ORIGINAL PAPER

Biomass derived silica containing products for removal of microorganisms from water

L. Zemnukhova · U. Kharchenko · I. Beleneva

Abstract The research is potentially attractive for converting silica rich biomass into useful materials. Silica containing specimens prepared by the thermal method and deposition from rice production waste (straw and husk) were evaluated for their ability to remove different strains of microorganisms from the water. The strains of microorganisms with different cell shape and size were chosen for investigation: *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and freshwater heterotrophic bacterial association. The results obtained have been discussed as compared to the physical and chemical parameters of the sorbents: their composition, specific surface value, pore size, zeta potential value and surface hydrophobicity. Our study showed that removal efficiency of SiO_2 specimens and alumosilicate sample prepared from rice husk to different strains of microorganisms was higher that of other specimens including commercial sorbent. The use of various processing schemes for rice production waste let to obtain efficient selective sorbents.

Keywords Rice husk · Mesoporous silica · Water purification · Microorganism removal

Introduction

Source water may contain microbiological and chemical contaminants that must be removed and/or inactivated during water treatment. A continuous increase of water quality standards imposes the development of both new water treatment methods and the optimization of well-known ones. Among various water treatment methods (flotation, precipitation, coagulation, evaporation, reverse osmosis, ion exchange, electrolysis and others) adsorption is supposed as the best one due to its inexpensiveness and easy operation (Ali and Gupta 2007; Ali 2012; Ali et al. 2012).

Since 1940s, activated carbon has been the main water industry's standard adsorbent for the purification of municipal and industrial wastewater. But high cost of production and difficulties in the regeneration of activated carbon limit the use of activated carbon for direct treatment of liquid industrial waste at large scale. It was shown that scrap tires, bark, tannin-rich materials, saw dust, petroleum wastes, rice hulls (RHs), fruit stones, coconut shell, peat moss, chitosan, algae, seaweeds, seafood processing wastes, resins can be used as organic precursors for development of low-cost adsorbents (Ali and Gupta 2007; Ali 2010; Ali et al. 2012). These adsorbents have been found to remove toxic metal ions and organic pollutants.

The involvement of renewable vegetable raw materials including great volumes of agricultural annual plant waste is an actual task. The principal quality of such raw material consists in its yearly reproduction, low cost and almost constant chemical composition for the same plant kind. Thus, multitonnage rice production waste is a perspective raw material for obtaining of amorphous silicon dioxide (silica) that was proved by researches from various countries (Della et al. 2002; Sergienko et al. 2004; Foo and Hameed 2009; Ahmaruzzaman and Gupta 2011). However, physicochemical properties of non-crystalline phases of biogenic SiO_2 are insufficiently studied that also may hinder from elaboration of ecologically and economically acceptable

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technologies for use of silica obtained from agricultural raw materials.

The abundance, availability and low cost of rice by-products make them good adsorbents for the removal of pollutants from wastewaters. It is known that thanks to high SiO_2 contents in rice straw (RS) and husk the products of their processing are efficient sorbents of heavy metals (Chuah et al. 2005), phenols (Mbui et al. 2002), pesticides (Hsu and Pan 2007), surfactants (Hosseinnia et al. 2006), fatty acids (Farook and Ravendran 2000; Kim et al. 2008) and other organic compounds. Literature data on adsorption of microorganisms by the products of the rice production waste processing are practically absent, although it would be logical to suppose that these products must have high adsorption ability relative to microbe populations.

