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RESEARCH ON THE INFLUENCE OF SILVER CLUSTERS ON DECOMPOSER MICROORGANISMS AND E. COLI BACTERIA

A. I. Piskaeva*, Yu. Yu. Sidorin, L. S. Dyshlyuk, Yu. V. Zhumaev, and A. Yu. Prosekov

Kemerovo Institute of Food Science and Technology,
bul'v. Stroitelei 47, Kemerovo, 650056 Russia,
*phone/fax: +7 (923) 606-33-73, e-mail: a_piskaeva@mail.ru

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Abstract: Modern methods of slime waste disposal utilized by wastewater treatment plants were analyzed. Domestic and foreign experience in the application of silver clusters in reducing pathogenic and conditionally pathogenic microorganisms inhabiting the waste sludge was studied. The main mechanisms of bactericidal and bacteriostatic effects silver clusters are capable of exerting on microorganisms were considered. Strains of microorganisms with the ability to recycle organic and inorganic substances present in the waste sludge into ecologically pure humus fertilizer in the course of their vital activity were selected. The effects of different concentrations of silver clusters on growth and development of the microorganisms decomposing organic compounds (*Microbacterium terregens* BSB-570, *Streptococcus termophilus* St5, *Lactobacillus* sp. 501 (2A4), *Rhodococcus erythropolis*, *Bacillus fastidiosus*, *Arthrobacter* sp. *(Arthrobacter paraffineus)* ATCC 15591, etc.), as well as on the *Escherichia coli* bacteria chosen as a model organism, were studied. For the first time, decomposers modified with silver clusters, i.e. resistant to high concentrations of silver clusters, able not only to grow, but also to reproduce normally and, consequently, to recycle the waste sludge, were obtained. Bacteriostatic and bactericidal concentrations of silver clusters with respect to decomposers and optimal concentration, at which the useful microorganisms are able to grow and reproduce actively and the pathogenic *Escherichia coli* die, were determined.

Keywords: silver clusters, nano-silver, destruction, pathogen, ion, nanoparticles, sludge, waste

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INTRODUCTION

Today, problems of environment protection and rational nature management, as well as improvement of ecological safety, are considered to be of paramount importance worldwide. Wastes formed in the process of industrial wastewater treatment belong to the most abundant contaminants of practically all components of the environment (surface and ground waters, soil, vegetative cover, and atmospheric air). These sediments, differing by chemical and physical properties, are called slimes. Very often, they are harmful for living organisms and the environment.

Most often, slimes formed in the course of wastewater treatment are buried at the industrial waste disposal sites upon slime treatment with bonding cement, bitumen, glass, or polymers. However, these waste disposal sites pose serious danger for the environment. Self-purification of the contaminated areas without human interference lasts for decades; besides, the inherent capacity of the environment for self-restoration decreases each year. Consequently, the problem of maximally efficient slime purification, which would take into account contaminant composition, economic, and ecological factors, becomes urgent [1].

In most cases, due to the lack in sufficient amount of specialized waste disposal sites meeting the requirements of construction norms and rules of the Russian Federation 2.01.28-85, the factories are forced to store wastes on their territories, often without adhering to the burial rules, which leads to soil and surface water contamination due to dissolving of slimes under the effects of atmospheric factors.

The amount of accumulated wastes and new wastes produced each year is so high that the slimes hold the first place in the extent of negative effect they produce on the environment and man, leaving behind such factors as noise, radioactive wastes, chemical fertilizers, and oil spills.

In most slimes, in addition to various organic and inorganic compounds, considerable amount of pathogenic microorganisms is contained. This natural slime microflora—thermotolerant coliform bacteria, *Escherichia coli*, *Chlostridium perfringens*, *Salmonella enteriditis*, *Salmonella virchow*, etc.—poses a great danger, as it possesses the ability to excrete toxic compounds affecting human body.

The aim of the work was to study the effect of silver clusters on microorganisms capable of waste decomposing in the course of their vital activity and on *E. coli* to determine antipathogen activity of silver clusters. The study was conducted to develop new microbiological method of waste slime purification and processing under conditions of Siberian Federal District.

