and apparatus 32

3.3 Silver nanoparticles characterization 32

3.3.1 TGA 33
3.3.2 XRD 33
3.3.3 TEM 33
3.4 Microorganism cultivation 33
3.4.1 Culture conditions 33
3.5 Silver nanoparticle solution preparation 33
3.6 Preliminary test on microorganisms 36
3.7 Test with straw bales 37
4. RESULTS 38
4.1 Yield of the silver nanoparticle synthesis 38
4.2 Silver nanoparticles characterizations 39
4.3 Concentrations of prepared solutions 41
4.4 Effects on microorganisms 42
4.4.1 Preliminary test on microorganisms 42
4.4.2 Test with straw bales 43
5. DISCUSSION 44
5.1 The efficiency of silver nanoparticles against microorganisms 44
5.2 Microorganisms in straw bale walls 45
5.3 The cost of production 48
5.4 The safety of silver nanoparticles 49
SUMMARY 50
ACKNOWLEDGEMENTS 52
REFERENCES 53
APPENDIXES 63

ACRONYMS AND ABBREVIATIONS

1.	Å	The Angstrom (0.1 nm)
2.	ATP	Adenosine triphosphate
3.	CNT	Carbon nanotubes
4.	DNA	Deoxyribonucleic acid
5.	ECA	Electronically conductive adhesive
6.	FCC	Face-centred cubic lattice
7.	HEK	Human embryo kidney cells
8.	HRTEM	High-resolution transmission electron microscopy
9.	nm	Nanometre
10.	nM	Nanomole
11.	NNI	US National Nanotechnology Initiative
12.	NSF	National Science Foundation
13.	PAH	Polycyclic aromatic hydrocarbons
14.	RH	Relative humidity
15.	RNA	Ribonucleic acid
16.	ROS	Reactive oxidant species
17.	SERS	Surface Enhanced Raman Scattering
18.	SPR	Surface plasmon resonance
19.	TGA	Thermogravimetric or thermal gravimetric analysis
20.	TEM	Transmission electron microscopy
21.	USA	United States of America
22.	XRD	X-ray diffraction

INTRODUCTION

Nanotechnology consists of the chemical, physical, biological and engineering sciences at the level of single atoms and molecules. Scope of nanotechnology research has grown over twenty years extensively and the need for nanomaterials will increase as miniaturization becomes more essential in various technological fields. The described tendency is illustrated by fact that national funding in the worldwide annual total public and private sector funding for nanotechnologies is about $13-14 billion [1]. For example, the United States of America (USA), with its $2 billion per year, the funding is second to only the space program investments [2, 3].

Global production of inorganic nanoparticles has increased exponentially. At the nanoscale, materials present new properties that can be used in a broad range of applications. Health sector and life sciences account for 18% of applications and 12% of all applications are formed by chemicals. In addition to latter two, the energy, communication and information technologies, transportation, and environmental applications, account for approximately 8-9% each. Finally construction, household products, defence and security, aerospace industry, personal care, food industry and textiles represent 1-6% of all applications [4]. Furthermore, it is estimated that by current year the proportion of products on the global market that have some kind of nanotechnology incorporated into their manufacturing process exceeds 15% mark [5].

Since nanotechnology growth is global trend, it will resolve many current and future obstacles, also in civil engineering and construction field. Construction field has always been, is now and probably will be essential part of technology research and development. The estimated earth's energy consumption of the building industry reaches up to 40%. Preceding segment includes operations from material extraction to building construction. For example, North America is known to be major world energy consumer and one third of its energy usage goes for housing [6]. Thus more energy efficient building technologies should be preferred and looked for. One option to make present field more sustainable is to concentrate on ecological building materials.

Although modern world appears to have distanced itself from “green” building materials, still about two-thirds of the world population lives in the buildings, which are constructed with non-industrial materials such as bamboo or earth [7]. If ecological materials are chosen to construct, it has the potential to reduce construction life cycle energy use and therefore the environmental impact of the building. Sustainable construction materials are for example straw bale, earth and clay and so forth [8]. For example straw is waste product, which decomposes poorly and its use avoids burning in the field or disposal in landfills. One the one hand advantages are its high sound and thermal insulation properties and being inexpensive locally sourced material. On the other hand the environment in the ecological materials is often suitable for many living organisms and possibility of mould development and presence of other vermin such as insects in the walls is probable [7]. Microorganism proliferation may bring inconvenience and discomfort causing many diseases [9].

Therefore straw should be treated with agents, which limit the spreading of harmful organisms. However if ecological material is treated with some kind of chemical, the environmental gain could be diminished. Furthermore chemicals tend to degrade or modify over time. Compared with other metal nanoparticles, silver exhibits higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cells [10]. Therefore silver nanoparticles as antibacterial and antifungal agents could be used to solve this matter appropriately.

The structure of the present thesis consists firstly of literature overview about sustainable building materials and silver nanoparticles. For information gathering the search from the different databases was conducted. To our knowledge this is a novel approach for application of silver nanoparticles, which is presented in current thesis and no similar research has been made. Secondly experimental part is presented, where silver nanoparticles synthesis, microorganism cultivation, tests methodology is described. Thirdly the results are analysed and present findings are compared with previous work in discussion part.

Following investigations will estimate the possibility of using silver nanoparticles as an antimicrobial and antifungal treatment for straw block implemented in green housing construction, instead of conventional available chemical treatments.

1. LITERATURE OVERVIEW

1.1 Sustainable construction

To reduce the proportion of construction field energy consumption, several steps can be set up towards it. One possible approach of the problem is focusing on sustainable building materials. By definition the sustainable building material is harvested, produced or manipulated to be a usable building with a way that has no negative impact on future generations during the material's life cycle and disposal [6].

"Green buildings" consist of these sustainable materials such as straw bale, cob, adobe, rammed earth, compressed earth block. The material choice should be based on the location of intended building. Material should be in the vicinity of construction and readily available. For example straw is basically a waste material from grain harvest and earthen construction takes material from available soils near the building [6].

During the harvest of various cereal (e.g. rye, wheat, barley, or rice), the heads of the grain are harvested, and the remaining stalks are left in the field to dry. Straw can be used for different agricultural and insulation uses, such as winter protection and livestock bedding. Usually there is an excess of straw. Straw as a waste product is mostly burned or cleaned from the field and utilized. Even though straw is a waste problem with its slow decomposition properties, the poor degradation in nature [11, 12] generates other opportunities, such as an option for being used as sustainable building material. The origin of straw bale construction is linked with Nebraska area in the USA in the late 1800's [8] and nowadays it is becoming once again popular. Dry cereal grain stalks are collected and made into compressed bales, which can be applied for construction as building blocks. This can reduce ecological impact, because only in China 90 million tons of straw is annually produced of which large amount is burned in the fields thereby inducing air pollution and participating to the global warming [12].

Straw is similar to wood in terms of structure. Likewise, it is considered as a natural composite consisting of polysaccharides (cellulose and hemicellulose) and lignin [13].

Hence, making building blocks out of straw appears to be a promising alternative. Bale size varies and depends on the packing density; one cubic metre usually can weigh from 70 to 130 kg. For illustrating the dimensions, most commonly used bales are approximately 381×584×1220 mm (15×23×48 inches) and accordingly with approximate volume of 0.27 m^3 [6].

Besides availability, straw has excellent sound insulation [14] and thermal insulation properties. Furthermore, the production of straw is a low energy process compared with other building materials [15]. However, there are also some risks involved with straw bale buildings. These include fire, relatively low compressive and flexural strength, aerobic or anaerobic decay. Also straws contain nutrients which can sustain life (e.g. rodents, insects, microorganisms), which can lower the living standards [16].

If conditions are kept in accordance of requirements, life inside straw bales could be prevented. Although, mould growth can occur in extreme conditions such as -5C^o, with a relative humidity (RH) level as low as 62%, it is generally not the case. Predominately, the optimum ranges of development are from +20^{o}C to +28^{o}C, and at RH levels of 95% and above [17]. In addition to humidity and temperature, two other factors can affect the rate of development of microorganisms and thereby the rate of straw decomposition. These two other important factors are nutrients contained in the straw and the amount of available oxygen. 15% of straw bale humidity mass content seems to be safe [18], which corresponds to 70% of RH. Over that, moulds begin to grow; yeasts usually need 80% RH and bacteria above 90% RH. Studies have shown that straw can withstand relatively high RH% and usually near 100% of RH is needed for complete degradation. Still the damage inside wall will occur before total deterioration and therefore the humidity should be kept as low as possible [19].

As described, home dampness may lead to proliferation of microorganisms, which can affect the construction and indoor air quality. Taken into account, that nowadays more than 80% of time is spent in an indoor environment [20], the air quality is of crucial importance. Spending long periods in poor air conditions, where airborne contaminants are present, can cause “The sick building syndrome” [21]. Microbial activities, which deteriorate the straw, release toxins that can affect the health of the persons, who are living in the house. In fact, the endotoxins coming from the cell wall of Gram-negative bacteria may cause severe

respiratory disorders [22]. Furthermore, mould growth associated with cellulose based materials and dampness causes symptoms, such as allergies, upper respiratory complaints, headache, eye irritation, epistaxis, nasal and sinus congestion, cough, "cold and flu" symptoms, as well as gastrointestinal problems [9, 23]. Naturally, chemicals applied against microorganisms can also be dangerous to environment and affect in the same way the inhabitant, therefore alternative solution against the sick building syndrome may be the use of nanomaterials.

The implementation of nanomaterials in construction industry is not rare. However, civil engineering being considerably traditional field is yet to discover the possibilities of nanotechnology. Currently, among worldwide nanotechnological applications construction field form over one per cent [4]. Properties, such as corrosion resistance, high chemical reactivity, durability and strength, are of particular interest to civil engineers. For example nano-reinforcement of construction elements is applied to reduce the need for steel [24]. Carbon nanotube (CNT) composite reinforced structures can increase the tensile stress value 50 to 150 fold compared with traditional steel-reinforced structures [25]. Furthermore CNT have a hardness equivalent to a diamond and can be additive in concrete to improve crack resistance. However, the cost of production of CNT remains quite high, limiting the possibility of applications [24].

Long-lasting properties of materials are essential for construction. Thus nanostructures are added to prevent deterioration under ambient conditions. Relatively small amounts of nanoparticles in materials or coatings can have a large effect on surface properties, such as resistance to wear, corrosion, prevention of condensation, mould and microbes, due to the high surface to volume ratio of these nanostructures. As stated before, one substantial application for nanoparticles is to keep environmentally friendly materials (e.g. wood, straw blocks, reed, flax, straw-clay mixture, etc.) free from all kinds of bacteria, fungi, viruses, algae and vermin [26].

One example, how inorganic nanoparticles can be used as antimicrobial agent, is the semiconductor titanium dioxide (TiO_2). This compound is known for its photocatalytic properties under UV light. After absorbing radiation, electrons have more energy to promote themselves into conduction band. Both electrons and formed electron holes move to the surface and produce reactive oxygen species such as superoxide anions, hydroxyl

radicals. These species damage cell membrane of bacteria and fungi and are the main reason of using TiO_2 nanoparticles for the prevention of the fungal damage in materials. For example it is efficient against fungi with the fastest destructive impact to wood (*Hypocrea lixii*, *Mucor circinelloides*). In the experiments the nano-treatment kept 100% of wood area safe after 50 days of contact. Fungi failed even to grow in the surrounding culture medium, because there is always some diffusion of particles. For the aesthetic point of view the treatment did not change even the appearance of materials [26]. However, antimicrobial photocatalysts, such as TiO_2, need regular radiation have an effect. Therefore, absence of UV radiation in terms of sunlight, limits the applicability indoors or inside constructions.