Interaction of microorganisms with solid materials is widely distributed in nature. This phenomenon attracts attention of the scientists that is connected, first of all, with possibility to influence on their physiological activity depending on the tasks, for example in biotechnology, as well as with perspective of purification of natural environment and human organism from undesirable microorganisms. The contact of microorganisms with solid surfaces is a result of a lot of physical, chemical and biological factors; the surface character is the most important among them. The interaction of microorganisms with zeolites (Kubota et al. 2008), clay minerals (Jiang et al. 2007; Rong et al. 2010), metal oxide (Li and Logan 2004; Rong et al. 2010), quartz, hematite and other minerals (Mills et al. 1994; Johnson and Logan 1996; Knapp et al. 1998) was determined in detail. There are some literature data on the interaction of bacteria (mainly yeast, nitrogen-fixing and methanotrophs) with such synthetic adsorbent as high-dispersed silicon dioxide and its modified forms (Kurdish and Chuiko 2003). Information on the microorganisms interaction with biogenic forms of amorphous silicon dioxide which might be obtained from multitonnage renewable rice production waste was not revealed.

The present work is dedicated to investigation of the removal and adsorption interaction of microorganisms with silica substances prepared from rice husk and straw as potential material for water purification. The research was carried out in the Institute of Chemistry FEB RAS in January–March 2013.

Materials and methods

Initial raw materials and preparation of silica substances

Husk and straw of the rice were preliminary prepared by the following way: the husk was sieved and the fraction with the particles not less than 2 mm was used; the straw was ground to the particles of 10–50 mm length; the material was washed with water and dried in the atmospheric air. The silica specimens were prepared by the schemes described earlier (Zemnukhova et al. 2005, 2006):

Scheme I—a weighed amount of the raw material was first ashed at 300 °C for removal of volatile substances and then was exposed to thermolysis at 600 °C;

Scheme II—a weighed amount of the raw material treated with 0.1 N hydrochloric acid solution at 90 °C for 1 h, filtered, washed with water, dried and then carbonized and annealed in a muffle furnace in air at 750 °C to the constant mass;

Scheme III—a weighed amount of the raw material was treated with 0.1 N sodium hydroxide solution at 90 °C for 1 h; the residue was separated from the solution; the latter was then used for deposition of silicon dioxide with concentrated hydrochloric acid; the sediment was washed with water to complete removal of sodium chloride and dried at 60 °C to the constant mass;

Scheme IV—a weighed amount of the raw material was treated with 1 N potassium hydroxide solution at 90 °C for 1 h; the residue was separated from the solution; the latter was then used for deposition of sodium alumosilicate with aluminium sulfate; the sediment was washed with water to complete removal of potassium sulfate and dried at 60 °C to the constant mass.

For comparison of adsorption ability, the initial rice husk (ground at a mill) and commercial activated charcoal were also studied. The list of the samples (Nos. 1–8) and their properties are presented in Table 1. All the samples were sieved and the fraction with 0.2 mm particles was selected for investigation. The substances prepared to research were identified by the chemical, IR spectroscopy (Shimadzu FT-IR Prestige-21 Fourier spectrophotometer, the frequency range 400–4,000 cm^{-1}, vaseline oil), X-ray (Bruker D8 ADVANCE diffractometer, CuK$_\alpha$-radiation0 and thermogravimetric (Q-1000 derivatograph) methods using standard techniques described earlier (Zemnukhova et al. 2005, 2006). Specific surface values (S_{sp}) and pore distribution by their sizes were determined by nitrogen adsorption at ASAP 2020 analyzer (Micromeritics Instrument Corporation).

Bacterial strains and media

The strains of microorganisms with different cell shape and size were chosen for investigation. The following test strains stored at the culture museum of A.V. Zhirmunsky Institute of Marine Biology Far Eastern Branch of Russian Academy of Sciences were used: *Escherichia coli* ATCC 15034, *Bacillus subtilis* BKM B501, *Candida albicans*

