



## EFFECT OF $Ca^{2+}$ AND $Al^{3+}$ IONS ON INTERACTION BETWEEN BACTERIA AND MINERAL SORBENTS

L. Globa, A. Gordienko, V. Lesichkov, S. Garbara

Key-words: calcium ions — aluminium ions — bacteria — mineral sorbents

Modern ways of water purification from microorganisms are not efficient enough because of their high aggregative and kinetic stability. The effect of removal of viruses and bacteria from water is increased by mineral sorbents during coagulation (1—3) and by their application as filters (4, 5). But there are still inadequate data about interaction between microorganisms and mineral sorbents.

Heterocoagulation of admixture and water clarification (6, 7) is observed in acidic medium with negative values of  $\zeta$ -potential of the mineral sorbent cells and particles being 20—30 mV. Coagulation of water admixtures with such widely used agent as aluminium sulfate is strained under these conditions. Optimum water clarification with aluminium sulfate depends on the medium ionic composition and is observed at pH 6,5—8,5 — isoelectric zone for aluminium hydroxide (1). At the same time bacterial interaction with mineral sorbents depends on the cation concentration and its type in the dispersion medium (8, 9).

So we intended to study the effect of the medium cations on bacterial interaction with mineral sorbents for destabilization of microbe dispersions.

### Material and methods

We used cultures *Escherichia coli* 163 and *Streptococcus faecalis* 134, cultivated on meat — peptone agar at 37 °C for 24 hours. Cells were then washed 3 times with distilled water in centrifuge at 6000 g for 15 minutes.

Colloid solutions of montmorillonite and palygorskite were prepared by Sabanin's method (10) from natural clays of Cherkassy deposit (USSR). Mineral fractions with particles  $\approx$  1—2 mkm and  $\ll$  1 mkm large were obtained by centrifugation.

Interactions between bacteria and minerals were studied in solutions of calcium and aluminium chlorides. Three types of samples were prepared in each experiment: two signal ones — suspensions of bacteria and mineral (with particles  $\approx$  1—2 mkm large); test ones — suspensions of bacteria in the mineral colloid solutions (with particles being  $\ll$  1 mkm large) of various concentrations. Bacteria and mineral were shaken for 15 min. Bacteria concentration remained the same —  $5 \cdot 10^7$  cell/ml. Electrophoresis rate and  $\zeta$ -potential of 50 cells of particles of mineral were determined using an apparatus for microelectrophoresis (11). Preparations for electron microscopic research were done by negative contrasting of 2 % phosphotungstic acid solutions (13).

## Results and discussion

Effect of the calcium ions content in dispersion medium on interaction of *Str. faecalis* cells and palygorskite was studied by microelectrophoresis.  $\zeta$ -potential of bacteria was found to be the same in calcium chloride solutions of  $10^{-5}$ — $10^{-4}$  mol/l at all the known concentrations of clay mineral (fig. 1). Further growth

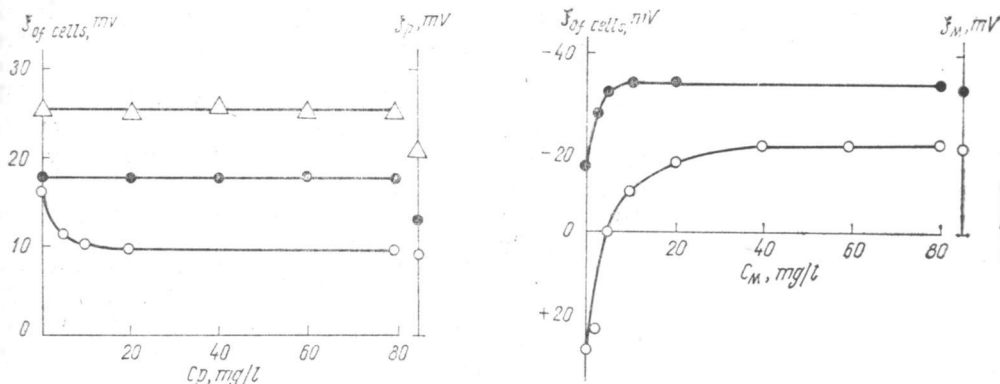


Fig. 1.  $\zeta$  — potential of *Streptococcus faecalis* 134 cells in colloid solutions of palygorskite with various concentrations ( $C_p$ ) and the palygorskite particles (P) at the calcium chloride content in the medium mol/l: ( $\Delta$ ) —  $10^{-5}$ ; ( $\bullet$ ) —  $10^{-4}$ ; (O) —  $10^{-3}$

Fig. 2.  $\zeta$  — potential of *Escherichia coli* 163 cells in colloid solutions of montmorillonite with various concentrations ( $C_M$ ) and the montmorillonite particles (M) at the aluminium chloride content in the medium, mol/l: ( $\bullet$ ) —  $10^{-6}$ ; (O) —  $10^{-5}$

of calcium chloride content to  $10^{-3}$  mol/l slightly decrease the negative  $\zeta$ -potential of the mineral with practically no changes in  $\zeta$ -potential of bacteria. But transformations in the nature of their interaction bring about concentrational growth of palygorskite in the medium along with decrease of the negative  $\zeta$ -potential of microbes. Bacteria acquire  $\zeta$ -potential which is characteristic of the mineral particles.