1.2 **Overview about nanoparticles**

The origin of the term nano is from the Greek word *νάνος* (nanos), which means dwarf. When it is used as a prefix, it describes a fraction of metric unit times 10^{-9}**.** As described nanometre (nm) is one billionth of a metre, illustratively seven silver atoms side by side. To give a comparison the DNA molecule is 2.5 nm wide, a protein approximately 50 nm, flu virus about 100 nm, bacteria and red blood cells about 2.5 μm (2500 nm) and human hair exceeding the 10 μm of diameter [27]. In general, material (nanoparticles, nanotubes, nanorods, nanoplatelets, etc.) is considered as nanomaterial, when at least one of the dimensions is less than 100 nm. Consequently nanostructures can be classified according to the hierarchical order of dimensionality. First classification group is zero-dimensional systems, which have all dimensions in the nanoscale, (e.g. ceramic nanopowders, nanoclusters or nanoparticles). Secondly there are one-dimensional systems, where one dimension is out of the nanoscale, such as nanofibres, nanowires, nanorods and hollow nanotubes. Thirdly two-dimensional systems have only one dimension in nanoscale and others are extended in the other two directions. Examples of these materials are nanodiscs, nanoprisms or layers such as nanofilms and nanomembranes [28]. Then there are three-dimensional nanosystems such as nanomaterials with improved features for example bulk material with nano-porous structure [29].

Zero-dimensional nanoparticles are of great scientific interest as they cover the gap between bulk materials and atomic or molecular structures. More specifically, the range of 1 to 20 nm is the bridge between small molecules with discrete energy states and bulk materials with continuous energy states [30]. When in nanoscale, the properties (electric, catalytic, magnetic, etc.) of the bulk material will be modified, and these properties are highly dependent on the size of the particle. Several common bulk materials have been found to have unconventional properties, when studied in the nanoscale. Comparison may be roughly drawn between behaviour of water molecule or a big lake filled with water. Difference in the processes which take place is vast. Subsequently reasons for interesting properties lie in the facts that nanoparticles possess a very high volume to surface aspect ratio, which affects their properties and leads to a higher reactivity. Furthermore, there is phenomenon called quantum effect, which begin to affect the behaviour of matter at the lower end of the nanoscale. The smaller the particle is the higher proportion of atoms it has on the surface of the material. For example 30 nm particles have 5%, 10 nm particles 20% and 3 nm particles 50% of its atoms on its surface. Hence surface effects, rather than bulk material properties, prevail [31].

Roots of nanotechnology lead back to 9^{th} Century, where Mesopotamian artisans added noble metal nanoparticles to glass and ceramics. Consequently the metallic glittering and light scattering of gold and silver nanoparticles have attracted the minds of people since the medieval times. The scientific explanation of optical effects induced by the noble metals was not really understood before the 19^{th} Century. In 1857, Michael Faraday published "Experimental relations of gold (and other metals) to light". Hypothesis was made that for example ruby colour of gold in colloidal solution was a result of the particle size approaching to the wavelength of light. However, necessary technology to prove that hypothesis was not yet available and was only proven to be correct later. The first concepts of engineering possibilities in nanoscale emerged in 1959, when ideas about machines built with atomic precision were introduced. It gradually started the development of novel approaches at the nanoscale in the scientific world [32].

Although nano-revolution started with the paper "An approach to the development of general capabilities of molecular manipulation" in the early 1980's, the breakthrough in the medicine field already started over a decade earlier in the end of 1960's. After some years of research and investments, the first nanoparticles for drug delivery were developed [32].

Certain metal nanoparticles have useful properties in that area. When reduced to the nanoscale, some magnetic materials exhibit a magnetic property named superparamagnetism. For example, decreasing the size of iron oxide to a few nanometres, the nanoparticle is reduced to single magnetic domain and then exhibits superparamagnetic property. In contrast to multiple domain ferromagnetic materials that retain their magnetism even after the removal of the magnetic field, superparamagnetic nanoparticles lose their magnetization and become highly dispersed when the magnetic field is switched off [33]. As mentioned, for clinical applications, this feature is important, because metallic nanoparticles tend to aggregate due to their ferromagnetic properties, which complicates biomedical applicability. Dispersion of superparamagnetic nanoparticles makes the foreign particle removal for the immune system easier [34].

Also advancements in transmission electron microscopy, atomic force microscopy and dynamic light scattering, made the increase in nanotechnology explode. At his very moment the nanotechnology is considered as the future to all technologies [33].

Bright future is also illustrated in the funding tendencies. Currently worldwide annual total public and private sector funding for nanotechnologies is about $13 to 14 billion. There are 1317 nanoproducts produced by 587 companies located in 30 countries [1]. Furthermore the contribution of nanotechnology to the global economy is expected to grow $3.1 trillion by the year 2015 [35]. Also, the National Science Foundation (NSF) estimated that at least two million workers will be employed in nanotechnology field by the aforementioned year [36].

However research in nanotechnology is rapidly increasing all over the globe, USA is still leading the worldwide development of nanotechnology with $18 billion. The sum has been appropriated by the Congress over the last decade and increasing, estimated annual funding from private sector with $3.5 billion [37]. Considering the aforementioned facts, USA priorities are important. The US government budget supplement for 2014 fiscal year provides $1.7 billion to the US National Nanotechnology Initiative (NNI) [3]. The NNI published a 2014 edition of its strategic plan. The latter describes vision, goals and strategies. Four goals remain the same [38]:

1. Advance a world-class nanotechnology research and development program.

2. Foster the transfer of new technologies into products for commercial and public benefit.
3. Develop and sustain educational resources, a skilled workface, and a dynamic infrastructure and toolset to advance nanotechnology.
4. Support responsible development of nanotechnology.

The NNI has 20 federal agencies and departments and the strategic plan emphasizes interagency activities in particular thematic areas; these are the Nanotechnology Signature Initiatives such as solar energy; sustainable manufacturing; next-generation electronics; informatics; sensors. These are national priority collaborations to accelerate the development in the field [38].

1.3 **Properties and applications of silver nanoparticles**

In general metal based nanoparticles have possible applications in many areas such as optical engineering [39], electrical engineering [40], medical theranostics [33, 34], environmental engineering [41] antimicrobial coating [42], etc. Commonly, metal nanoparticles have a wavelength below the critical wavelength of light, which is due to their dimensions. Therefore metal nanoparticles appear transparent and could be used in many applications especially in coatings [27]. Coating development is one of the key directions for research in metal nanotechnology [42]. The fact that metal nanoparticles can be induced to merge into a solid at relatively low temperature, often due to small size without melting, is contributing element to improvement of the coatings for different applications [43, 44].

Noble metals appear to be the most promising metal nanoparticles due to their unique properties. Since the first encounters to mankind, gold and silver have been associated with the prosperity of civilizations. The bulk scale noble metals show minimal reactivity, which explains their noted use in jewellery [45]. However the nanodimensional silver and gold reveal distinctive properties from the bulk form [46, 47].

The significance of silver nanoparticles is illustrated by the fact that these are one of the most produced materials in nanoindustry. Silver production is close to 10 000 tonnes

annually and silver nanoparticles represent approximately 5% of it, which corresponds to 500 tonnes a year [48]. Furthermore, silver is found to be second most referenced nanomaterial after carbon in the scientific literature, making it the leader for commercial applications among all metals [49]. The cause of this interest in silver nanoparticles is related to their unusual optical, electronic, and chemical properties, which are highly dependent on their size, shape, composition, crystallinity, and structure [50].

1.3.1 **Optical properties and applications**

Silver, similar to few other noble metals, has useful optical properties, which have attracted the extensive research interest. Light interacts intensively with the free electrons in silver nanostructure and this leads to a collective excitation of the conduct electrons. As a result, the electromagnetic field on the surface of the material is also enhanced leading to an increase of the down-falling and the scattered light intensity. This phenomenon is called surface plasmon resonance [51]. Since silver is the only material whose plasmon resonance can be tuned to any wavelength in the visible spectrum, the material has universal optical applications. Furthermore, the optical excitation of plasmon resonances in silver nanoparticles is the most efficient mechanism known by which light interacts with matter. Subsequently the light interaction cross-section compared to geometric cross-section can be up to ten times greater, which means the silver nanoparticles capture much more light than is physically falling to them [43].

Aforementioned properties are essential for many measuring and biosensor applications. For example high-resolution microscopes are constructed to use short wavelength plasmons with amplifying high energy [52]. Moreover silver, can enhance the Raman scattering 10^6-10^{15} fold, which leads to very sensitive analytical detection down to single molecule levels [53].

1.3.2 **Electrical properties and applications**

Silver nanoparticles demonstrate an ability to improve the optical absorption and thus, to increase electric field in the photoactive layer. Silver nanoparticles are therefore used in photovoltaic applications [54]. The potential of silver nanoparticles in that field has led to the development of solar cells and coatings using silver nanoparticles for solar energy absorption [55, 56]. Solar cells have been demonstrated as a credible alternative to

conventional energy production. Solar cells represent a clean and renewable method contributing to more sustainable energy management [54].

Silver nanoparticles are used in electronics for their electric conductivity. Silver is among single metals the one that present the lowest electrical resistivity and differing from other metals because its oxide form is in fact also conductive. These properties are main reason why silver as conductive filler has been replacing hazardous tin-lead conductive adhesive materials in electronic connects [57, 58]. Furthermore, silver nanoparticles melting point can be significantly reduced due to high surface energy of the particles. This contributes to the easy fabrication of micro-interconnections using for example ink-jet printing of silver nanoparticle suspensions and taking advantage of low sintering temperatures [59].

Other electronic applications of silver nanoparticles include semiconductors [60], single-electron transistors [39], high-density data storage devices [40, 61], optical wireless interconnections [62], etc.

1.3.3 **Antibacterial properties and applications**

For thousands of years, silver has been used for wounds, burns and water treatment. However, in the 1940's the penicillin was introduced and the use of silver for the treatment of bacterial infections minimized [63, 64]. Still nowadays it is not unusual to find silver used as antibacterial agent in burn wounds bandages too. Silver is also used in dental work [65]. Moreover, with the development of antibiotic resistance among the common pathogens, it is necessary to develop a new generation of antibacterial agents [66]. Since, silver is reported to be antibacterial to over 150 different pathogens [30], silver appears as the most appropriate candidates to fill with its unique antibacterial properties once again the need in medical field [67].

Compared to all antimicrobial nanomaterials, silver nanoparticles are the most widely implemented. Silver nanoparticles can be found in over 100 consumer products with great variety [68, 69]. Examples of disinfectant applications are household agents such as antibacterial nanopaint [70], household devices such as washing machines and refrigerators coated with silver [71], and different silver coated medical devices [41, 72] or photoactive disinfectants [73, 74].

In the modern world, one essential antibacterial application is for the purification of groundwater, where silver nanoparticles can be used as water purification agents [75]. Moreover, for wastewater treatment iron(II,III)oxides (Fe_3O_4) combined silver nanoparticles are already used. These nanoparticles can be easily removed after using magnetic field to avoid contamination of environment [76]. Another way for water purification is to use polyurethane (PU) foam coated with silver nanoparticles. Particles inserted in PU are stable and cannot be washed away even after extended period of using. That material can also be developed to be used for an antibacterial packing or air filtration [77]. In addition to PU foam, activated carbon fibres are also coated with silver nanoparticles and used as air filters. These have efficient removal of hazardous gaseous pollutants and therefore have potential for large scale production [78].

Wide spread of silver nanoparticles as an antimicrobial agent is due to its high efficacy. Concentrations of 35 ppb of silver itself have been reported to already show a bactericidal effect. This corresponds to 35 micrograms per kilogram [79]. Silver nanoparticles take it a step further, whereas as little as one gram of silver nanoparticles may change hundreds of square metres of surface to antibacterial area [27]. Still, one fact that has to be kept in mind is the silver nanoparticles behaviour in liquids. In fact, their low colloidal stability induces aggregation and sedimentation in solutions. Therefore if solution is not stabilized, these unique biocidal effects can suffer from this problem [46].

Nanoparticles have large surface area to volume ratio, which gives its high reactivity. The antimicrobial effect is related to the size of the silver nanoparticles. The smaller nanoparticles present higher antimicrobial activity due to the increasing release of silver ions into the solution and more efficient contact with pathogens [67]. The bactericidal effect is stated to be highest for nanoparticles with diameter in the 1 to 10 nm range [80]. Subsequently, under 5 nm of diameter, if the nanoparticles are uncharged, these present the ability of entering into the bacterial cells easily. Within that range they are sufficiently small to pass through transmembrane pores for transport across cell membranes [81]. Also the scope of that size has the best interaction with HIV including HIV-1 preventing the virus biding to the host cells, implying that the attachment to the viruses is also size-dependent [82, 83].