Table 1 Sorbent specimens prepared from RH and RS

Nos.	Composition	Preparation scheme	Contents (%)				
			SiO_2	C	H_2O	S_{sp} ($m^2\ g^{-1}$)	d (nm)
1	RH		15.0	~ 40	<0.5		
2	(SiO_2 + nC) from RH	Scheme I	47.5	52.0	<0.5	260	4.2
3	SiO_2 from RH	Scheme II	99.4	–	<0.5	231	4.4
4	$SiO_2 \cdot nH_2O$ from RH	Scheme III	88.5	–	11.3	479	3.9
5	SiO_2 from RS	Scheme II	97.5	–	<0.1	35	19.2
6	$SiO_2 \cdot nH_2O$ from RS	Scheme III	90.0	–	8.9	31	20.1
7	$KAlSi_xO_y \cdot nH_2O$ from RH	Scheme IV	57.3	–	11.5	106	13.2
8*	Activated charcoal	Commercial products	<0.5	>90	–	487	4.7

* The specimen 8 contains admixture of calcium and potassium carbonates

KMM 455, *Pseudomonas aeruginosa* KMM 433 and *Staphylococcus aureus* ATCC 21027.

The strains have been cultivated on tryptose-soya agar (Caso-agar, Merck) for 24 h at 28 °C. To obtain a natural microflora association of heterotrophic bacteria, a water sample from Kai river (Nha Trang, Vietnam) was taken. Water samples (0.1 mL) were plated in Petri dishes with tryptose-soya agar and cultivation was carried out under the above-described conditions. Bacterial colonies were suspended in 0.05 M NaCl solution for obtaining of the suspension with optic density $OD_{670} = 0.300$.

Tests of removal efficiency and bacteria adsorption

Intensity of the microorganisms removal by the specimens was studied by means of bacterial suspensions filtration through a certain amount of the powder. In all tests, 0.5 cm^3 of the sample powder was placed onto a filter paper and then 10 mL of suspension containing 10^8 cells mL^{-1} was passed through it. The cell concentration in the suspension after filtration was estimated by the spectrophotometer according to the preliminary obtained calibration plots. The percentage of the adsorbed cells was calculated by the following equation:

$$I = \frac{C_0 - C_1}{C_0} \cdot 100\ \%,$$

where C_0 and C_1—concentrations of the bacterial cells before and after interaction, respectively (cells mL^{-1}).

We also carried out adsorption test where filtration could not occur. To determine the effect of contact time and pH onto microorganisms adsorption, 0.5 cm^3 of the silica sample was mixed with 20 mL of 0.05 M NaCl containing 10^8 bacterial cells mL^{-1}. The mixture was shaken for appointed time and then centrifuged at 4,000 rpm^{-1} for 15 min. The cell concentration in the suspension after centrifugation was estimated with spectrophotometer.

All experimental data of adsorption tests are given as the mean of three independent experiments with three replicates.

Determination of zeta potentials

The zeta potential of test specimens and bacteria was measured in 0.05 M NaCl at pH 7. The mass concentration of specimens was 0.2 g L^{-1} and concentration of bacteria was 10^7 CFU mL^{-1}. The particles of test powders and bacteria were dispersed on a shaker at 200 rpm for 15 min. The samples were allowed to stand for 15 min to let lager particles to settle. An aliquot taken from the supernatant was used to measure the zeta potential. The measurements were performed by means of laser Doppler electrophoresis using ZetaSizer Nano ZS (Malvern, UK). The experimental reproducibility was tested 3 times.

Determination of surface hydrophobicity

Hydrophobicity of bacteria was determined via the bacterial adhesion to hydrocarbon (BATH) test (Hollender et al. 2002). The BATH assay is based on the affinity of bacterial cells for an organic hydrocarbon; more hydrophobic bacteria display greater affinity for the hydrocarbon such as hexadecane. Bacterial cells were suspended in 0.05 M NaCl to give absorbances 0.4 at 600 nm (A_0). To 2 mL of cell suspension, 0.5 mL of hexadecane was added and the suspension was vortexed for 30 s. After each phase was allowed to separate for 10 min, the absorbance (A_t) of the aqueous phase was measured. Percentage hydrophobicities were determined from:

$$\frac{A_0 - A_t}{A_0} \cdot 100\ \%.$$

This method was applied to silica and charcoal samples.

