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BIOCIDAL ACTIVITY OF THE PRECIPITATED SILICA WITH SURFACE COMPOUNDS OF Ag, Cu AND Zn

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By modification of the highly dispersed silica surface with amino complexes of silver, copper and zinc from aqueous solution with further heat treatment, silica nanocomposites containing surface compounds of silver, copper and zinc were synthesized. The concentration of the silver in the samples was 1-2 wt%, and the copper and zinc - 1-5 wt%. Biocidal activity of the composites was tested against 18 different strains of micromycetes, phytopathogenic bacteria and algae. It was shown, that the highest efficiency is observed in the samples of silica modified with silver/copper and silver/zinc.

Introduction

It is known that microorganisms have both positive and negative effects on the environment. In particular, there are many causative agents of many diseases in warm-blooded animals and plants, species with toxigenic properties and corrosion activity in different materials and buildings. A malignant bacteria causes significant damages to the human health and therefore to the economics in general. The main tools to combat the harmful actions of microorganisms are antibiotic compounds and chemical agents, including composites those which contains silver, copper, zinc and etc. The biocidal activity of copper, zinc and silver was well known even in ancient times. New directions in study of those composites have appeared due to recent developments in the nanotechnology, which caused an interest in investigation of the bioactivity of the metal and oxides nanoparticles for there use in industry, biotechnology and medicine [1-9].

Special attention is paid to creation of new advanced materials and coatings, resistant to the biodeteriorations or with biocidal activity against the microorganisms. In this regard, an interesting approach is to utilize silica materials (silica gels, zeolites, aerogels, microspheres and etc.) as substrate for bioactive nanoparticles deposition. The bioactivity of metallic silver nanoparticles in various materials was widely studied; furthermore, the possibilities of transformation of the silver nanoparticles into the oxide form on the silica surface and influence of this process on the biocidal activities have been reported [10-16]. The antimicrobial effect of the silica matrix modified with nanoparticles of metals or ions of silver, copper, zinc has been reported [17-22]. Such materials can be used as the bioactive adsorbent alone as well as a part of polymer composites and textile fabrics with biocide properties.

Various methods of chemical modification of silica surface with silver, copper and zinc, were previously reported. However, majority of the traditional methods are multi-stage and involves the silver salt reduction to the zero valence state; formation of stabilized sol and precipitating of the obtained nanoparticles in the pores or on the silica surface [23-25]. The possibility of chemical modification of the silica surface with ammine complexes of silver, copper and zinc is extremely perspective to obtain bioactive nanocomposites [26-32]. In case of the fumed silica, this method is simple enough to obtain the composite powder with nanoscale content of the biocidal metals.

The aim of this work was to synthesize a highly dispersed solid of the precipitated silica modified with silver, copper and zinc, and to study their bioactive properties against the wide range of microorganisms (micromycetes, algae and bacteria).

Materials and methods

Precipitated silica NewSil-125 (China), silver nitrate $AgNO_3$ (GOST 1277-75, 99.7%), copper acetate monohydrate $Cu(Ac)_2{\cdot}H_2O$ (GOST 5852-70, 98%), zinc acetate tetrahydrate $Zn(Ac)_2{\cdot}4H_2O$ (GOST 5823-78 97%), and aqueous solution of ammonia 25% (Chemlaborreaktiv Ltd, Ukraine) were used to prepare modified silica. All chemicals were used without further purification.

Ammonia complexes of the metal salts were prepared by dissolving of metal salts in distilled water with further addition of the ammonia solution. Initially, the metal hydroxides precipitate, but with further addition of ammonia the insoluble hydroxide transforms into the transparent solution of metal-ammine complex i.e. silver diammine, copper tetraammine and zinc tetraammine complexes [30, 33].

Utilizing metal-ammine complexes to prepare the silica-based biocide composites allows modification of the precipitated silica with two metals at the same time. In this case, ammonia was added into solution of the two salts in distilled water until transparent solution of metal-ammine complexes was formed.

In a typical preparation of the modified silica the ammonia complex of metal was added to 3g of the precipitated silica under stirring at the room temperature in order to obtain a homogeneous suspension. Then the suspension was aged for 0.5 h and placed into the thermostatic oven at 200 °C for 2 hours to evaporate ammonia and to dry the sample.

The ratio of the initial components in the synthesis and the calculated content of the metal content in the samples are given in Table 1.

Table 1. Composition and reagent consumption for the modification of 3 g of precipitated silica*.