E. coli was chosen as a model organism since this rod-like bacterium is one of the best studied prokaryotic

microorganisms and one of the most important subjects in biotechnology and microbiology. *E. coli* is well adapted to growth and proliferation under laboratory conditions [2].

Silver clusters (silver nanoparticles) are a type of colloidal silver of highest quality more homogeneous and of smaller size than classic colloidal silver preparations [www.vector-vita.com].

The authors have elaborated an original, so-called AGL (ARGENTUM LYSOL), method of synthesis of stable silver nanoparticles with mean diameter in the range of 1–2 nm (particles of this size may be referred to clusters). The particular feature of the method is the use of a natural polymer gelatin as a ligand instead of the standard povidone. Also, synthesis according to the standard technique (chemical synthesis using silver nitrate), or AGM synthesis (ARGENTUM MEDICAL) allowing to produce silver nanoparticles 1–10 nm in diameter was performed. Silver clusters produced by NPTs "Vector-Vita" with particle size distribution in the range of 1–8 nm are an example of the technique utilization. Therefore, monodispersity of the closest analogue of silver clusters is almost three times lower. Figure 1 presents size distribution curves for particles synthesized according to AGM and AGL methods.

On the whole, silver clusters contain highly dispersed metallic silver particles in the nanometer size range. The terms "silver clusters", "nano-silver", and "silver nanoparticles" are essentially synonymous. However, silver clusters differ considerably by their physicochemical properties from other nano-sized forms of silver. Their small size provides for the high efficiency, stability, and safety [3].

Owing to their unique physical and chemical properties that are defined by the high surface area-to-volume ratio and other size effects, antibacterial and antiviral properties of silver clusters are enhanced considerably, if compared to other metals, while silver as such remains absolutely safe for human health upon appropriate application.

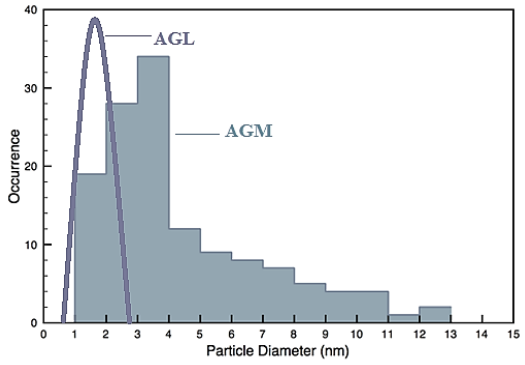


Fig. 1. Size distribution of silver nanoparticles produced by two methods of synthesis, AGL and AGM.

In view of a rather wide spectrum of antibacterial effect of silver-based preparations, there is a chance that the bactericidal effect is exerted not only against pathogens, but against normal microflora of human body as well. However, the theoretical assumption is not applicable to silver clusters. Solutions of metallic silver in the form of clusters not only have been proven to be safe for the organism, but also are used in preventive and therapeutic doses in place of antibiotics. Silver preparations are administered to normalize microbiocenosis and suppress disease-causing microorganisms. Although, today there is no shared opinion on the reasons of such selectivity, the researchers agree that silver administration in the first place results in suppression of pathogenic flora, which promotes development of normal flora.

Biocidal effect of silver clusters is explained by three main mechanisms: interference in electron transport, binding to bacterial DNA, and interaction with cell membrane.

Complex formation with sulfhydryl groups may inactivate enzymes on cell surface and alter respiration processes in cell membrane. The DNA-bound silver ions block transcription, and binding with cell surface components interrupts bacteria respiration and adenosine triphosphate (ATP) synthesis [4]. In *Candida albicans* yeast (and not in *E. coli*) irreversible interaction of silver ions with cysteine residue of phosphomannose isomerase interrupts synthesis of cell wall, which, in turn, leads to loss of essential nutrients [5]. Silver clusters suppress phosphate consumption, repress DNA functions, and inhibit transmembrane transport of organic and inorganic substances [6, 7].

