Eradication of *Listeria* from water suspensions using octadecylammonium derivatives of montmorillonite

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Summary

A series of octadecylammonium derivatives of montmorillonite (ODM) with different contents of the organic matter were prepared, characterized and tested at eradication of *Listeria innocua* from water suspensions. ODM containing octadecylammonium at 100% of the ion-exchange capacity of montmorillonite exhibited a high efficiency of eradication of *L. innocua* from water suspensions during a contact time of 10 min, with $I_{50} = 0.03$ mg·ml^{-1} at a bacterial suspension density of 10^4 CFU·ml^{-1}, and $I_{50} < 0.01$ mg·ml^{-1} at a bacterial suspension density of 10^3 CFU·ml^{-1}. The compound was effective also at devitalization of *L. innocua* in water suspensions during a contact time of 12 h, with minimum inhibitory concentration of 0.1 mg·ml^{-1} at a bacterial suspension density of 10^7 CFU·ml^{-1}. Montmorillonite modified with octadecylammonium at 20% of the ion-exchange capacity exhibited an efficiency of eradication of *L. innocua* from water suspensions during a contact time of 10 min at I_{50} of 3 mg·ml^{-1}. Because this compound sediments in water, it may be a candidate for further study at microbiological decontamination of water in food processing environments.

Keywords

montmorillonite; octadecylammonium; *Listeria*

Contaminated food processing environment, namely, food-contact surfaces, air and water, may cause contamination of food products. A food safety issue is in particular the contamination of the food processing environment by pathogenic bacteria, because this may be a main route of contamination in certain types of food products such as cheese, meat and fish products. Eradication of microorganisms from the food processing environment is important and various sanitation techniques using various sanitizing agents have been developed and implemented in the food industry. However, the currently available agents are not effective in all situations and new agents with an improved efficiency are necessary [1–4].

Organically modified clays are a group of chemicals with a wide range of properties and applications. Some of them are industrially produced in large amounts, including alkylammonium derivatives of montmorillonite. These have been developed already in 1940s [5, 6] and are used as antisedimentation agents. Besides other properties, they have been found to be effective at devitalization of bacteria [7]. Out of alkylammonium derivatives of montmorillonite, we focused on octadecylammonium derivatives, which are well defined from the structural point of view, and are cheap to produce. Octadecylammonium salts exhibit moderate antimicrobial properties [8], but bound in the form of organoclay are thought to act rather by adsorbtion [9].

In this study, a series of octadecylammonium derivatives of montmorillonite with different contents of the organic matter were prepared, characterized and tested at eradication of *Listeria innocua* from water suspensions. *L. innocua* was chosen as a model for the pathogenic species *L. monocytogenes*, which has similar properties in the environment but is safer to work with. Strains of the both *Listeria* species are frequent contaminants of the processing environment in the food industry [10, 11].

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MATERIALS AND METHODS

Octadecylammonium derivatives of montmorillonite were prepared as described previously [12]. Briefly, a suspension of montmorilllonite (particle size, 5–10 μm), separated from bentonite of Stará Kremnička – Jelšový Potok, Slovakia by sedimentation in an automatic apparatus [13], was mixed with a solution of octadecylamine (ODA; p. a.; Lachema, Brno, Czech Republic) acidified with concentrated acetic acid at a 30% excess (based on stoichiometry). The suspension was incubated with mixing at 60 °C for 2 h and the solid product was separated by filtration, dried at 40 °C and homogenized to a fine powder using mortar and pestle. A series of octadecylammonium derivatives of montmorillonite were prepared, containing ODA at 5–100% of the ion-exchange capacity of montmorillonite. Individual products were characterized on the basis of basal spacings, determined by X-ray diffraction analysis using a Philips PW 1050 instrument (Philips, Eindhoven, The Netherlands) and computer programmes PhilMet 1.1 and Kuch D001 of Dr. Ľubomír Kuchta from Department of Inorganic Chemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia.

A commercial octadecylammonium derivative of montmorillonite, Organobentonit, was obtained from Rudné Bane, Banská Bystrica, Slovakia.

Eradication of *Listeria* cells by octadecylammonium derivatives of montmorillonite (ODM) was studied in water suspensions. *Listeria innocua* CCM 4030 was grown in Tryptone-soya-yeast extract broth (TSYEB; Oxoid, Basingstoke, United Kingdom) at 37 °C for 16–18 h with shaking and diluted with 0.9% (w/v) water solution of NaCl to a suspension density of 10^3 CFU·ml^{-1} and 10^4 CFU·ml^{-1}, respectively. A volume of 10 ml of this bacterial cell suspension was pipetted in a 15 ml tube and a weighed amount of ODM was added. The suspension was incubated at 37 °C with shaking (2 Hz) for a defined time period (usually 10 min) and centrifuged at 110*g* for 5 min. Then, 0.1 ml of the supernatant was spread on Tryptone-soya-yeast extract agar (TSYEA; Oxoid), the agar plates were incubated for 24 h at 37 °C and the colonies were counted. For control (100% for calculation of the rate of eradication), ODM was incubated in 0.9% (w/v) water solution of NaCl, centrifuged at 110*g* for 5 min and 0.1 ml of the supernatant was spread on TSYEA. Afterwards, 0.1 ml of the respective suspension of *L. innocua* was spread on the plate, the plate was incubated for 24 h at 37 °C and the colonies were counted. All experiments were carried out in duplicate.