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Results and discussion

Characterization of test specimens

According to the X-ray data, the specimens Nos. 2–8 are amorphous; the sample No. 8 contains a small admixture of potassium and calcium carbonate. The data of Table 1 show that SiO_2 contents in the specimens (except No. 8) vary from 15 to 99.4 % Carbon contents in the samples No. 1 and No. 2 equal approximately 40–52 %. Water contents determined by the thermogravimetric method in all the silica specimens depend on the initial raw material and the substance preparation method and are in the range approximately 0.1–11 % (Table 1). All the samples are mesoporous: the pore diameter varies from 3.9 to 20.1 nm. However, it should be noted that all silica have a little amount of macro- and micro-pores that results in various
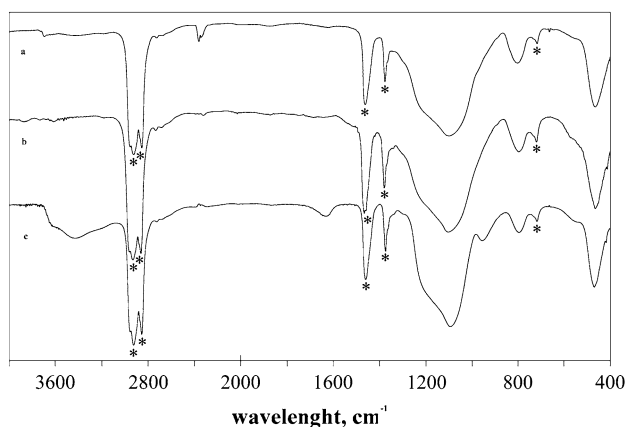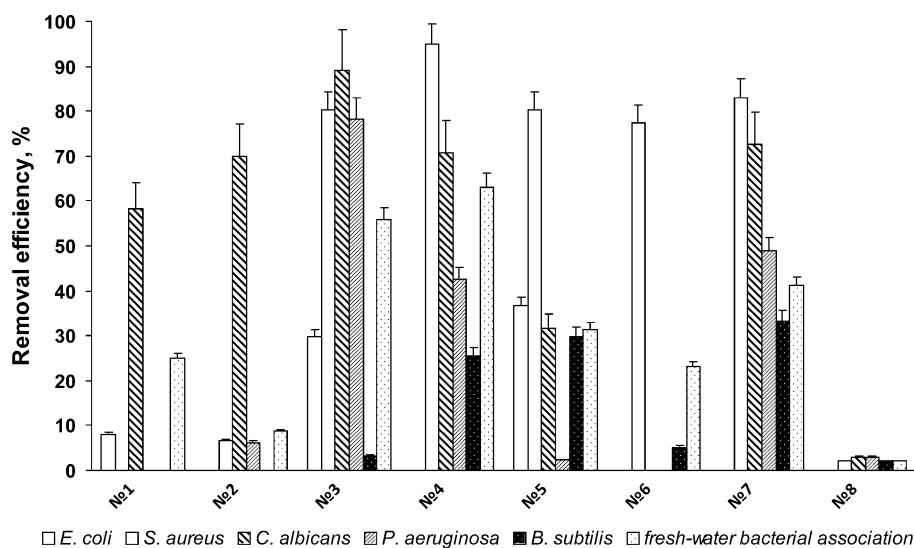

Fig. 1 FT-IR spectrum of amorphous silica from rice husk (*a* No. 3) and straw (*b* No. 5) prepared by the scheme II and deposited by the scheme III (*c* No. 4)

defects in the porous structure of the amorphous specimens obtained from rice waste. Amorphous silica specimens from rice husk have the most homogeneous pores (Zemnukhova et al. 2012). The specific surface values differ in considerable degree: the samples Nos. 2–4 and No. 7 prepared from RH have the largest values (106–479 $m^2 g^{-1}$). The specimens No. 5 and No. 6 obtained from RS have a lower S_{sp} value (<40 $m^2 g^{-1}$).