One of the antibacterial effects is induced by silver the presence of ions. The bonding of silver ions to tissue proteins induces structural changes in the bacterial cell wall and nuclear membrane when released [80, 84]. The dismantling of the respiratory chain of bacteria leads to cell distortion and death [85, 86]. Silver can also bind to bacterial DNA and RNA, thus denaturing and inhibiting bacterial replication [84, 87]. In addition to Ag ion shedding the nanoparticles themselves may impact the bacterial cells. Subsequently, membrane permeability, potential and energy transport (ATP) levels are affected due to expression of crystal defects, which increases the surface reactivity [88, 89].

Comparing one to another, silver nanoparticles and dissolved silver ions may have 1000 fold difference between their antibacterial efficiency. In the case of silver nanoparticles, these already have an antibacterial effect in nanomole range, the latter needs a concentration in micromole range [90].

Biocidal effect is still greater when ambient conditions support the silver ion generation. However, it was stated that in anaerobic conditions, where ions could not be generated, no measurable effect on examined bacteria, compared to similar conditions with ions, was noticed [91]. Consequently, particles themselves do not always affect biological activity of the microbes and toxicity may be dependent on the presence of air. It is predominately generalized that antibacterial properties of silver nanoparticles are defined by parameters such as size, shape, surface coating and charge. However, all the aforementioned are factors which affect the ion release rate, extent, location and timing, thereby are responsible for toxicity [92].

Increasing market for applications of nanotechnology in biomedical science, electronics, cosmetics and pharmaceutical industry has already exceeded 60 000 tons of engineered nanoparticles in a year. Such increase has potential also to raise the release of nanoparticles into environment [50]. Even though metallic silver has antibacterial and -fungal properties, it is been viewed to be a minimal health risk and relatively non-toxic to mammalian cells. Nonetheless, it should be taken into account that in nanoscale many materials show significant increase of toxicity compared to bulk size material and as silver nanoparticles are toxic to certain bacteria and fungi, they could still be toxic also to the whole environment, human body included [93, 94].

1.4 Toxicity and environmental concern

In the 1970's was the peak of silver levels released in the environment. It was predominantly induced by photographic industry. The estimated amount was around 2.5 million kilograms of silver. Soon after that, silver use in the photographic industry was declined and concentrations have decreased ever since [95]. Due to the commercialised nanodimensional silver products that have spread to everyday life within the last decade, toxicity problems of silver and especially nanoparticles have risen on the agenda [50].

Aforementioned specific antibacterial properties of silver nanoparticles may have a flipside. This characteristic, which is useful in many fields, turns problematic for the environment. Despite very efficient bacterial membrane disrupting ability of silver nanoparticles, these are also causing production of free radicals via accepting easily electrons. Reactive oxidant species (ROS) are being produced by these free radicals. ROS compounds lead to oxidative stress and then damage DNA and other proteins [96, 97]. Consequently, the ROS are one of the main reasons for the toxicity of silver nanoparticles. This phenomenon can unfortunately have a supporting role for the growth of some bacteria [82].

Engineered nanoparticles become a toxicity concern with diameters lower than 30 nm. In that range many particles have dramatic crystalline changes in the structure and therefore unique properties will prevail [44]. The crystalline defects occurring on the surface of the nanoparticles are the fundamental cause of the ROS when in contact with biomolecules [88] and also smaller the particle, more ions it will release due to the higher reactivity of the surfaces [80].

Ultra-fine particles produced by combustion have been a problem for a long time due to industrial revolution, spreading air pollution. Black carbon, which can also be nanoscale, may carry very toxic, often carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAH) [90]. In addition to seemingly imminent atmospheric pollution, nanodimensional silver also draws intention to some potential effects on health. In fact, its daily use in healthcare and hygiene spray products should be carefully controlled. Particles of diameter lower than 2.5 μm can get through breathing down to the alveoli.

Commercialized nanoparticles are way under 2.5 μm as nanoparticles are by definition between 1 and 100 nm in size. Therefore, health risk using silver nanoparticles is a reality [68]. Also the oxidative stress induced by silver nanoparticles due their catalytic activity, could lead to cancer or other diseases and complete toxicity study is therefore necessary [98]. Furthermore, possibility of inhaled nanoparticles entering the brain through translocation via nose nerves is worrying fact favouring the penetration across blood brain barrier [99, 100].

Obviously, the respiration is only one way nanoparticles to enter an organism. Skin for example plays major role providing protection to the underlying organs. Even though evidence shows that nanoparticles can penetrate into human hair follicle upper regions or outer layer of skin. Nonetheless, the penetration of the barrier of intact skin without chemical enhancers, electromagnetic field or skin treatment has not been reported yet [101, 102].

Also the gastrointestinal tract is a gateway to the organism. Particles which are over 2.5 μm cannot reach alveoli, however these particles can easily go through gastrointestinal way. Liver is the first checkpoint before spreading in the bloodstream, and toxicity against liver has been reported in vitro [103, 104].

As described many ways exist to penetrate the human body. However the buffering capacity of organism should not be underestimated. For example due to daily oral incorporation people may have some silver in the bloodstream. The concentration of 200 μg in one kilogram, depending on the form of the silver in the bloodstream, is considered as normal [105]. Also if silver ion is bounded, it is no more active [106]. However, the quantity of silver ions exceeding over 300 ppb (300 μg per kg) in the bloodstream damage the liver and kidney and also form of leukopenia has been reported [107, 108]. Consequently, the harmful potential concentration differs very little from the harmless and may be different for everyone.

In contrast with human body, there are more primitive organisms that can tolerate a lot less silver contamination in the environment. For example, increasing the amount of silver nanoparticles can also interfere with our modern waste water treatment systems. In fact, essential nitrifying microorganisms involved with waste water treatment are extremely

sensitive to silver nanoparticles. As the amount of silver grows, biological nutrient removal from the waste water with conventional methods could suffer [109]. The sensitivity to silver compounds of different bacteria is common phenomenon. In addition to bacteria, free silver ions toxicity is reported against a wide variety of organisms; planktonic species such as algae, [110] zooplankton [111] and fish [112].

It has already been pointed out that size is one of the most important factors when getting in contact with nanoparticles. In addition to the aforementioned, for example, laboratory rats became extremely affected with 15 nm diameter silver nanoparticles that were found to present the highest toxic response on alveolar macrophages in comparison to larger nanoparticles (i.e. 30 to 55 nm) [113]. Yet the shape difference appears to be a significant factor in terms of toxicity. Silver nanoparticles have variety of shapes which behave differently in interaction with various organisms. For example, triangular silver nanoparticles are stated to have stronger biocidal action against Gram-negative bacterium *Escherichia coli* than spherical or rod-shaped nanoparticles. This could be mainly attributed to the arrangement of atoms in the crystalline structure present on the surface of the nanoparticle. Recent studies showed that 1 μg of triangular nanoparticles had similar effect with twelve-fold amount of spherical particles and 50 to 100 times more rod-shaped particles are necessary to get the similar result [114].

Furthermore, it was observed that silver nanoplatelets are more toxic compared to some other shapes. The comparison of the toxicity between nanowires and spheres to nanoplatelets showed that the latter were several times more toxic to zebrafish embryos [88].

The comparison of the two mechanisms, in terms of toxicity, showed different result among the data published in the scientific literature. It is presently stated that there is no reason to consider silver nanoparticles more dangerous to ecosystems than silver ions. Therefore the environmental risks do not exceed risks related to contamination by soluble silver salts [115, 116]. According to the data presently known, it is even considered that ionic silver can be around 10 000 times more toxic than nanoparticles in water. Therefore, it appears that the release of silver in the environment in form of nanoparticles may be less relevant compared to the release of ionic silver coming from nanoparticles [48]. Furthermore, silver nano-compounds are poorly water soluble and therefore silver ions

released into surroundings should be in low concentrations **[**117].

To point out more comparison, organisms that are living in the salty water are more likely to bioaccumulate silver under equivalently contaminated conditions than the ones living in fresh water. Difference between freshwater organisms and marine organisms is due to chloro-complex concentrations, which let the silver ions be free [118].

In contrast with previous statements, silver nanoparticles do appear being significantly more toxic than silver ions towards *E. Coli* bacteria [119]. The fact that fish have shown difference in uptake of silver compounds from the aquatic environment indicates that the impact of silver nanoparticles may differ a lot compared to silver ions. For example, one study shows that silver nanoparticles aggregates were incorporated into blood vessels, skin, brain, heart and yolk, whereas silver ions concentrated in organelles, the nucleus and the yolk only [112]. To sum it up, it can be implied that silver nanoparticles have stronger impact for suppressing autotrophic organisms (e.g. some bacteria), whereas silver ions affect more heterotrophic species [109].

Toxicity aspect of current work should not be excluded. Although, there is still room for debate about correlation between ion release and actual damage, all this depends on the given organisms. It could be said that toxicological effects are rather caused by crystalline defects of the silver nanoparticles. As spherical particles, which are the present shape of the nanoparticles used in research work, do not have so many defects in the structure than other shapes, it is therefore expected that the toxicity of these nanoparticles should not be a critical problem [88]. However, the study of their toxicity is presently investigated in collaboration with the Biomedicum of the University of Tartu.

1.5 **Silver nanoparticles syntheses**

A wide range of applications in aforementioned fields depend on the ability to synthesize particles with exact chemical composition, shape, size, and monodispersity [46]. Usually those features are major reasons for the choice of the method of synthesis. Therefore, the great majority of synthesis processes are not environmentally friendly, with only 24% of the reported methods relying on “green” techniques. The reason of this fact lies in the high

controllability of non-green methods that provide monodisperse silver nanoparticles with peculiar characteristics [120]. Since harmfulness appear to be an inevitable phenomenon accompanying nanoparticles, syntheses methods could be chosen considering the safety and environmental impact.

1.5.1 **Conventional bottom-up syntheses**

In recent years many methods of silver nanoparticles synthesis have been developed. These methods include chemical and photoinduced reduction, template, electrochemical methods, microwave-assisted synthesis [83], and so forth [121]. Top-down syntheses, where particles are created from the bulk material, may cause the surface imperfection and are not considered as the most reliable and this leads to the decrease of particles applicability. Therefore, bottom-up syntheses, where nanoparticles are formed from the smaller structural units, are more commonly used and preferred.

In the conventional bottom-up synthesis, the first step consists of choosing the metal salt precursors. In the silver nanoparticles production, the most widely used silver salt is silver nitrate ($AgNO_3$). That particular precursor accounts for over 80% of all synthesis generated. Low cost and chemical stability are the properties responsible for that interest. Solvents can be organic or inorganic, but over 80% of synthesis use water as main solvents. In a third step a reducing agent is necessary. This reducing agent could be chemical, biological, plant extract or irradiation method that provides the free electrons needed to reduce silver ions and to form silver nanoparticles. The principle is, that stronger reducing agents such as sodium borohydride ($NaBH_4$) produce narrow range of small monodispersed nanoparticles, whereas weaker agents, such as ascorbic acid, produces larger particles with larger size distribution. Another important component is the stabilizing agent or capping agent, which is used in the synthesis process to prevent nanoparticles from aggregation and to control the final size of the nanoparticle. Stabilizing agents have electrostatic repulsion force coming from surface charge or steric properties, which keep nanoparticles apart from each other. Sodium citrate ($C_6H_5Na_3O_7$) is one of the most commonly used electrostatic capping agents and is present in almost 30% of all syntheses. The choice and concentration of the stabilizing agent affects greatly the nanoparticle properties [89].

1.5.2 **Green synthesis**

Due to the growing demand researchers are looking for synthesis methods which are at the same time inexpensive, rapid, safe, with narrow size distribution, morphology and more particularly environmentally friendly. Therefore, emphasis is now being placed on greener methods in which toxic reducing agents, such as potassium bitartrate and sodium borohydride, are replaced with agents that do not produce any hazardous by-products. The present investigations focus on microorganisms and plants.

The three main concepts in the preparation of the nanoparticles using green approach are the choice of the solvent medium, preferably water, an environmentally friendly reducing agent and the nontoxic material for the stabilization of the nanoparticles [122]. Furthermore, the synthesis processes should occur near ambient temperature and pressures and a pH close to neutral. Therefore, the biological systems appear to be the most suitable for natural chemistry conditions. Hence, it is reasonable to use biological approaches to produce inorganic materials in low impact mode.

Currently there are three main directions of investigation based biological synthesis method for producing silver nanoparticles. Firstly microorganisms, such as different silver-resistant bacteria that are able to bioaccumulate silver from the mediums inside their body, were studied. One example is bacterial strain *Pseudomonas stutzeri* AG259 that can accumulate ions from the soil and is able to release nanoparticles down to 200 nm from their cell [123].