The effect of silver on a microbial cell occurs in two steps: 1) adsorption and 2) active transport of ions inside cell. Up to 90% of internalized silver ions are retained in the membrane; cell metabolism is disturbed as a result of inactivation of enzymes and transport proteins (permeases). Electron microscopy studies demonstrated that silver ions caused morphological changes in bacterial cells [8].

Silver ions inhibit consumption and exchange of phosphates in *E. coli* and cause loss of accumulated phosphate, as well as mannitol, succinate, glutamine, and proline. The effect of Ag^+ is blocked by thiols and, to a lesser extent, bromide. In the presence of *N*-ethylmaleimide, Ag^+ does not cause phosphate leakage but still inhibits exchange between intracellular and extracellular phosphates [9]. Another mechanism of silver ion effect, especially at low concentrations, was reported in the work [10]. The authors demonstrated that low concentrations of Ag^+ caused massive leakage of protons through membrane of *Vibrio cholera*, which ultimately resulted in complete deenergizing and, most probably, death of a cell.

Authors of the work [11] studied the ability of bacteria to consume Ag^+ ions from solutions by the examples of *Bacillus cereus, B. subtilis, E. coli*, and *Pseudomonas aeruginosa*. Consumption of Ag^+ from solution by the bacteria occurred rather efficiently: approximately 89% to the total Ag^+ was removed from a 1 mM solution.

In the work [12], oligodynamic effect of silver on *Bacillus subtilis* (1 strain), *Enterobacteriaceae* (26 strains), *Legionellaceae* (13 strains), *Micrococcaceae* (6 strains), and *Pseudomonas aeruginosa* (4 strains) was studied. *B. subtilis* and *Legionellaceae* demonstrated the highest susceptibility. The effect of small amounts of

silver on various bacteria groups differed considerably: non-pathogenic microorganisms were less susceptible than *Staphylococcus aureus.*

Silver clusters possess pronounced antibacterial effects if compared to other metals. Bactericidal effect of silver clusters was found to be 1750 times stronger than that of carbolic acid and 3.5 times stronger than that of mercuric chloride and lime chloride [13].

The studies demonstrated that susceptibility of various pathogenic and non-pathogenic organisms to silver was different. Pathogenic microflora is much more sensitive to silver ions than the non-pathogenic microflora.

MATERIALS AND METHODS

Strains of microorganisms decomposing organic compounds and possessing the ability to adsorb silver clusters on their surface, including *Microbacterium terregens* BSB-570, *Streptococcus thermophilus* St5, *Lactobacillus* sp. 501 (2L4), *Rhodococcus erythropolis*, *Bacillus fastidiosus,* and *Arthrobacter* sp. (*Arthrobacter paraffineus*) ATCC 15591, were subjects of the work.

Adaptation of the microorganisms and their modification with silver clusters was performed by cultivation of the strains at various concentrations of silver clusters (from 0 to 400 μg/mL) in liquid nutrient medium.

Solutions containing silver clusters 1–2 nm in diameter at concentration of 10000 μg/mL were prepared at the chair of Bionanotechnology of the Kemerovo Institute of Food Science and Technology. The solutions were introduced into nutrient medium containing cultures of decomposer microorganisms and in the medium for cultivation of *E. coli*.

Nutrient medium for cultivation of *M. terregens* BSB-570, *R. erythropolis*, *B. fastidiosus*, and *Arthrobacter* sp. *(A. paraffineus)* ATCC 15591 contained (g/L) yeast extract, 5.0; pepton, 15.0; sodium chloride, 5.0; and distilled water, 1.0 L. Temperature of cultivation was set to 30–34°C. Duration of cultivation was 3 days.

Nutrient medium for cultivation of *S. thermophilus* St5 and *Lactobacillus* sp. 501 (2L4) contained (g/L) papain digest of soybean flour, 5.0; peptic digest of animal tissue, 5.0; yeast extract, 2.5; beef extract, 5.0; lactose, 5.0; ascorbic acid, 0.5; magnesium sulfate, 0.25; and distilled water, 1.0 L. Temperature of cultivation was 40°C. Duration of cultivation was 2 days.