Analysis of IR absorption spectra of the specimens Nos. 2–6 showed the presence of the adsorption bands at 467–470, 798–804 and 1,095–1,101 cm^{-1} related to the valent and deformation vibrations of Si–O–Si siloxane bonds in amorphous silicon dioxide. The typical IR absorption spectra of amorphous silica prepared from rice husk and straw by the scheme II and via precipitation by the scheme III are shown in Fig. 1. The spectra of the specimens No. 2 and No. 3 also display slightly intensive bands at 3,182, 3,400 and 3,694 (valent) and 1,624 cm^{-1} (deformation) (Fig. 1a) typical to vibrations of O–H bonds and molecules of sorbed water; the bend at ∼958 cm^{-1} indicates to insignificant amount of Si–OH silanol bonds. The spectra of the sample No. 5 prepared from RS by the same way (Fig. 1b) has neither such bands nor bend. The IR spectra of the silica samples prepared from rice husk and straw by the scheme III differ from the first two specimens in the presence of the band of middle intensity at 958 cm^{-1} showing straightly to vibrations of Si–OH silanol bond and in more intensive bands of O–H bonds at 3,182, 3,431, 3,645 and 1,637 cm^{-1} (Fig. 1c). The IR spectra of the specimens studied correlate with the contents of sorbed water (Table 1). Thus, the specimens No. 4 and No. 6 are characterized by a larger number of Si–OH silanol bonds than samples No. 2 and No. 3 and the specimen No. 5—by their complete absence. The results show that chemical composition and surface parameters of the

Fig. 2 Removal efficiency of the specimens relative to different microorganisms

□ *E. coli* □ *S. aureus* ⊠ *C. albicans* ▨ *P. aeruginosa* ■ *B. subtilis* □ *fresh-water bacterial association*

Fig. 3 Effect of contact time on adsorption *E. coli* and freshwater bacterial association
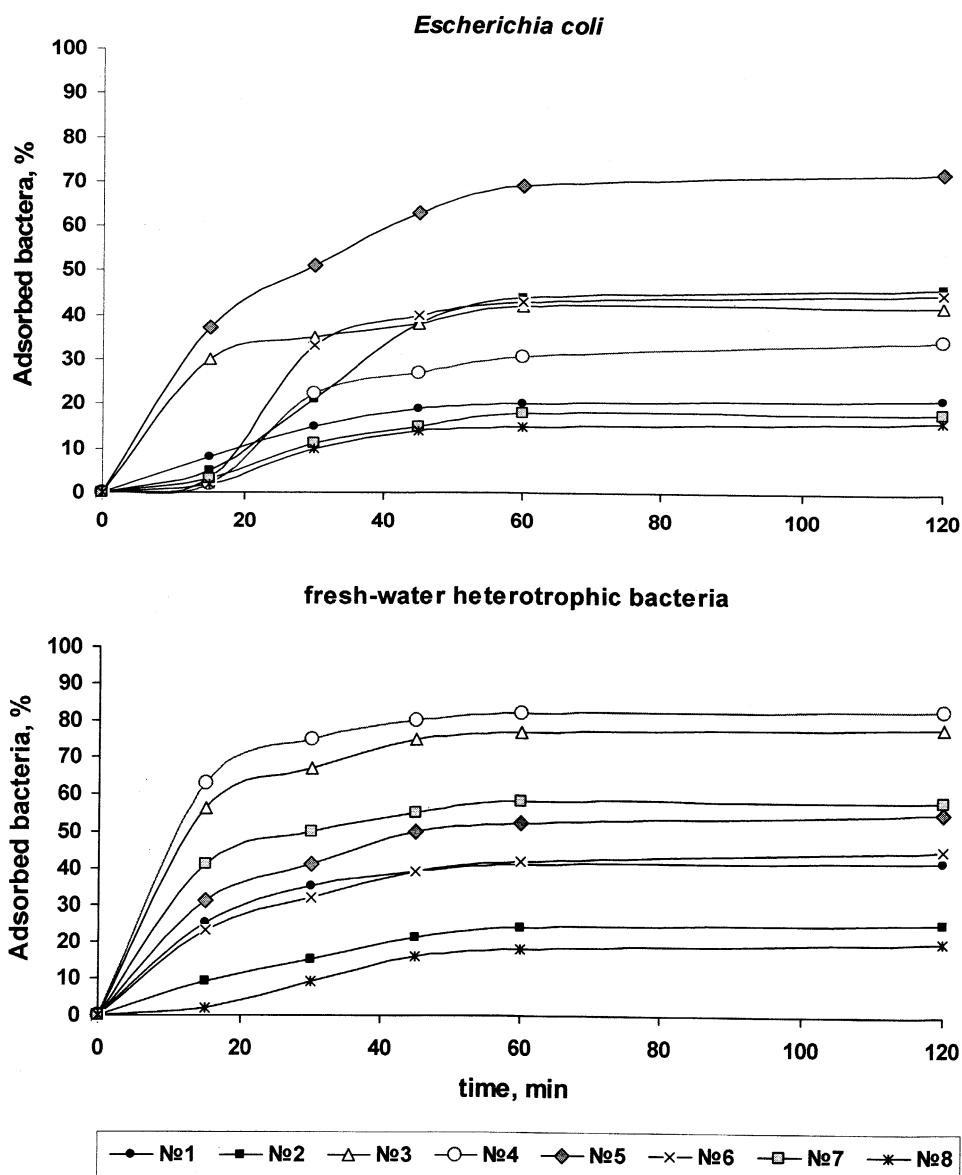
amorphous silicon dioxide depend on both the kind of initial raw material and the method of a substance preparation from it.