Interaction of *Str. faecalis* cells and palygorskite under these conditions was studied by electron microscopy. It was established that there had been no bacterial interactions with the mineral particles at  $10^{-4}$  mol/l calcium chloride concentration in the dispersion medium. Particles of palygorskite not bound with bacteria could be observed. But the rise of calcium chloride concentration to  $10^{-3}$  mol/l facilitates bacterial interaction with the mineral. Particles of palygorskite are sorbed on the bacterial cell surface, and a layer or "shell" of the mineral particles around a bacterium. In other words, bacterium-mineral complex is formed.

So, data of microelectrophoretic studies were proved by the results of electron microscopic investigation of interaction between *Str. faecalis* cells and palygorskite in calcium chloride solutions. Microorganism-mineral complex with electrokinetic properties of the mineral particles is formed due to the increased interaction within the system. The same complex are brought about by the interaction of *E. coli* cells and montmorillonite in calcium chloride solutions.

Interaction between *E. coli* cells and palygorskite in these solutions was also studied. Bacterial  $\zeta$ -potential and that of the mineral particles were disco-

vered to be rather alike. Under such conditions we failed to detect the palygorskite particles effect on electrokinetic properties of *E. coli* cells by microelectrophoresis.

Microelectrophoretic study of interaction between *E. coli* cells and montmorillonite in aluminium chloride solutions showed that higher salt concentrations in dispersion medium caused lower or neutral negative surface charge, or even re-charge of bacteria and the mineral particles. *E. coli* cells in montmorillonite solutions have the value of  $\zeta$ -potential, characteristic for the mineral particles at aluminium chloride concentration in dispersion medium two orders lower than that in calcium chloride solution (fig. 2).

We have got similar results of the study of interaction between *E. coli* cells and montmorillonite in aluminium chloride solutions and the data on the hydrogen ions effect upon the interaction in the system (6, 7). Bacterium — clay mineral complexes are formed at the negative values of  $\zeta$ -potential of cells and the sorbent particles  $< 30$  mV. But data on bacterial interaction with mineral sorbents in calcium chloride solutions differ from the results achieved in dispersion media with the above mentioned cations. This interaction isn't pronounced even at the negative values of  $\zeta$ -potential of cells and mineral particles  $< 20$  mV. Bacterium — clay mineral complexes are formed when calcium chloride concentration is  $10^{-3}$  mol/l and content of palygorskite is  $< 20$  mg/l. These data allow us to consider the given concentration of calcium chloride to be the condition for an active interaction between bacteria and mineral sorbent.

Microorganisms have greater aggregative and kinetic stability than mineral admixtures in water due to hydrophilic properties of their cells surface and electric charge (14). Dispersion of *E. coli* and *Str. faecalis* cells may be regarded as a rather stable one. We transformed these bacteria into isoelectric state by decreasing their pH and polyvalent cations in the medium (12, 15), but all this hasn't resulted in the cell coagulation. Contrary to our results, but according to other authors (14), some microorganisms may aggregate while their negative  $\zeta$ -potential is decreased to 15 mV.

We were able to destabilize microbe dispersions only in the presence of mineral sorbents. Microbe cell-mineral sorbent complexes are formed during intensive bacterial interaction with clay minerals. Surface properties of these complexes are characteristic for those of the mineral particles, and their aggregative stability is lower than that of microbe dispersion, which makes bacterial water purification by coagulation easier. Decrease of  $\zeta$ -potential of the montmorillonite particles to 10 mV is enough for the efficient mineral removal from water during coagulation in the volume.

Therefore, microorganism-mineral sorbent complexes are necessary for the microbe dispersion destabilization. We have established that the formation of such complexes is not specific and that they are formed due to application of *E. coli* and *Str. faecalis* cells (12, 15), and palygorskite or montmorillonite, which all differ from each other by their composition, structure, physical and chemical properties (16).

Composition of dispersion medium, concentration, and the type of cation in particular, has a strong effect on the efficiency of interaction between microorganisms and highly-dispersed minerals. Bacterial interaction with mineral sorbents may be controlled by changing the dispersion medium composition. Since it is possible to obtain bacterium — mineral sorbent complexes, stable microbe dispersions may now be replaced by inorganic dispersions that are better removed from water by traditional coagulation technique (1, 2).