Secondly, methods based on fungi nanofactories were investigated due to their high binding capacity and intracellular uptake. Compared to bacteria, they are easier to handle in the laboratory for production, needing less sophisticated equipment for getting filtrates from the colloidal broths [124, 125]. Certain fungi secrete large amounts of enzymes that are involved in reducing process of silver ions [126]. Often the low speed of synthesis makes it non-profitable compared to the other methods. However, one of the most common moulds *Aspergillus fumigatus* has been reported to synthesize silver nanoparticles as fast as a matter of minutes [127]. This demonstrates the potential of fungi for large scale silver nanoparticle production.

The third possibility is using the plants that provide large variety of species, which can help produce silver nanoparticles. Living plant systems themselves [128] as well as extracts from different plant parts [129, 130] can contribute to greener synthesis methods. Plants consist of biomolecules that act both as reducing and capping agents, helping to form more stable and shape-controlled nanoparticles. Some examples of the compounds involved are polysaccharides, alkaloids, phenolics, different proteins, and so forth [131].

Greener syntheses are still under investigation to reveal their potential. Every year several new organisms and methods are described and published. Choosing among the three main directions is dependent on the expectations. For example microorganisms can be easily manipulated genetically to get certain characteristics. However, large-scale production is still problematic using bacteria. In addition, the control of the synthesis is also more difficult [126]. Moving from prokaryotes to eukaryotes, fungi represent many advantages. They are easier to handle, have high binding capacity and their secretion of enzymes is intensive [125]. However, the drawback relates to the fact that eukaryotes are not that well genetically manipulated than simpler organisms [27]. Finally plants present many advantages over the two other categories; they are readily available, well explored and need no certain cultivation and preparation [132]. Furthermore, implementing plants is safer because no potential pathogens can be found among them. Also richness of some certain biomolecules makes the syntheses faster, which is an important aspect if we consider larger scale production [131].

1.6 **Utilization of nanoparticles**

The effect of nanoparticles on the environment is still not yet fully understood. It is then important to take some precautions, when manipulating them or releasing into the environment. Many studies have shown the toxicity aspect of nanoparticles and thus releasing them into the environment should be avoided if possible. The European Union chemical law; Regulation Evaluation, Authorization and restriction of chemical substances (REACH), has stated that the afterlife of produced nanomaterial remains the responsibility of the manufacturer himself.

Since basically no regulations and little information is available on how to handle discarded nanomaterials, main of the waste goes to sewage, in the atmosphere or finds its way to landfill (50%) [48] [133].The fate of nanowaste in municipal solid waste landfills is rather unknown and very little information is given by the municipalities [133].

The nanomaterial waste needs more advanced methods of landfill disposal and recycling compared to conventional waste material. Noble metal nanoparticles are a specific case of interest and their recycling is economically valuable. The recycling cycle includes actions of sorting, dismantling, chemical or physical recovery. It is presently more difficult to estimate the cost of the recycling of products that require some latter steps to recover nanomaterials. Presently, the combustion of carbon-rich materials appears a reliable option and is intensively used. The possibilities of using microorganism and plant uptake are really promising options and new greener remediation methods are under investigation [134].

For example, three options are available for the extraction of nanoparticles from the waste-water. The first option is the coagulation of the nanoparticles with chemicals or electricity. The second possibility is the floatation and filtration. The last option is to use biological-based method [135].

In terms of the present work, the best option for recycling the nanoparticles that will be used as antibacterial and antifungal agents in straw bales appears to be straw bale combustion without previous shredding. The recycling process can be achieved using “cigar burner” technology (figure 1), where shredding the bales is not necessary, as they go directly into the furnace [136].

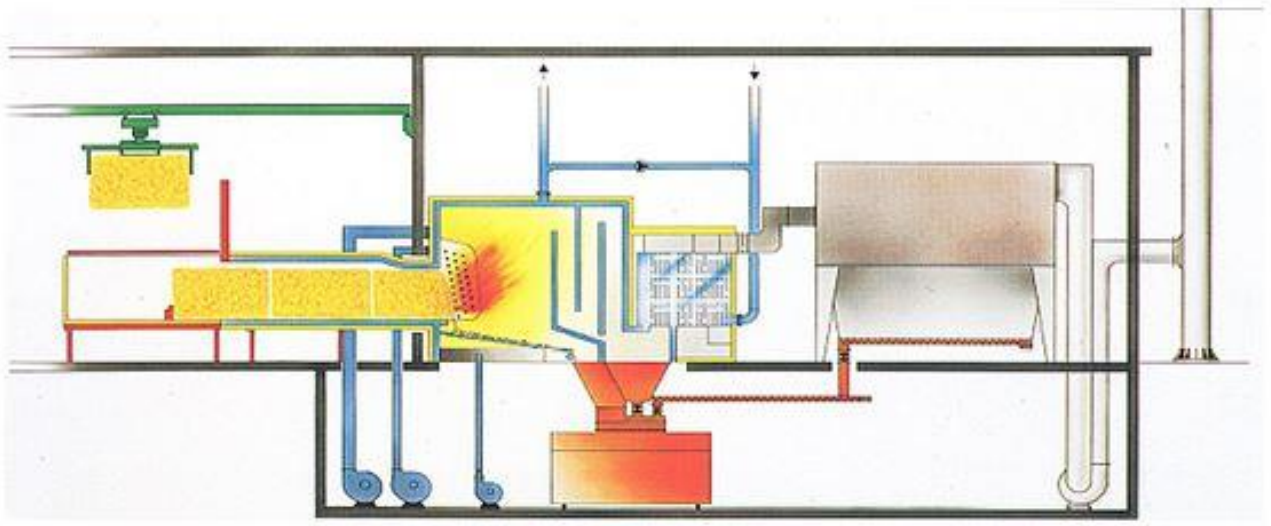

Figure 1. “Cigar burner” technology for big bales [136]

The next step could be somehow to extract silver nanoparticles from the dried ash. One possibility would be the use of column extraction, which is similar to filter systems. Layers of porous gel inside the column let through particles with different sizes, after a while each layer has captured and contains particles with different size and fraction. If the size of the nanoparticles is known, the extraction from the specific gel layer is simple [137].

2. THE OBJECTIVES

Exploiting sustainable building materials might be the future way to reduce the high energy consumption of construction field. However, ecological materials tend to be suitable environment for several living organisms and it causes issues for the house residents. Bacteria and fungi may take advantage of the favourable conditions and then may proliferate in the house walls. All the illness symptoms that are induced by different toxins and spores produced by microorganisms, are quite difficult to handle. So the development of microorganisms inside the building materials should be avoided. Since the treatment using chemicals may diminish the ecological effect, not many options remain for tackling the problem. In addition, chemical products are relatively expensive and degrade or evaporate over time depending on their nature. The objective of the current thesis is to investigate the potential use of silver nanoparticles for solving this specific problem. More particularly, the investigation of silver nanoparticles effectiveness on microorganisms, such as bacteria and fungi, will be performed. Moreover, the safety aspect will also be discussed covering the toxicity and utilization part. A study of the toxicity of the silver nanoparticles that will be used for this application is simultaneously made in collaboration of Biomedicum of the University of Tartu. Finally, the cost-effectiveness of this method will be also discussed in the conclusion.

The methodology includes following tasks to achieve the objectives:

1. Research of publication on silver nanoparticles and straw bale construction.
2. Synthesis of silver nanoparticles.
3. Characterisations of silver nanoparticles.
4. Growth fungi and bacteria in vitro.
5. Preparation of solution containing different concentrations of silver nanoparticles.
6. Test of the efficacy of biocidal action against bacteria and fungi in vitro.
7. Test of the antimicrobial efficacy of silver nanoparticles in straw bale samples.
8. Analyse the cost-effectiveness of current method.

3. MATERIALS AND METHODOLOGY

3.1 Research strategy and inclusion criteria

The research articles used in the present thesis are from databases such as Google Scholar, Scopus (ScienceDirect), Springer, ASCE Library (American society of civil engineers) and others. Finding the relevant articles the following keywords were used: silver nanoparticles, green synthesis, antibacterial properties, green home construction, straw bale, etc.

3.2 Silver nanoparticle synthesis

3.2.1 Materials

The silver nanoparticles were produced by sol-gel method using a recent patented method. The method cannot be fully described and is based on the utilisation of silver acetate ($(CH_3COO)Ag$) precursor (STREM 99%) purchased from Sigma Aldrich. The silver metal nanoparticles were rinsed with ethanol (CH_3CH_2OH) and dichloromethane (CH_2Cl_2) purchased from Sigma Aldrich. Distilled water was produced at TUT Tartu College.

3.2.2 Methods and apparatus

The procedure for synthesizing silver nanoparticles was carried out in a glove box. The reaction mixture was transferred into a stainless steel autoclave and carefully sealed. Thereafter, the autoclave was taken out of the glove box and heated in a furnace (Memmert) at 200ºC for 48 hours. The resulting suspensions were centrifuged (high speed brushless centrifuge MPW-350); the precipitates were thoroughly washed with ethanol and dichloromethane; and subsequently dried in air at 70ºC. As a result dry and pure silver nanoparticles were produced.

3.3 Silver nanoparticles characterization

Characterisations were carried out in collaboration with the University of Oslo.

3.3.1 **TGA**

The thermogravimetric (TG) analyses were carried out. The thermal history of the silver nanoparticles was evaluated on a Rheometric Scientific STA 1500 TGA instrument with a heating rate of 5^{o}C/min under flowing air atmosphere. The samples were heated from room temperature to 800^{o}C.

3.3.2 **XRD**

X-ray diffraction (XRD) patterns were obtained using Bruker D5000 XRD instrument equipped with a Braun position sensitive detector, both using Cu (kα=1.54056) radiation as the X-ray source.

3.3.3 **TEM**

Transmission electron microscopy (TEM) studies were carried out on a JEM2010F operating at 200 kV and disposing a point to point resolution of 1.9 Å.

3.4 **Microorganism cultivation**

3.4.1 **Culture conditions**

Fungi and bacteria were separated from straw during the previous research of microorganisms populating in rye straw. These have been recultivated periodically. According to the recipe, liquid media was autoclaved under 120^{o}C and poured on Petri dishes to dry over-night. Then randomly isolated microorganisms were plated with a spatula on the agar plates. The cultures were grown on the liquid broth media under a 25^{o}C atmosphere. All necessary substances (Fluka) are purchased from HNK Analüüsitehnika.

3.5 **Silver nanoparticle solution preparation**

In order to get desired concentrations of silver nanoparticle in solutions, structural information and the size of silver nanoparticle synthesised are necessary. Crystal structure of silver was found to be face-centred cubic lattice (FCC). Taken the aforementioned into consideration, it is possible to calculate, how many atoms are in one synthesised silver nanoparticle and thereby find the weight of silver nanoparticles you need to prepare 200

nanomole (nM) per litre, 500 nM/L and 1 μM/L of concentration solutions for testing the antimicrobial properties.

The first step is to determine, how many atoms are in one unit cell. As the cubic structure has six faces and for each a half representing in the unit cell, these are multiplied and added to the corner atom quarters, which are eight (figure 2).

$$Z_a = f \times \frac{1}{2} + c \times \frac{1}{8} \quad \text{(Eq. 1)}$$

Where: Z_a – number of atoms in unit cell

f – number of faces in unit cell

c – number of corners in unit cell

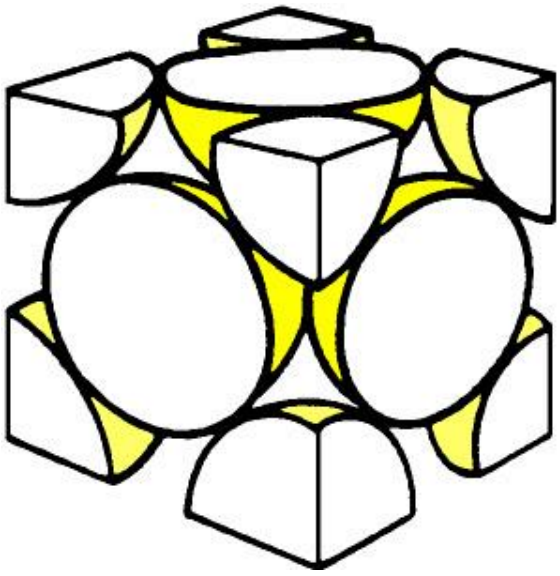

Figure 2. Scheme of FCC unit cell [138]

Text step is to determine the length of the unit cell, which is side of the cube "a". Since the unit cell diagonal is four fold atomic radii (figure 3), the Pythagorean Theorem helps to find the length of the side of the cube.