Nutrient medium for cultivation of *E. coli* contained (g/L) pancreatic hydrolysate of fish flour, 10.0; enzymatic peptone, 10.0; lactose, 10.0; yeast extract, 5.0; purified bile, 1.0; neutral red, 0.05; crystal violet, 0.001; and agar, 13.0. Temperature of cultivation was 37°C. Duration of cultivation was 3 days.

During the whole process of cultivation, samples were monitored with an AxioVert.A1 (Carl Zeiss AG) microscope at ×40 and ×100 magnification each 8 h during the first day and each 12 h, the following days. Final concentrations were selected taking into account decomposer survival and complete death of *E. coli* culture.

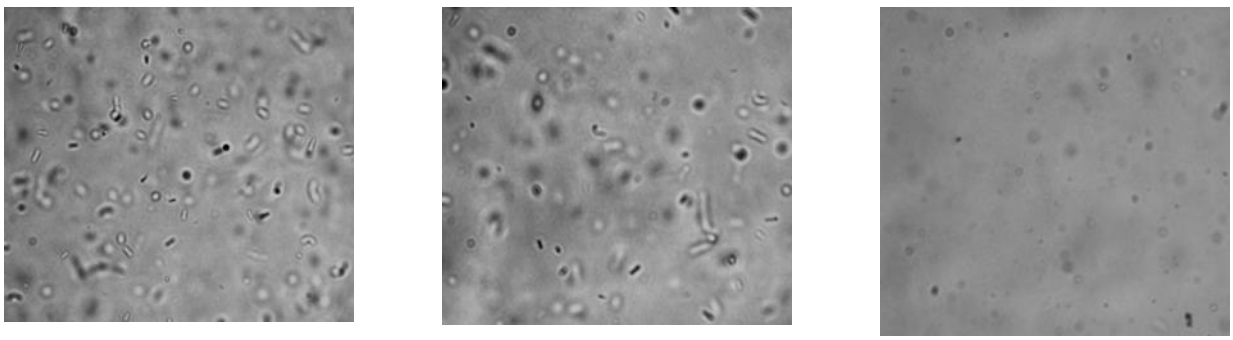
RESULTS AND DISCUSSION

Results of the study of the effect of various silver cluster concentrations on decomposer microorganisms are presented in Table 1.

Figure 2 demonstrates the results of the *Microbacterium terregens* BSB-570 microscopy.

Table 1. Threshold concentrations of silver clusters for decomposer microorganisms

Strain	Concentration, μg/mL		
	bacteriostatic	bactericidal	optimal
Microbacterium terregens	100	400	50
Streptococcus thermophilus St5	200	500	100
Lactobacillus sp. 501	200	450	100
Arthrobacter paraffineu	50–100	250	50
Bacillus fastidiosus	50–100	300	50
Rhodococcus erythropolis	50–100	300	50



(a) (b) (c)

Fig. 2. *Microbacterium terregens* BSB-570 strain in the medium containing silver clusters at concentration of (a) 50 μg/mL, (b) 150 μg/mL, and (c) 300 μg/mL at ×100 magnification.

Analysis of the data presented in Table 1 showed that silver cluster concentration of 50 μg/mL is optimal for most decomposer microorganisms. At this concentration, they are capable of normal growth and proliferation and thus processing of the compounds comprising waste slimes.