Removal of microbial cells from the water

The results of the microorganisms removal efficiency of specimens are presented in Fig. 2. As it can be shown, the adsorption spectra of these specimens to different microorganisms are not identical. The specimens No. 3 and No. 5 adsorbed all five strains of microorganisms and freshwater bacterial association. The sample No. 3 sorbed 30 % of *E. coli*, 80 % of *S. aureus*, 89 % of *C. albicans*, 78 % of *P. aeruginosa*, 55 % of association and only 3 % of *B. subtilis*. The specimen No. 5 has a higher adsorption ability to

E. coli and *B. subtilis* as compared to No. 3 but much slighter to other microorganisms.

The samples No. 4 and No. 7 demonstrated adsorption ability to four strains and microbial association being quite inert to fecal indicator bacteria *E. coli*. It should be noted that the removal efficiency of commercial activated charcoal No. 8 was very weak. It may be connected with the experimental conditions: a short time of the microorganisms interaction with the adsorbent did not allow to the former to be fixed onto the carbon material surface.

The comparison and the analysis of data presented in Table 1 and Fig. 2 showed that there are some difficulties in establishing of the correlation between physical and chemical parameters of silica samples and their adsorptive characteristics in relation to bacteria. As described by

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Stevik et al. (2004), the mechanisms involved in the removal of microorganisms from the water through porous media are straining and adsorption. Straining is determined by the size of the material and the bacterial cells. Larger cells are removed effectively by filtration, whereas smaller cells can penetrate deeper into the material. The results presented in Fig. 2 show that removal efficiency of *S. aureus* cells with size of less 1 μm and *C. albicans* cells with size of up to 10 μm was much higher than others.

In order to determine the intensity of adsorption interaction between microorganisms and silica and carbon samples, we carried out the batch adhesion tests under various time of contact and pH conditions and estimated the zeta potentials and surface hydrophobicity of samples and cells.

Adsorption kinetic was performed for all studied strains at pH 7.0. Results showed that maximal adsorption of microorganisms on samples occurred within the initial 30 min for *B. subtilis*, *C. albicans* and *S. aureus,* and no significant increase in adsorption was observed after this time (results not presented). Adsorption of *E. coli*, *P. aeruginosa* and freshwater bacterial association was completed after 60 min. Figure 3 shows results of adsorption kinetic for *E. coli* and freshwater heterotrophic bacteria association. These data are conformed to results presented in Fig. 2, testifying high removal efficiency of sample No. 5 for *E. coli* cells and samples No. 3 and No. 4 for bacteria association. The removal efficiency of carbon sample No. 8 is within 15–18 % after 1 h of bacteria cells contact with it.