Sample	Initial salt	Salt content, mg	Water, ml	Aqueous solution of ammonia, ml	Metal content, wt%
№2	$AgNO_3$	50	9	1	1
№3	$AgNO_3$	100	9	1	2
№4	$Cu(Ac)_2{\cdot}H_2O$	600	8	3	5
№5	$Zn(Ac)_2{\cdot}4H_2O$	130	10	1	1
№6	$Zn(Ac)_2{\cdot}4H_2O$	660	7	4	5
№7	$AgNO_3/Cu(Ac)_2{\cdot}H_2O$	100/120	10	1.5	2/1
№8	$AgNO_3/Zn(Ac)_2{\cdot}4H_2O$	100/130	10	1.8	2/1
№9	$Cu(Ac)_2{\cdot}H_2O/Zn(Ac)_2{\cdot}4H_2O$	120/130	8.5	1.5	1/1

*Sample №1 is the initial precipitated silica

NewSil-125 is a white powder with silica content over 98% and values of specific surface area is about 110-140 m^2/g. Surface silanol groups of the silica react with amino complexes of the metals in the aqueous solution via ion exchange reaction with the formation of the surface metal amino compounds. A thermal treatment at 200 °C leads to evaporation of ammonia and the subsequent cooling in the ambient condition forms the hydroxo-complexes or chemisorbed molecules of metal hydroxide on the silica surface [30-32]. Obtained samples of silica modified with silver, copper and zinc were tested for biocidal activity against micromycetes of phytopathogenic bacteria and algae. The pristine precipitated silica was used as a blank test (sample №1).

In this research 12 cultures of microscopic funguses (8 of them with corrosion activity (*Penicillium funiculosum* 16721, *P. chrysogenum* 16719, *P. aurantiogriseum* 16244, *Aspergillus terreus 16184*, *A. niger* 73001, *Aspergillus oryzae* 16718, *Paecilomyces variotii* 16724 and *Trichoderma viride*16516) and 4 with toxic activity (*P. urticae* 811, *Fusarium oxysporum* 220, *Myrothecium verrucaria* 324 and *Stachybotrys chartarum 526*)) were used to test the biocidal properties of synthesized composites. *Stachybotrys chartarum* 526 is known as the initiator of the so-called "sick building syndrome" appearance [34]. Along with this, three strains of pathogenic bacteria and three strains of green algae were tested too. The cultures of microscopic funguses and phytopathogenic bacteria are from Ukrainian Collection of Microorganisms (UCM), and cultures of green algae are from National Herbarium of Ukraine (NHU).

Biocidal activity was determined by the wells method in Petri dishes using appropriate agar medium at 26 ± 1 °C. Test cultures were introduced into the molten broth (≈40°C) in amounts of $\times10^6$, 1×10^6 and 1×10^2 cells/ml, for fungi, green algae and phytopathogenic bacteria, respectively.

Nanocomposites (10 mg of the powder) were placed in holes on the agar surface in Petri dish. All experiments were performed under sterile conditions and in triplicate. The biocidal efficacy was assessed by measuring of the diameter of the zones of inhibition of culture growth (bright zone) and expressed in mm as the average for three experiments (Table 2 and 3).

Results and discussion

According to the obtained data (Table 2), the highest inhibition effect on the micromycetes growth has a silver/copper and silver/zinc nanocomposites (samples №№ 2, 3, 7, 8). It should be noted that nanocomposites with high concentration of zinc (sample № 6) shows the selective activity against toxigenic strains of *P. urticae* 811 and *M. verrucaria* 324. Moreover, those samples act equally according to the corrosion-active and to the toxigenic strains. Slightly greater sensitivity in the investigated micromycetes was established for *Penicillium* and *Aspergillus.* High sensitivity of *Penicillium* and *Trichoderma* strains as to copper (sample №4) and zinc (sample№6) must be noted too.