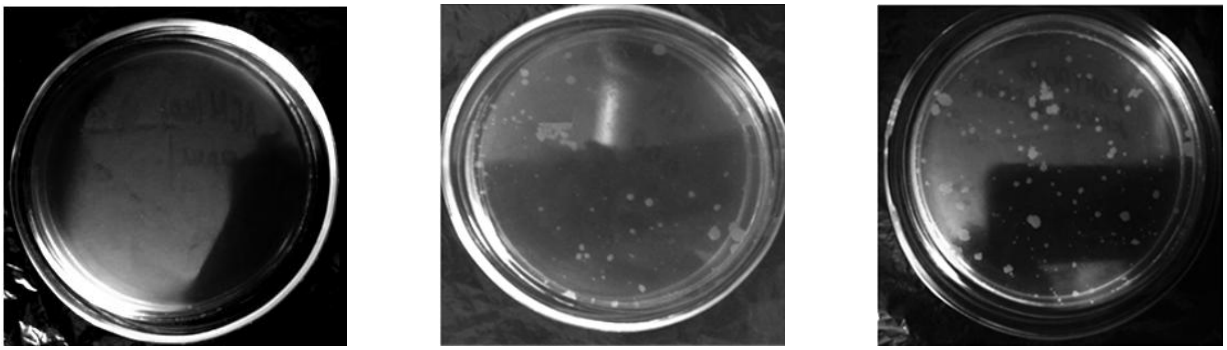
According to Fig. 2, cultivation of the *Microbacterium terregens* BSB-570 strain at concentration of silver clusters of 150 μg/mL resulted in irreversible processes of culture death. However, they proceeded very slowly and only upon constant increase of silver concentration. The culture is capable of proliferation even at silver concentration of 250 μg/mL. Concentration of 50–100 μg/mL was found to be optimal.

According to the data on the effect of silver clusters on *E. coli* growth on a dense nutrient medium (Table 2, Fig. 3), concentration of 100 μg/mL was found to be lethal for the culture. At 50 μg/mL, the number of colonies decreases more than twofold.

Table 2. The effect of various concentrations of silver clusters on growth of *Escherichia coli*

Silver cluster concentration in the medium, μg/mL	Number of *E. coli* colonies	
	after 24 h	after 36 h
100	0	0
50	79	104
Control (0)	196	254

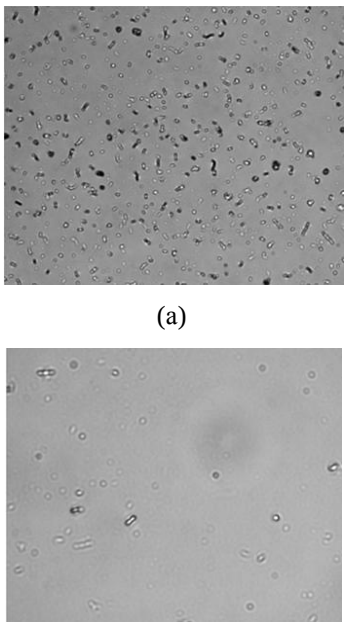
Figure 3 presents *E. coli* strains obtained after 72 h of cultivation at 37°C on nutrient media with varying concentration of silver clusters.



(a) (b) c

Fig. 3. Cultivation of *E. coli* on a dense nutrient medium at concentration of silver clusters of (a) 100 μg/mL and (b) 50 μg/mL; (c) control samples grown in the absence of silver.

Figure 4 demonstrates the results of microscopy investigation of the *E. coli* culture.



(a) (b)

Fig. 4. Microscopy analysis of *E. coli* culture at ×100 magnification, after 24 h cultivation: (a) control sample (no silver) and (b) silver clusters concentration of 100 μg/mL.

As follows from Fig. 4, after 24-h cultivation, the number of bacteria decreased considerably. The ability to proliferate was practically absent. Therefore, at silver cluster concentration of 100 μg/mL in the nutrient medium no *E. coli* growth occurs. At the same time, this concentration does not cause bactericidal or bacteriostatic effect on the decomposer microorganisms. At this concentration, they are capable of normal growth and proliferation.

Basing on the results of the work, we can conclude the following:

1. The effect of various concentrations of silver clusters (from 0 to 450 μg/mL) in the liquid nutrient medium on growth and proliferation of decomposer microorganisms was studied.
2. Microorganisms decomposing various organic and inorganic compounds modified with silver were obtained.
3. Bacteriostatic and bactericidal concentrations of silver clusters were defined for all decomposer microorganisms under study.
4. The efficiency of the effect of the silver clusters against pathogenic bacteria was proven by the example of *Escherichia coli*.
5. Concentrations of silver clusters allowing for growth and active proliferation of decomposer microorganisms and death of *E. coli* bacteria were chosen. The optimal concentration of silver clusters was found to be 100 μg/mL.

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