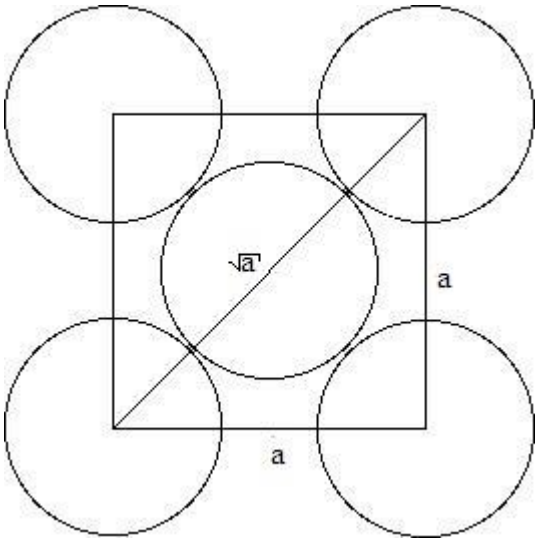

Figure 3. FCC cube side length

$$a = \frac{Ag_r \times 4}{\sqrt{2}} \quad \text{(Eq. 2)}$$

Where: a – length of the side of cubic unit cell;

Ag_r – atomic radius of silver.

The volume of the unit cell is volume of a simple cube.

$$V_{unit} = a^3 \quad \text{(Eq. 3)}$$

Where: V_{unit} – volume of unit cell

a – length of the side of cubic unit cell

Since, synthesised silver nanoparticle is spherical the volume of the nanoparticle can be calculated with the formula for the volume of the sphere.

$$V_p = \frac{4}{3}\pi r^3 \quad \text{(Eq. 4)}$$

Where: V_p – volume of nanoparticle;

r – radius of a nanoparticle.

When both the volume of the unit cell and volume of the nanoparticle is known, the number of unit cells in one nanoparticle can be calculated.

$$Z_u = \frac{V_p}{V_{unit}} \quad \text{(Eq. 5)}$$

Where: Z_u – number of unit cells;

V_p – volume of nanoparticle;

V_{unit} – volume of unit cell.

Next the number of atoms in one nanoparticle can be calculated.

$$Z = Z_u \times Z_a \quad \text{(Eq. 6)}$$

Where: Z – number of total atoms in nanoparticle;

Z_u – number of unit cells;

Z_a – number of atoms in unit cell.

After the number of atoms in one nanoparticle is known the weight of one mole of silver nanoparticles can be calculated.

$$M_{AgNP} = M_{Ag} \times Z \quad \text{(Eq. 7)}$$

Where: M_{AgNP} – weight of one mole of silver nanoparticles;

M_{Ag} – molar mass of silver;

Z – number of total atoms in nanoparticle.

Although an error bar of 10-20% should be considered, because it is in fact not possible to know exactly the concentration of the nanoparticles in the prepared solution. It is due to size distribution and every particle varies slightly from another and even if the size distribution is quite sharp, small variations can induce an important difference compared to the average size of the nanoparticle. Also agglomeration of the silver nanoparticles is possible and should be taken into consideration, even if the solution is prepared using magnetic stirring and ultrasonic bath to induce the dispersion.

3.6 **Preliminary test on microorganisms**

Bacteria and fungi were randomly isolated from untreated straw bales. Ten strains of fungi and ten strains of bacteria were cultured on the surface of solid nutrient media. 100 μL

drops of three different concentrations of silver solution were dropped to the agar plates on marked places. After absorption cultures were put into the incubator for 14 days at 25^{o}C. Agar plates were marked from “A” to “J” for bacteria and same for fungi (figure 4).

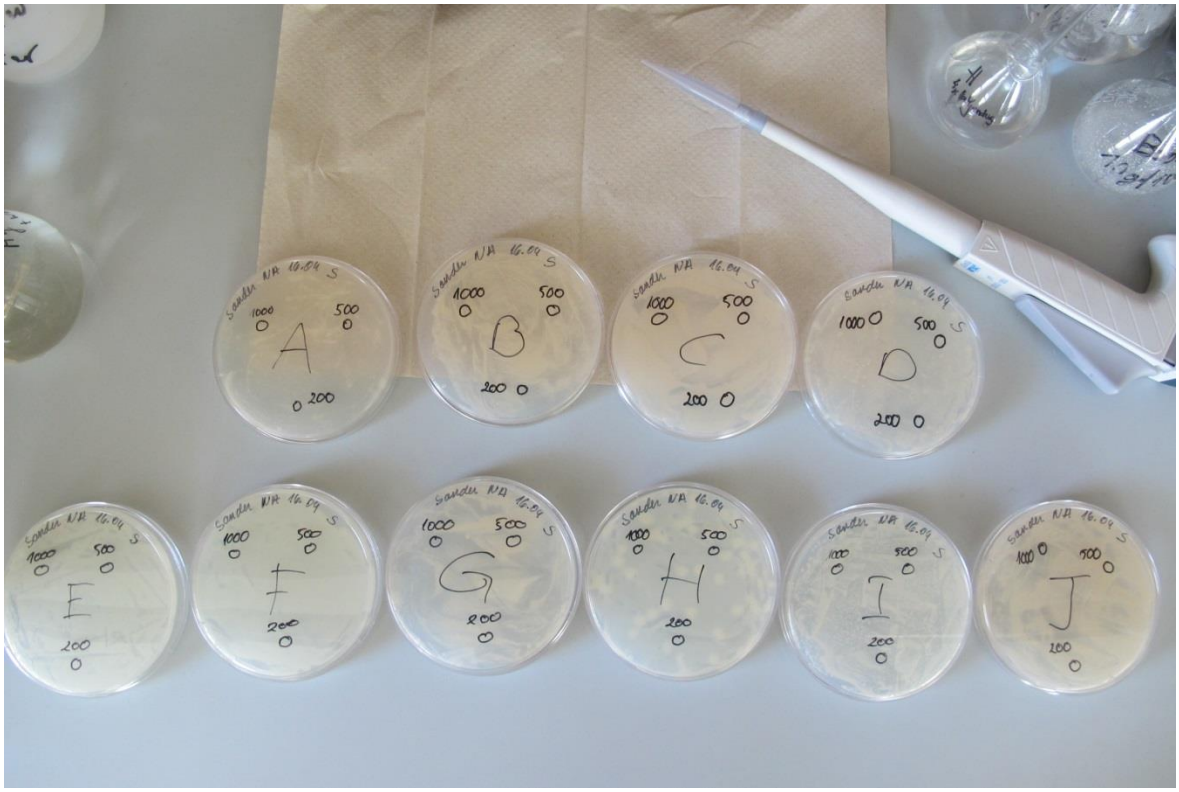

Figure 4. Marking of microorganism agar plates

3.7 **Test with straw bales**

Three test blocks were prepared from rye straw with approximate measurements of 70×250×50 mm (figures 8-10 in the appendixes 1). Two out of three straw bale samples were treated; first one was left untreated but simply soaked into water, the second was dipped into solution of pure ethanol. The third straw bale sample was soaked with solution containing 100 mg of silver nanoparticles dissolved in 1 litre of pure ethanol. The alcohol was let to evaporate and samples were placed into outdoor conditions without being in direct sunlight. Microorganism cultures were let to grow on straw for 14 days then from each bale a sample was taken. The pieces of the straw bale samples were printed to the agar plates. After 96 hours under 25^{o}C, colonies were fully developed and taken for staining and identification.

4. RESULTS

4.1 Yield of the silver nanoparticle synthesis

During the investigations, five syntheses were performed for producing silver nanoparticles. These syntheses enable the calculation of the yield of the production, which is an important parameter in case of a possible scaling-up of production. The precursor used, during the syntheses was silver acetate with molar mass of 166.92 g/mol. Silver atom has molar mass of 107.86 g/mol. The proportion between the weight of the precursor and silver atom is 64.61% (eq. 8).

$$\frac{107.86\ g/mol}{166.92\ g/mol} = 0{,}6461 \qquad \text{(Eq. 8)}$$

This calculation shows that 1 g of silver acetate precursor should produce 0.6461 g of silver nanoparticles in the case of a yield of 100%. The yields of five syntheses were calculated and are presented in the Table 4.1. The average yield of syntheses performed was 95.3%.

Table 4.1. Yields of silver nanoparticles syntheses performed with silver acetate

No	Weight of the precursor, g	Weight of the calculated silver, g	Weight of the syntesised silver, g	Calculated yield , %	Average yield
ETCAg001	0.6420	0.4148	0.3928	94.70	**95.3%**
ETCAg002	0.6045	0.3906	0.3774	96.63	
ETCAg003	0.5517	0.3565	0.3351	94.01	
ETCAg006	0.4820	0.3114	0.2967	95.27	
ETCAg007	0.7946	0.5134	0.4924	95.91	

The values presented in table 4.1 show that the yield is higher in the case of higher start amount of precursor. This suggests that in the case of a higher amount of precursor, the losses during the rinsing step of the silver nanoparticles are lower.

4.2 **Silver nanoparticles characterizations**

The crystalline structure of the silver nanoparticle was confirmed by X-Ray diffraction (XRD) and electron transmission microscopy. Thermogravimetric analysis (TGA) was performed to estimate the possible presence of surfactant and organic coating on the surface of silver nanoparticles. Electron micrograph of spherical silver nanoparticles is shown in figure 5. Silver nanoparticles are 1.8 to 4 nm in diameter with an average size 3 nm. TEM images revealed that particles are easily dispersed in ethanol and surfactant-free. A typical XRD pattern of silver nanoparticles is presented in the figure 6 and it shows that synthesised silver nanoparticles are highly crystalline. TGA, performed on a typical silver nanoparticles sample, showed no weight loss or gain during the heating in 800^{o}C (figure 7), which demonstrates a high purity of the nanoparticles. It seems that the silver nanoparticles are surfactant-free, with no organic species on their surface. The fact that no weight increase is observed till 800^{o}C demonstrates a high stability of the nanoparticles that do not oxidise under air even at high temperature.

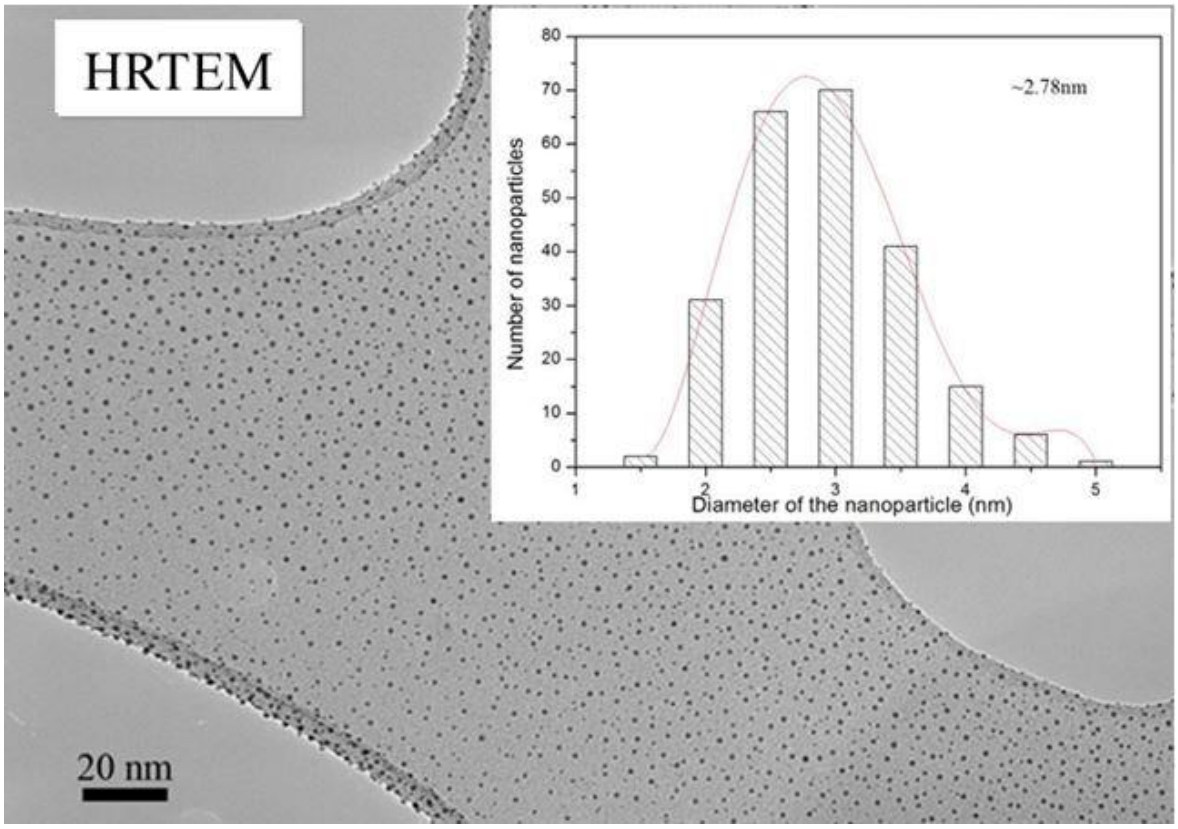

Figure 5. HRTEM image of silver metal nanoparticles dispersed in ethanol on a carbon grid (inset: size distribution diagram showing a sharp size distribution with an average diameter of 2.8 nm)