RESULTS AND DISCUSSION

A series of octadecylammonium derivatives of montmorillonite (ODM) containing octadecylammonium (ODA) at 5–100% of the ion-exchange capacity of montmorillonite were prepared. ODM containing 5% (w/w) ODA (designated ODM-5), 10% (w/w) ODA (designated ODM-10) and 20% (w/w) ODA (designated ODM-20) were powders, sedimenting in water. ODM with higher contents of ODA were non-sedimenting compounds, due to their low density and the strongly hydrophobic character. Individual ODM were characterized on the basis of determination of basal spacings (Tab. 1). The determined values of basal spacings suggest [14] that the three ODM with lower contents of ODA, i. e. 5% (w/w), 10% (w/w) and 20% (w/w) ODA, represented an organoclay complex with alkylammonium cations distributed in the interlayer space in a monolayer along the basal layer of montmorillonite. Other samples, ODM with higher contents of ODA, were heterogenous mixtures, containing an organoclay complex with a paraffin-type arrangement of the amine as the major component, and a double-layer complex as a minor component.

The prepared octadecylammonium derivatives of montmorillonite were tested at eradication of *L. innocua* cells from water suspensions. In order to eliminate the possible antimicrobial effect of unbound octadecylammonium, which might have affected the results, the supernatants from centrifuged respective ODM suspensions were used as controls throughout the experiments.

First, ODM were tested in short-term experiments. This experimental setting modelled the potential practical application of ODM at regular decontamination of food processing plants. In a preliminary experiment, effect of the duration of the contact between ODM containing ODA at 100% of the ion-exchange capacity of montmorillonite (ODM-100) and the bacterial suspen-

Tab. 1. Basal spacings (d_{001}) of octadecylammonium derivatives of montmorillonite.

Sample	ODM-5	ODM-10	ODM-20	ODM-100	Organobentonit
d_{001} [nm]	1.378	1.353	1.382	5.100	2.831

Tab. 2. Short-term eradication efficiency and long-term devitalization efficiency of unmodified montmorillonite and of octadecylammonium derivatives of montmorillonite.

Clay sample	I_{50} [mg·ml-1]		MIC [mg·ml-1]
	in a suspension of 10^4 CFU·ml-1	in a suspension of 10^3 CFU·ml-1	in a suspension of 10^7 CFU·ml-1
Unmodified montmorillonite	6	< 0.5	> 10
Organobentonit	8	0.05	1
ODM-100	0.03	< 0.01	0.1

I_{50} – the concentration bringing about 50% eradication of bacterial cells compared to the control at a contact time of 10 min, MIC – minimum inhibitory concentration at a contact time of 12 h.

Tab. 3. Short-term eradication efficiency of montmorillonite modified with various amounts of octadecylammonium.

Concentration [mg·ml-1]	Eradication efficiency [%]			
	ODM-5	ODM-10	ODM-20	ODM-100
0.5	2.69	4.81	26.85	75.05
1	4.79	12.99	32.77	87.93
2	10.46	18.82	43.68	92.69
4	23.50	36.68	58.62	100.00
8	42.76	75.00	84.62	100.00

sion on the eradication efficiency was studied. When ODM-100 concentrations of 0.05 mg·ml-1 or greater were used, measurable eradication was observed at 10 min of contact, while the prolongation of the contact up to 30 min had no significant positive effect (data not shown). Using the contact time of 10 min, eradication was studied with ODM-100, Organobentonit and unmodified montmorillonite used in different concentrations. With increasing concentrations, eradication efficiency increased, which facilitated calculation of I_{50}, the concentration bringing about 50% eradication of bacterial cells compared to the control. A similar experiment was done at the contact time prolonged to 12 h at a high density of the bacterial suspension, 10^7 CFU·ml-1, which modelled the situation at thorough decontamination of food processing plants. In this experiment, growth of the bacterial culture was assessed on the basis of turbidity and the devitalization efficiency was expressed as the minimum inhibitory concentration (MIC). Results of both short-term and long-term experiments are presented in Tab. 2. It is demonstrated that ODM-100 was the most effective from the tested clays in both applications.

However, ODM containing ODA at 100% of the ion-exchange capacity of montmorillonite (ODM-100) is non-sedimenting in water and, for practical applications, a sedimenting compound would be preferred. For this reason, ODM containing 5% (w/w) ODA (ODM-5), 10% (w/w) ODA (ODM-10) and 20% (w/w) ODA (ODM-20) were tested in a short-term contact experiment at a density of *L. innocua* suspension of 10^3 CFU·ml-1. Results summarized in Tab. 3 demonstrate that the eradication efficiency of ODM increased with the contents of ODA.

CONCLUSIONS

Octadecylammonium derivatives of montmorillonite were efficient at eradication of *L. innocua* from water suspensions during a short-term (10 min) contact, and were efficient also at devitalization during a long-term (12 h) contact. Montmorillonite modified with octadecylammonium at 20% of the ion-exchange capacity exhibited a high bacterial eradication efficiency and, being a compound sedimenting in water, may be a candidate for further study at microbiological decontamination of water in food processing environments.

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