It is known that in the process of the microorganisms interaction with solid surfaces, the important role belongs to electrostatic forces which can be estimated by zeta potential value. These values for the specimens Nos. 3-6 and *E. coli* cells (Table 2) show at neutral pH, the specimens surface has negative charge as well as *E. coli* surface. Therefore, no attractive electrostatic forces could be expected in contact of the bacteria with specimens. Nevertheless, the specimens No. 3 and No. 5 sorbed 42 and 69 % microorganisms, respectively, after 1 h. The adsorption ability of samples No. 4 and No. 6 for *E. coli* was 30 and 43 %, respectively, after 1 h although their zeta potentials slightly differ from those of No. 3 and No. 5.

Some studies reported that the electrostatic interaction was an important factor for adsorption of bacteria onto dispersed materials (Jiang et al. 2007; Kubota et al. 2008), while other studies did not (Hrenovic et al. 2009, 2011). Our results show that zeta potential cannot be the parameter for predicting the adsorption of bacterial species onto disperse silica.

Surface hydrophobicities of bacterial cells and samples are presented in Table 3. The results show that samples No. 2 and No. 7 were very hydrophobic with adhesion to hexadecane at 100 and 55 %, respectively. On the other

Table 2 Zeta potentials of test specimens and *E. coli* suspension (the data provided are mean values ± standard deviation)

| Test suspension	Zeta potential (mV)
No. 3	−16.4 ± 1.2
No. 4	−9.63 ± 1.3
No. 5	−42.2 ± 1.5
No. 6	−38.9 ± 1.8
E. coli	−27.2 ± 1.1

Table 3 Percentage of silica samples and bacterial cells adhering to hexadecane

Samples	Percentage of hydrophobicity
No. 1	35 ± 5.17
No. 2	98 ± 7.41
No. 3	5 ± 0.25
No. 4	6 ± 0.33
No. 5	0
No. 6	0
No. 7	55 ± 6.15
No. 8	0
S. aureus	3 ± 0.12
E. coli	9 ± 0.25
C. albicans	20 ± 2.2
P. aeruginosa	5 ± 0.13
B. subtilis	7 ± 0.34

hand, slight changes in hydrophobicity of bacterial cells were seen. Only *C. albicans*, which was well adsorbed onto sample No. 2, showed high hydrophobicity compared with other species. Not all bacteria behaved as expected from the hexadecane test, which indicates that interactions other than hydrophobicity may also play a role in the adsorption of bacteria. It is known, that the process of bacterial attachment to solid surfaces comprises three components (bacterial surface, substratum and liquid medium), all of which have been shown to affect the thermodynamics of adhesion (van Loosdrecht et al. 1987).