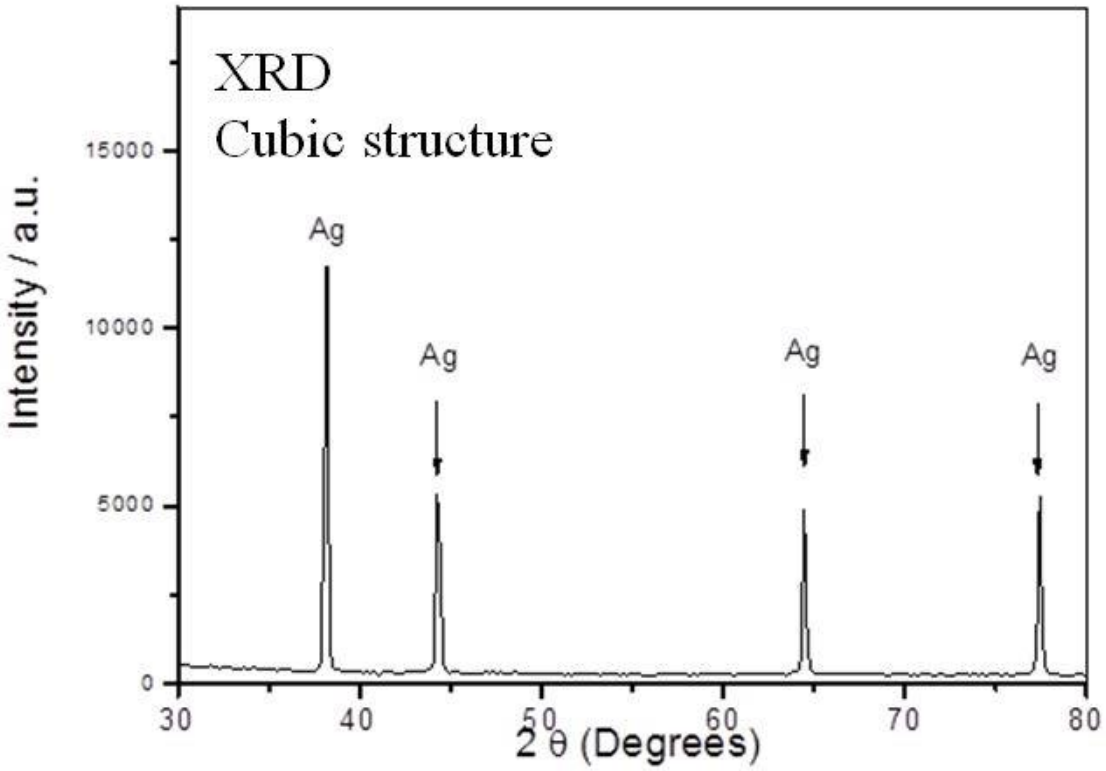

Figure 6. XRD pattern of silver metal nanoparticles showing a face-centred cubic structure

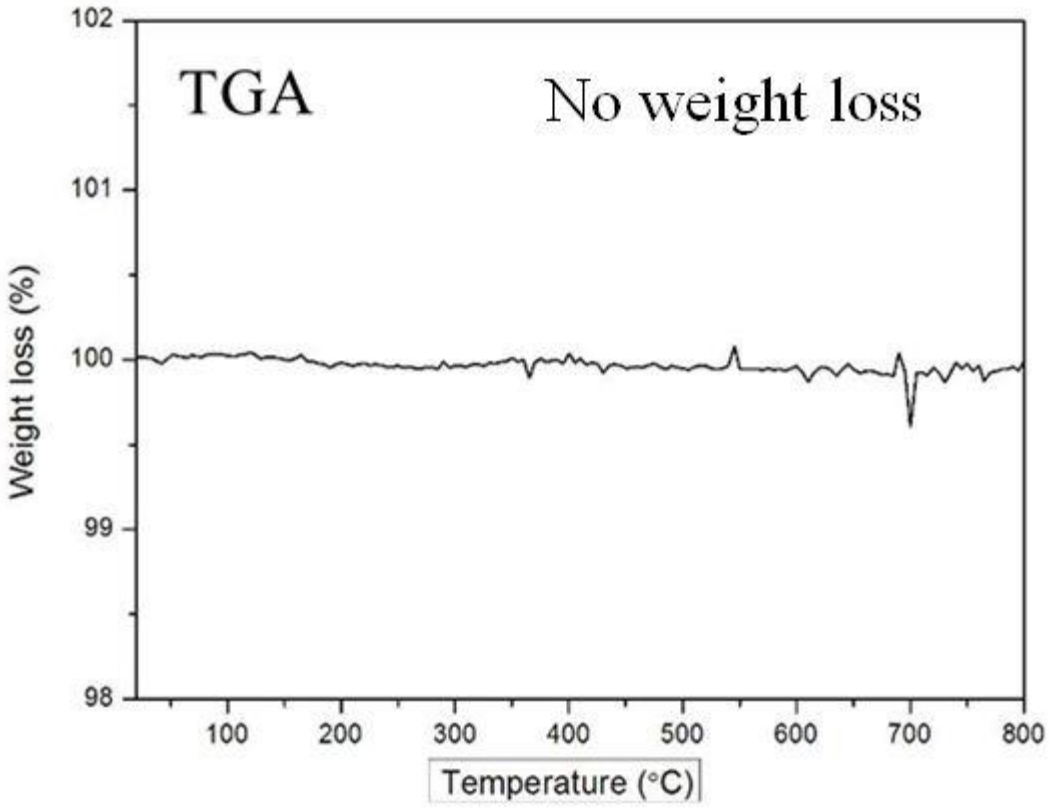

Figure 7. TGA of a sample of silver metal nanoparticles showing no weight loss

4.3 **Concentrations of prepared solutions**

The aim of the calculations was to find the weight of synthesised silver metal nanoparticles powder needed for three different concentration solutions. For the solvent the distilled water was chosen to get the results of the toxicity against bacteria and fungi. However, the dissolution of the metal nanoparticles in water solution is not as efficient as in ethanol solution, the use of a magnet stirrer and an ultrasonic bath (Bandelin Sonorex Digitec) was necessary to improve their dissolution. The first step was to calculate the number of atoms in one FCC unit cell. Cube has six faces and eight corners therefore (eq. 1) four atoms are in one unit cell.

$$6 \times \frac{1}{2} + 8 \times \frac{1}{8} = 4 \qquad \text{(Eq. 1)}$$

Next step was to determine the length of the side of the unit cell (eq. 2), where silver atom radius is 0.144 nanometres.

(Eq. 2)

$$\frac{0.144 \times 4}{\sqrt{2}} = 0.407\ (nm)$$

When side of the cube is known, the volume of the cube is its side length third power (eq. 3).

$$0.407^3 = 0.0676\ (nm^3) \qquad \text{(Eq. 3)}$$

Since, synthesised nanoparticles were spherical and with the diameter of 3 nm, the volume formula is the volume of simple sphere (eq. 4).

(Eq. 4)

$$\frac{4}{3}\pi\left(\frac{3}{2}\right)^3 = 14.137\ (nm^3)$$

To find the number of unit cells in one nanoparticle, both previously calculated volumes have to be divided (eq. 5).

(Eq. 5)

$$\frac{14.137}{0.0676} = 209.129\ (unit\ cells\ in\ nanoparticle)$$

If the number of unit cells in a nanoparticle is multiplied with the number of silver atoms in unit cell (eq. 6) the total number of atoms in nanoparticle can be found.

$$209.129 \times 4 = 836.518\ (atoms\ in\ 1\ nanoparticle) \qquad \text{(Eq. 6)}$$

Next step is to find the weight of one mole of silver nanoparticles (eq. 7), where M_{Ag} is 107.868 g/mol.

$$107.868 \times 836.518 = 90\ 233.77\ (g/mol) \qquad \text{(Eq. 7)}$$

1 kg of silver nanoparticles corresponds to 11.08 μmol, which is deduced from eq. 7 and accordingly 1 mg of silver nanoparticles corresponds to 11.08 nmol. Since, 3 concentrations were chosen: 200 nmol, 500 nmol and 1μmol. The solutions were prepared in volumetric flask of 200 ml: 4 mg, 10 mg and 20 mg of silver nanoparticles were dissolved respectively. To get the exact measures the (Kern&Sohn GmbH) laboratory analytical electronic balance was used with maximum capacity of 120 g and precision of 0.1 mg.

4.4 Effects on microorganisms

4.4.1 Preliminary test on microorganisms

After 14 days, colonies of bacteria and fungi had fully developed. Overall impact was noticed with four agar plates, which three were bacteria and one was fungus. The 1 μM concentration was efficient with all four. In case of fungus "H" all concentrations were efficient to prevent any fungal development (figures 12-14 in the appendix 2). A similar result was observed in the case of bacteria "E" (figures 17-18 in the appendix 2). In the case of bacteria "J", only concentrations of 500 nM and 1 μM affected the colonies of bacteria (figures 19-20 in the appendix 2). For bacteria "D" only the solution of 1 μM concentration was efficient to prevent the development of the bacteria (figure 22 in the appendix 2).

It can be stated that preliminary in vitro tests with silver nanoparticle solutions showed more effect on bacteria compared to fungi.

4.4.2 **Test with straw bales**

After 14 days, microorganisms were isolated from the straw samples. Results of the first sample, containing only water content, showed extensive growth of the microorganism colonies (figure 23 in the appendix 3). Many different species of fungi and fewer species of Gram-negative bacteria and Gram-positive bacteria were present (table 4.2). Second test sample, taken from the bale treated with ethanol, contained also many species of microorganisms (figure 24 in the appendix 3) mostly Gram-negative bacteria (table 4.2). The third straw bale that was treated with the ethanol solution containing 1 μM/L of silver nanoparticles was also investigated (figure 25 in the appendix 3). The results of the third sample were different from the first two samples and agar plates only containing Gram-negative and Gram-positive bacteria colonies as seen in table 4.2. Third sample in fact did not contain any fungi.

Table 4.2. Microorganism species found in the straw samples after 2 weeks in outdoor conditions

	Untreated wet straw	**Straw with ethanol**	**Straw with ethanol silver nanoparticle solution 1μ M/L**
Fungi families	*Aspergillus, Penicillum, Cladosporium*	*Aspergillus*	No fungal activity
Bacteria families	*Streptomyces, Pseudomonas, Firmicutes*	*Pseudomonas, Firmicutes*	*Streptomyces, Firmicutes, Pseudomonas*

As seen in the table 4.2, the ethanol solution containing silver nanoparticles with a concentration of 1 μM/L had satisfying fungicidal effects, when applied on straw bales, although agent did not hinder the bacterial activity.