Table 2. Antifungal activity of silica nanocomposites containing surface compounds of silver, copper and zinc (diameter of the zones of inhibition, mm)

Sample	*Penicillium funiculosum* 16721	*P. chryso-genum* 16719	*P. aurantio-griseum* 16244	*Aspergillus terreus* 16184	*A. niger* 73001	*A. oryzae* 16718	*Paecilomyces variotii* 16724	*Trichoderma viride* 16516	*Penicillium urticae* 811	*Fusarium oxysporum* 220	*Myrothecium verrucaria* 324	*Stachybotrys chartarum* 526
№1 (silica)	0	0	0	0	0	0	0	0	0	0	0	0
№2	30	22	32	18	22	15	16	20	36	18	16	31
№3	33	20	46	26	26	16	19	22	32	28	18	30
№4	15	0	20	0	0	0	0	16	35	24	0	21
№5	0	0	0	0	0	0	0	0	0	0	0	0
№6	20	0	0	0	0	0	0	0	30	0	23	0
№7	52	45	51	28	30	25	30	30	49	23	28	39
№8	30	25	32	22	23	19	20	26	42	25	26	28
№9	0	0	0	0	20	0	0	0	28	0	0	0

The improved biocidal activities of the mix-metal samples can be explained by differ in biostatic action. In particular, if the silver composites are characterized by biocidal activity, the others have static activity. It has to be noted that, the active agents together with the suppression of fungal growth inhibited sporogenesis. Using silver together with copper or zinc compounds in the sample synthesis increases the biocidal activity of the final silica composites. All these characteristics of active composites can be utilized in prospective protective agents by incorporating of such composite within the building element structure or into the anticorrosion coating. It opens a way to depress the growth of the *Stachybotrys chartarum* fungus and to protect the buildings from the so-called "sick building syndrome" by treatment with active nanocomposites.

High activity of nanocomposites was shown against to phytopathogenic bacteria and green algae (Table 3), which also gives an opportunity to use them to treat the agricultural crops diseases. A green algae overgrowth of the structures and communications within the buildings at high humidity also can be averted by the active nanocomposites application.

Table 3. Biocidal activity of silica nanocomposites containing surface compounds of silver, copper and zinc (diameter of the zones of inhibition, mm).

Sample	*Pectobacterium carotovorum* 8636	*Pseudomonas syringae pv. syringae* 8523	*Agrobacterium tumefaciens* 8628	*Chlorella vulgaris* 190	*C. vulgaris* 191	*C. kessleri* 200
№1 (silica)	0	0	0	0	0	0
№2	55	25	33	34	29	24
№3	32	25	31	40	38	25
№4	19	34	20	29	28	23
№5	26	33	14	0	0	0
№6	0	0	22	29	31	27
№7	54	35	41	48	45	42
№8	47	29	34	54	44	53
№9	26	24	19	22	20	18

In prospective this work needs additional studies, in particular extending the range of test cultures and in development of technology of the nanocomposites application.

Conclusions

A high biocidal efficiency of the precipitated silica modified with silver, copper and zinc has been shown. An increase in the biocidal activity of the modified silica when silver is combined with copper or zinc was discovered.

The resulting material can be used as filling compounds for organic and inorganic polymer coatings of the building constructions imparting a high bio-resistance.

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БИОЦИДНАЯ АКТИВНОСТЬ ОСАЖДЕННОГО КРЕМНЕЗЕМА С ПОВЕРХНОСНЫМИ СОЕДИНЕНИЯМИ Ag, Cu И Zn

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Модифицированием поверхности высокодисперсного кремнезема амино-комплексами серебра, меди и цинка из водного раствора с последующей термообработкой, синтезированы кремнеземные нанокомпозиты, содержащие поверхностные соединения серебра, меди и цинка. Концентрация серебра в образцах составляла 1–2 %, а меди и цинка – 1–5 %. Биоцидная активность полученных композитов была изучена по отношению к 18 различным штаммам микромицетов, фитопатогенных бактерий и водорослей. Установлено, что наибольшая эффективность наблюдается в образцах кремнезема, модифицированного серебром/медью, а также серебром/цинком.

БІОЦИДНА АКТИВНІСТЬ ОСАДЖЕНОГО КРЕМНЕЗЕМУ З ПОВЕРХНЕВИМИ СПОЛУКАМИ Ag, Cu ТА Zn

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Модифікуванням поверхні високодисперсного кремнезему аміно-комплексами срібла, міді та цинку з водного розчину з наступною термообробкою, синтезовано кремнеземні нанокомпозити, що містять поверхневі сполуки срібла, міді та цинку. Концентрація срібла в зразках становила 1–2 %, а міді та цинку – 1–5 %. Біоцидну активність одержаних композитів вивчали по відношенню до 18 різних штамів мікроміцетів, фітопатогенних бактерій і водоростей. Встановлено, що найбільша ефективність спостерігається в зразках кремнезему, модифікованого сріблом/міддю, а також сріблом/цинком.