The results show that the samples No. 3, No. 4 and No. 7 have the best sorption properties. These samples were chosen to determine the effect of pH on bacterial adsorption. Freshwater bacterial association *E. coli* and *B. subtilis* were used to further characterization of adsorption under various pH conditions. Figure 4 shows the extent of bacterial adsorption on the samples No. 3, No. 4 and No. 7 as a function of pH. Adsorption ability of studied samples was higher at acidic values of pH and decreased with pH from 5 to 9. The received results are confirmed by the data, testifying that pH shift into acidic values allows to reduce the energy barrier interfering interaction of microorganisms sorbents (Kurdish and Chuiko 2003; Husmark and Rönner
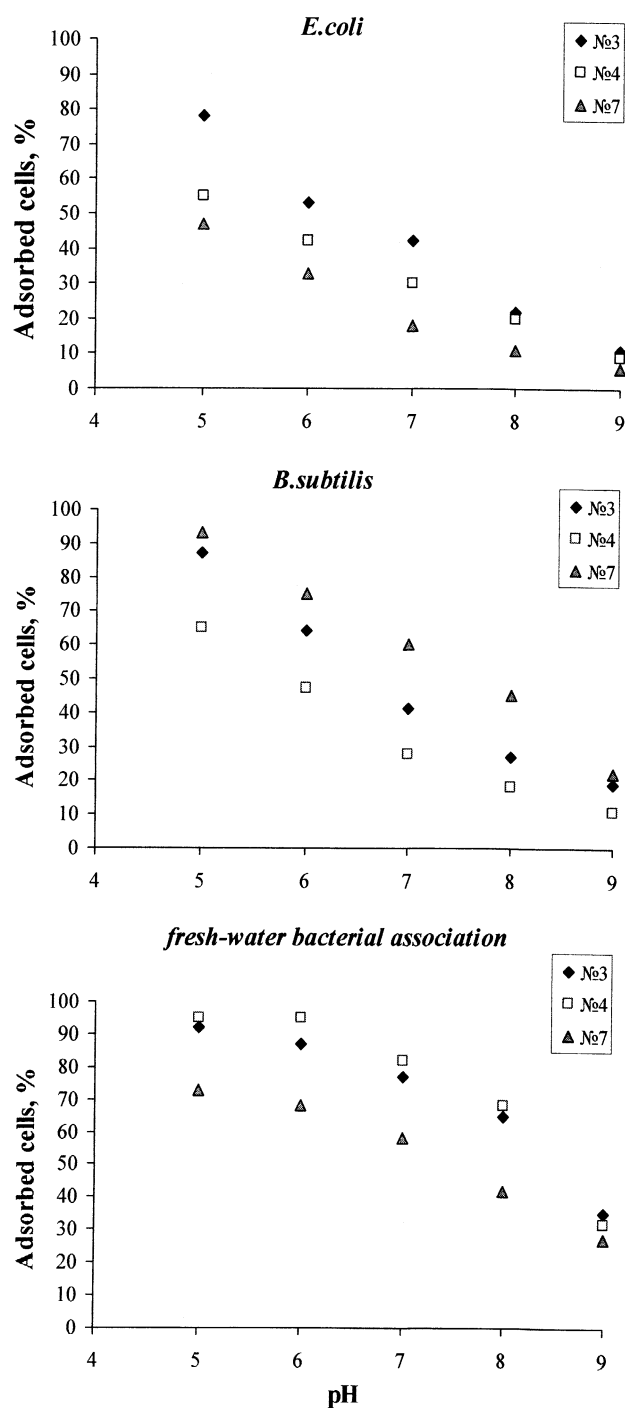
Fig. 4 Adsorption of the *E. coli*, *B. subtilis* and freshwater bacterial association onto samples No. 3, No. 4 and No. 7 under different pH conditions

1990). In the acidic electrolyte, the electrostatic repulsion is less than in the neutral and alkaline electrolyte because of the reduction of the double electric layer diffusion part.

The data presented in Fig. 4 show that the arrangement of additional conditions (decrease of solution pH)

can increase the removal efficiency of studied mesoporous samples significantly. Thus, the removal efficiency of sample No. 3 for *E. coli* and *B. subtilis* rises in two times at pH 5. For freshwater heterotrophic bacterial association, the acidification of solution leads to adsorption of 92–95 % bacterial cells to the mesoporous silica samples surface.

Conclusion

The investigated silica products from RS and husk processing have different physicochemical parameters and adsorption ability which are determined by the kind of initial raw material and the way of substance obtaining from it. Removal efficiency of SiO_2 specimens (No. 3 and No. 4) and alumosilicate sample (No. 7) prepared from rice husk to different strains of microorganisms was higher that of other specimens including commercial sorbent. The amount of bacterial cells adsorption on these samples increased with decreasing pH. Our study showed that use of various processing schemes for rice production waste let to obtain efficient selective sorbents.

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