5. DISCUSSION

5.1 The efficiency of silver nanoparticles against microorganisms

The results of straw bale tests showed microbial activity on all agar plates from the three different samples. The main difference was between untreated and two treated straw bale samples. On two samples that were treated with alcohol practically no fungal activity was observed or it was trivial compared to watered sample, from where many fungal colonies dominated over bacteria on the agar plates. Samples treated with ethanol solution containing silver nanoparticles kept the agar plates 100% free from fungi and plates from pure ethanol straw sample let only one fungal species to grow (*Aspergillus*). However, that particular fungus is quite problematic, when proliferating inside residential buildings [23]. Additional difference between treated samples was that straw bale treated with silver nanoparticle ethanol solution had Gram-positive bacteria (*Streptomyces*) that was not found in the plates from sample treated with pure ethanol.

Among the bacteria, the Gram-negative bacteria were predominantly represented, whom only the competing fungal colonies did restrain on the untreated straw sample. Previously mentioned *Streptomyces* did not colonise the pure ethanol treated sample probably due to the minor spreading of fungus *Aspergillus*, which was enough to hinder the Gram-positive bacteria to proliferate. Due to complete efficacy of ethanol solution containing silver nanoparticles against fungi, some species of bacteria there could develop, which would probably be dominated by fungal species. Supposedly this was the case with *Streptomyces* spreading in the nanotreated samples.

Although, results from the preliminary tests in vitro implied, that silver nanoparticles may be more efficient against bacteria compared to fungi, the quite opposite revealed in the test with straw bales. The inefficiency of silver nanoparticles ethanol solution against bacteria could be explained by the fact, that in a stressful environment, which the straw apparently is due to constant microbial competition, bacteria tend to grow the protective biofilm around them. Supposedly this slime layer does not enable the silver nanoparticles to affect the bacterial colonies. In the case of preliminary tests, bacteria were isolated from each

other and not competing, therefore no stress response was needed and silver nanoparticles had greater effect on them.

High efficiency of ethanol solution containing silver nanoparticles could be explained by the fact that fungi do not usually have the ability to protect themselves by generating biofilm layers. In preliminary tests the fungi were affected less intensively due to absence of stress driven by competition with other microorganisms and therefore the defence mechanisms were not disturbed. Latter could be one explanation to the phenomenon, and in aggressive environment the silver nanoparticle ethanol solution could have been the final addition of stress to affect the fungal colonies.

The test should definitely be repeated with the same samples in outdoor conditions for a longer period of time. Then the long-term effect of silver nanoparticles can be evaluated. Also the repetition of the test is necessary to find the optimal concentrations, which also could penetrate the biofilm of bacteria and thereby inhibit them. Another option that will be studied in near future is to combine the silver nanoparticles with another type of metal nanoparticles that can present a more efficient antibacterial effect than the silver nanoparticles studied in this thesis.

Since fungi apparently dominate over bacterial colonies in the environment and can be more problematic inside the walls of residential buildings, the ethanol solution with silver nanoparticles could be one option for keeping the straw bale houses more convenient to live in. Although according to the results of current experiments, the 1 μM/L ethanol solution of silver nanoparticles is not sufficient to hinder the proliferation of bacteria and its application will not prevent the possible health risks linked to the bacterial activity inside straw bale walls.

5.2 **Microorganisms in straw bale walls**

The first and foremost concern with straw bale construction is its durability and construction load bearing. Since straw is a natural composite material similar to wood consisting of more than 80% of cellulose and hemicellulose and the remaining 20% is mainly lignin and water soluble components, straw should be a suitable material for

construction [139]. Also the fact that straw is poorly degradable in the fields due to its basic constituents, suggests that it can be efficiently applied in construction. However, there are still certain micro-organisms that are known for their straw degrading abilities [140]. Although, it is stated in the literature, that the most wide spread microorganisms present on different straw are fungus *Neocallimastix frontalis*, which reduces the tensile strength of straw and therefore can be real problem for straw bale constructions, cellulolytic bacteria *Ruminococcus flavefaciens*, whose co-culture with latter reduced significantly the resistance to shearing [141], these microorganisms were not found in the present experiment.

As stated, straws consist predominantly of cellulose [140]. In terms of ability to efficiently degrade cellulose the list of bacteria (i.e. *Clostridium* sp., *Brevibacillus* sp., *Bacterium* sp., *Bacillus fusiformis*, *Cytophaga* sp., *Clostridiales bacterium*, *Ruminobacillus xylanolyticum*, *Clostridium hydroxybenzoicum* [140]) is quite exhaustive and therefore represents a threat to straw construction elements [11]. Fortunately, these microorganisms were not found in the present experiments. This suggests, that although currently produced silver nanoparticle solution with a concentration of 1 μM/L was insufficient for limiting the growth of bacteria, most destructive bacteria are not present in our climate therefore there is more stability in straw bale construction.

Even if the degrading microorganisms are hindered, still the toxic microorganisms may be a real health concern. The harmfulness of microorganisms is expressed through different toxins, which they produce to compete with other species over food supplies. Different species have developed hazardous compounds to destroy the competition and prevail. For example, yeasts produce ethanol from sugar and *Penicillum* produces well-known antibacterial agent called penicillin. Even though the vital functions of microorganism could be more easily limited, these chemical compounds produced by these microorganisms may be extremely persistent and toxic causing various health problems [24] (e.g. eye irritation, upper respiratory infections, headache, “cold and flu” symptoms, nasal and sinus congestion, gastrointestinal complaints). These main health problems are all linked to the most common microorganisms (Fungi: *Stachybotrys chartarum* indoor mould, *Aspergillus*, *Penicillum*, *Cladosporium*, *Ulocladium*, *Geomyces pannorum* and *Sistronema brinkmannii*, bacteria: *Legionella*) proliferating in damp buildings [23]. As experiments of current investigation showed, if moisture damage would occur in a straw

bale house, many of these microorganisms could be present and with an exception of bacteria, all can be restrained with current the antifungal method.

In comparison, an already constructed straw bale house was found to a contain number of pathogens and producers of toxins, according to the experiment, which was carried out in in 2014 in Estonia, near Võrtsjärve. From indoors, a white rot fungus (*Sclerotina*) was found, which is known to decompose lignin and has a toxic effect if digested. In addition, the ascospores produced by this fungus may induce allergies and asthma. Furthermore, inside the wall many harmful toxin producers were found (*Aspergillus*, *Paecilomyces*, *Cladosporium*), whose life cycle produces harmful compounds if inhaled [142]. The current straw bale experiment showed a significant efficiency of the silver nanoparticles to prevent the development of all these discovered fungi and is therefore suitable for the given environment.

The preliminary test in vitro showed that only a few microorganisms were affected by low silver nanoparticle concentrations (i.e. 200 nM/L). This could imply that species present in rye straw in Estonia need greater amounts of silver nanoparticles to prevent their proliferation than compared with some other microorganisms stated in the literature [67]. Moreover, the efficiency is also very dependent on the specific size and shape of the silver nanoparticles. Therefore, further experiment using higher concentrations were performed. However, the amount of silver nanoparticles used for these experiments, when compared with other antibacterial and antifungal agents, contained still a very small amount of reagent (table 5.1).

Table 5.1. Comparison of antibacterial and antifungal agents on the market with silver nanoparticle solution 1 μM/L [142-145]

	AgNPs + Ethanol	**Boric acid**	**Sodium hypochlorite**	**Biotol**
Applied concentration, g/L	0.1	2.25	0.5	1.0
Micro-organisms affected	Fungi	Fungi	All micro-organisms	All micro-organisms
Cost of 1 litre, €	1-1.5	1.6	2.3	10

The efficiency of silver nanoparticles can be compared with more conventional biocides, such as boric acid, sodium hypochlorite and Biotol. The comparison is presented in the table 5.1. As presented, the silver nanoparticle solution needs significantly less amount of active agent for a biocidal effect, being also more environmentally friendly than these aforementioned chemicals [142].

5.3 **The cost of production**

The profitability of using the antibacterial and antifungal solution of silver nanoparticles on straw bales remains one of the most important factors for future larger scale production plans. Since the biocidal efficacy part was discussed, the other main obstacle could be the cost of production for these silver nanoparticles. The cost of the usual antifungal and antibacterial chemicals used for the treatment of straw ball cost €1.6 per litre in case of slightly safer boron compounds and really hazardous sodium hypochlorite, which costs €2.3 a litre if bought in large quantities (table 5.1). All these mentioned agents are quite polluting [146, 147].

It should be taken into account that the comparison of existing chemicals with the solution of silver nanoparticle is still at a laboratory scale of production and general cost should decrease in the case of higher scale of production. As stated before the average yield in the laboratory conditions was 95.3% and henceforth yield of 95% is considered as standard.

1g of acetate precursor produces 0.646g of silver metal nanoparticles with a yield of 100% (eq. 8). Taken this into account the 95% yield we need 1.629 g of acetate for the production of 1 g of silver metal nanoparticles. The production of 1 g of silver nanoparticles at the laboratory scale cost around €7.71, consisting of precursor (€5.15) and solvent (€2.56). In addition to latter, there will be other expenses and estimated cost of the synthesis of 1 g of silver metal nanoparticles is around €10-12. 1 g is the necessary amount for preparation of 10 litres of solution that contains 1μM/L of silver metal nanoparticles and demonstrates an efficient antifungal activity. This means that the self-cost for the preparation of 1 litre is around €1-1.5 as showed in the table 2. It is also necessary to add the price of the solution in which the nanoparticles will be dissolved. This solution is

usually ethanol and can be used several times for the treatment of multiple straw balls. The cost of current method is comparable and slightly cheaper, suggesting the cost-effectiveness of present option.

Furthermore, one of the by-products of the present synthesis is ammonia (NH_3), which is valuable gas in many fields. For example market value of 25% water solution of ammonia is 7.3€ a litre [148]. It makes the current treatment option even more profitable, because the process of metal nanoparticles induces the production of ammonia in the gas and liquid form. In the case of scaling up the process it should be possible to extract this ammonia from the by-product and recycle it for the future commercialization.

5.4 The safety of silver nanoparticles

In case of using some sort of biocide for the treatment of straw bale some questions about the safety aspect can be raised. In fact, the high antimicrobial efficiency of different agents is often related to harmfulness or even toxicity to humans or the environment. On the contrary, in our study, the silver nanoparticles concentrations that demonstrate a high efficacy against fungi in straw bales and against bacteria in vitro seem to be relatively harmless to human cells. This is in fact the usual difference between the silver nanoparticles compared to other antimicrobial metal nanoparticles. Many other metal nanoparticles often present higher overall toxicity against all that is in contact with them [10]. Furthermore, in the present case, the synthesised nanoparticles are spherical, which according to the literature seem to be less toxic compared to other shapes of nanosized silver, such as nanoplatelets or triangular nanoparticles [88, 114].

In collaboration with Biomedicum of Tartu University the toxicity of currently synthesised silver nanoparticles was tested on human cells. The first results of toxicity tests on human embryo kidney (HEK) cells show that 500 nM/L of presently synthesised silver nanoparticles do not seem to present toxicity. This implies that on the environmental perspective, the applicability of silver nanoparticles as antifungal and antibacterial agent is suitable for ecological residential buildings such as homes that include straw bales in their construction.

SUMMARY

The objective of the present master thesis was to investigate the possibility of using the antibacterial and antifungal properties of silver nanoparticles on ecological construction material such as straw bale.

The silver nanoparticles were produced by sol-gel method using a recent patented method. Synthesised silver nanoparticles were examined and characterised with TGA, XRD methods and using high resolution transmission electron microscopy. It was found that the current silver nanoparticles were spherical with the diameter of 3 nm. Subsequently, it was demonstrated that it is possible to dissolve synthesised silver nanoparticles in different solutions (diluted water, ethanol and methanol) at desired concentrations.

Preliminary tests using different concentrations of silver nanoparticles were performed on isolated microorganisms from straw. Silver nanoparticle solution with a concentration of 1 μM/L was found the most efficient for straw bale treatment. Subsequently, three samples from dried rye straws were prepared as a confirming test. The first sample was soaked into water, the second sample was treated with pure ethanol and third sample was treated with silver nanoparticles (1 μM/L) in ethanol solution. After the evaporation of ethanol, the three samples were stored into outdoor conditions covered from direct sunlight and rainfall.

The straw block test lasted for two weeks after which pieces of samples were taken for microorganism isolation and further cultivation in agar plates. After four days of incubation the developed colonies were identified and examined. Although the preliminary test in vitro showed more efficient inhibition of bacterial species than fungal by silver nanoparticle ethanol 1 μM//L, the straw bale tests in the outdoor environment showed the efficiency only against the fungal species. However antibacterial properties of silver nanoparticles in this case were not proven, the 100% efficiency against fungal colonies shows the possible use of silver nanoparticle 1 μM/L ethanol solution as an effective fungicide.