Thus we have investigated the mechanism of interaction between microorganisms and mineral sorbents in dispersion media with various ion compositions, and have improved our knowledge of the microbe dispersion destabilization and intensification of the bacterial coagulation removal from water by using such highly-dispersed mineral sorbents as montmorillonite and palygorskite.

Natural resources of mineral sorbents are enormous and they find still wider application in water purification these days (16). Their ability to destabilize microbe dispersions in the presence of coagulating ions provides for further advance of natural and waste water treatment.

## REFERENCES 1

1. Бабенков, Е. Д. Очистка воды коагулянтами. М., Наука, 1977. 356 с. — 2. Кульский, Л. А. Теоретические основы и технология кондиционирования воды. Киев, Наукова думка, 1983. 528 с. — 3. Лесичков, В. С., Л. И. Глоба, Л. Г. Кириллова. — In: Internat Congr. Infect. Parasit. Dis., VII. Varna, 2—6. X. 1978. Reports. Vol. 3, 555—558. — 4. Капрелян, Г., В. Лесиченко, В. Русев, Н. Енчев. *Сб. науч. тр. СНРБ — клон Варна*, 1, 1977, № 1, 76—82. — 5. Rusev, V., V. Eftimova, V. Gardevska, G. Kargelyan, V. Lesichkov, N. Enchev. *Scr. sci. med.* 13, 1976, 118—120. — 6. Глоба, Л. И., А. С. Гордиенко, С. В. Гарбара, М. Н. Ротмистров, А. С. Кузема. *Хим. и технол. воды*, 1981, № 6, 549—551. — 7. Глоба, Л. И., А. С. Гордиенко, С. В. Гарбара, М. Н. Ротмистров. *Микробиол. ж.*, 1983, № 2, 22—26. — 8. Звягинцев, Д. Г. Взаимодействие микроорганизмов с твердыми поверхностями. М., Изд. Московского университета, 1973. 176 с. — 9. Кокина, А. Г., Н. А. Лукашевич, А. К. Душкевич, Н. В. Новицкая. *Гиг. и сан.*, 1978, № 7, 21—24. — 10. Коузов, П. А. Основы анализа дисперсного состава промышленных пылей и измельченных материалов. Л., Химия, 1974, 280 с. — 11. Глоба, Л. И., А. С. Гордиенко. *Мед. техн. (М.)*, 1980, № 2, 50—51. — 12. Глоба, Л. И., А. С. Гордиенко, М. Н. Ротмистров. *Хим. и технол. воды*, 1981, № 5, 466—468. — 13. Бирюзова, В. И., В. Л. Боровягин, В. П. Гилев, Н. А. Кисилев, А. С. Тихоненко, Ю. С. Ченцов. Электронно-микроскопические методы исследования биологических объектов. М., Издательство АН СССР, 1963. 204 с. — 14. Бърдаров, С., К. Марков. *Микробиология. С., Медицина и физкультура*, 1972. 508 с. — 15. Гордиенко, А. С., Л. И. Глоба, М. Н. Ротмистров. *Докл. АН Укр. ССР*, сер. Б, 1978, № 5, 426—428. — 16. Тарасевич, Ю. И. Природные сорбенты в процессах очистки воды. Киев, Наукова думка, 1981. 208 с.

## ВЛИЯНИЕ ИОНОВ $\text{Ca}^{2+}$ И $\text{Al}^{3+}$ НА ВЗАИМОДЕЙСТВИЕ БАКТЕРИЙ С МИНЕРАЛЬНЫМИ СОРБЕНТАМИ

Л. Глоба, А. Гордиенко, В. Лесичков, С. Гарбара

### РЕЗЮМЕ

Концентрация и тип катиона дисперсионной среды, а также содержание в ней глинистого минерала монтмориллонита или пalyгorskита существенно влияют на взаимодействие клеток *Escherichia coli* 163 и *Streptococcus faecalis* 134 с минеральными сорбентами. В оптимальных условиях на поверхности микробной клетки коагулируют высокодисперсные частицы минерала. Они образуют слой, окружающий бактерию в виде «оболочки». Такие комплексы бактерия — минеральный сорбент приобретают электрокинетические свойства, характерные для частиц минерала. По сравнению с бактериальными клетками, комплексы имеют пониженную агрегативную и кинетическую устойчивость. Выявлен механизм destabilизации микробных дисперсий с помощью минеральных сорбентов, перспективы использования минералов и состава дисперсионной среды для повышения эффективности коагуляционной очистки воды от микроорганизмов.