In addition to antibacterial and antifungal efficiency study, the cost for the preparation of a 1 μM/L of silver nanoparticles solution preparation was calculated. The cost for the synthesis of the silver nanoparticles necessary for the preparation of solution is quite cheap, compared to similar antibacterial and antifungal agents on the market. Low producing cost and possibility of producing ammonia as by-product of the synthesis, makes the current synthesis cost-effective. The preliminary test results on human body did not show any toxicity for the half-concentration of the current solution (500 nM/L) on HEK cells. The environmental toxicity impact of silver nanoparticles could be prevented by correctly recycling the materials of construction after they have expired. The building can be demolished and the treated straw bales can be combusted and silver nanoparticles can then be extracted and reused.

According to present findings, it can be stated that the ethanol solution containing 1 μM/L of silver nanoparticles appears to be suitable for fungicidal applications in straw bales. Further testing is necessary to find the optimal concentrations that can also affect bacteria in straw bales. Also further tests are needed with the current straw samples to evaluate the long-term effect of silver nanoparticles. At the same time the toxicity tests should continue with different concentrations to confirm the safety aspect.

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APPENDIXES

Appendixes 1

Figure 8. Depth of a straw bale samples

Figure 9. Width of straw bale samples

Figure 10. Length of straw bale samples

Appendixes 2

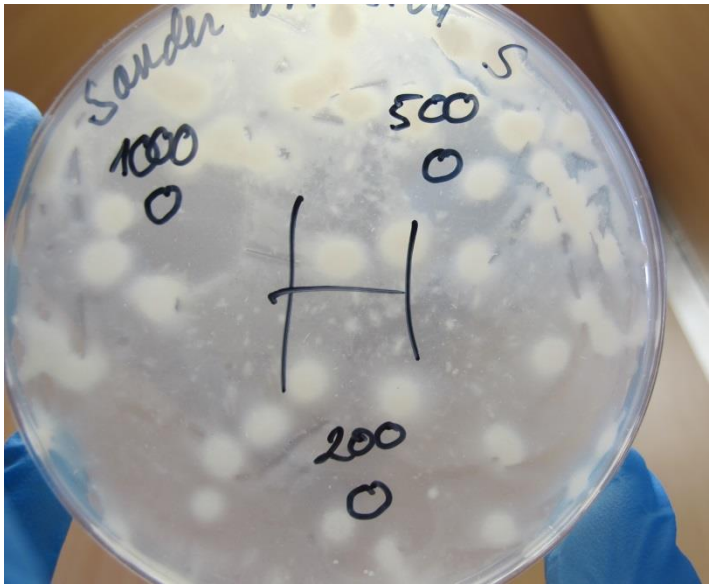

Figure 11. Fungus "H"

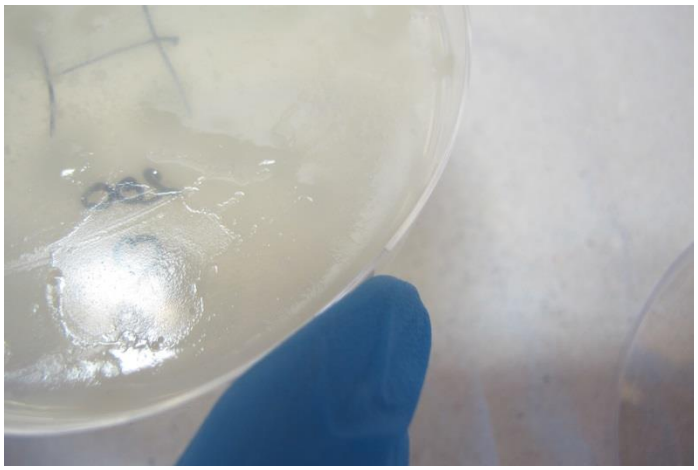

Figure 12 Fungus "H" 200 nM

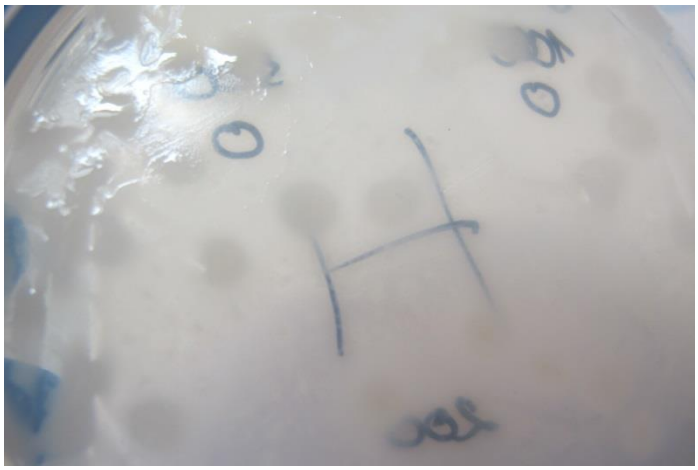

Figure 13. Fungus "H" 500 nM

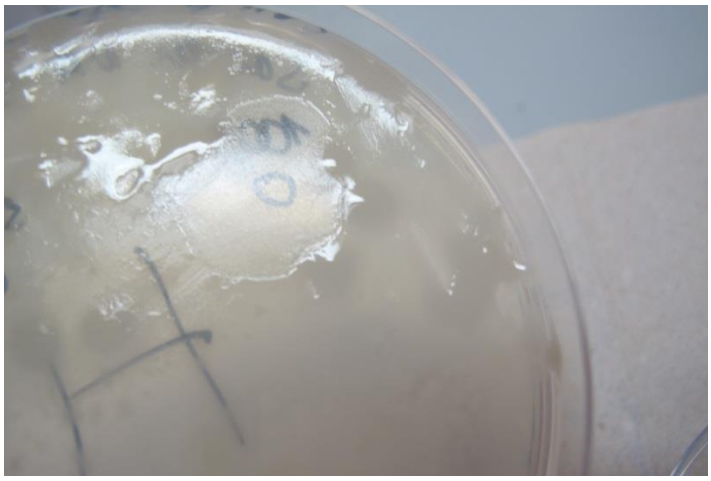

Figure 14. Fungus "H" 1 µM

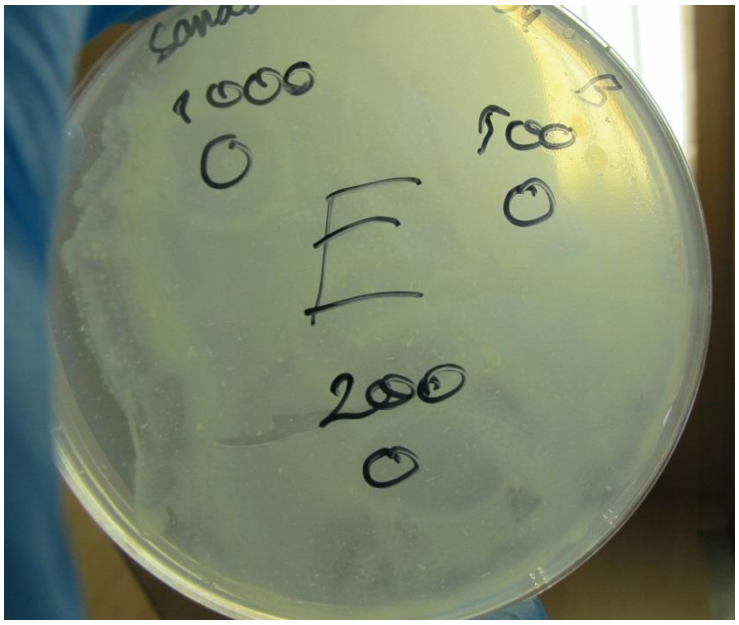

Figure 15. Bacterium "E"

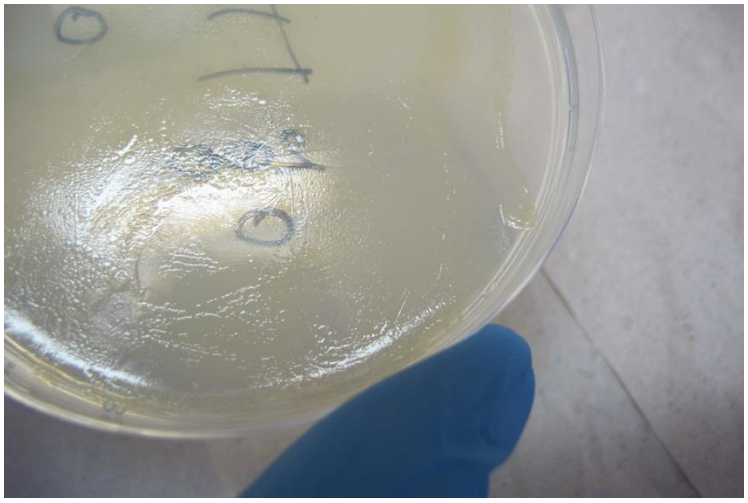

Figure 16. Bacterium "E" 200 nM

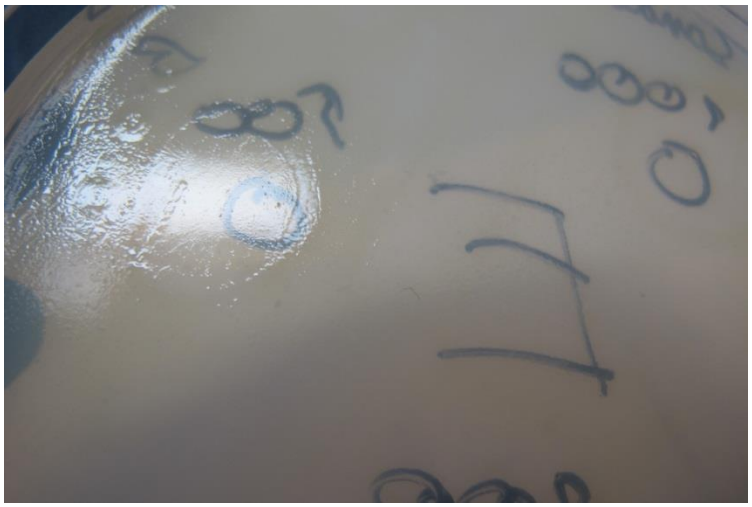

Figure 17. Bacterium "E" 500 nM

Figure 18. Bacterium "E" 1 µM

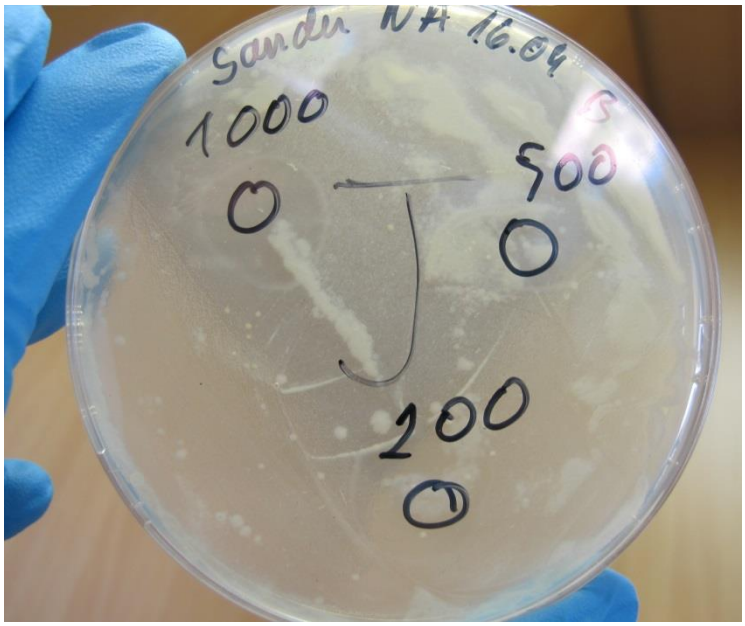

Figure 19. Bacterium "J"

Figure 20. Bacterium "J" 1 µM

Figure 21. Bacterium "D"

Figure 22. Bacterium "D" 1 μM

Appendix 3

Figure 23. Untreated straw

Figure 24. Straw treated with ethanol

Figure 25. Straw treated with 1 μM/L AgNP ethanol solution