

Integration, persistence and control of hygienically relevant bacteria in iron oxide incrustations in wells

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Abstract

Groundwater can become contaminated by hygienically relevant microorganisms and pathogens, for example, in consequence of heavy rainstorms, flooding of wells or hydraulic short circuits between surface water and groundwater. In case of an entry of hygienically relevant microorganisms into the aquifer, or by direct ingress, these organisms can reach drinking-water wells. If incrustations like oxides or oxyhydroxides of Fe(III), which represent the most common incrustation type in Germany, exist in the water well, the microorganisms encounter a very large and porous surface to colonise. Aim of the present study, therefore, was to find out if hygienically relevant bacteria can integrate and persist in ochreous incrustations, and if the water phase, in contact with the ochre, can become contaminated by those bacteria. Furthermore, the effectiveness of disinfection, using hydrogen peroxide, against hygienically relevant bacteria attached to ochre was studied.

Escherichia coli, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Legionella pneumophila* und *Aeromonas hydrophila* were selected as examples of hygienically relevant bacterial species. The persistence of those bacteria was investigated in microcosm experiments, using suspensions of ochre from dewatering and drinking water wells and, in addition, the corresponding well water, as well as in experiments with ochre containing columns under flow-through conditions. The disinfection effectiveness of hydrogen peroxide, against *E. coli*, *E. faecalis* or *P. aeruginosa* attached to ochre was tested for H₂O₂ solutions of up to 34 g/l.

Overall, a part of the population of all target bacteria survived in ochre for up to 14 days in a culturable state, both in microcosm and flow-through experiments. In microcosm experiments, *L. pneumophila* showed the least and *E. coli* the most pronounced mean reduction of culturability of about one and three log units after 14 days, respectively, both in ochre suspensions and well waters. An even larger portion of the populations of the investigated target bacteria survived under flow-through conditions, attached to ochre, as in the ochre suspension microcosms. Considering all target bacteria, the mean log reduction after 14 days was only about 0.5; *P. aeruginosa* showed no reduction at all, whereas the amount of *E. coli* decreased by 1.1 log units. Over the whole period of the experiments (14 days), target bacteria, originating from the contaminated ochre, were detected in the water phase of the ochre containing columns.

The hydrogen peroxide concentration officially recommended for well disinfection, about 150 mg/l, was ineffective against hygienically relevant bacteria (*E. coli*, *E. faecalis* or *P. aeruginosa*) attached to ochre. Even the addition of a single dose of a more than 200 times higher concentrated H₂O₂ solution (up to 34 g/l; contact time: 24 h) resulted only in a minor decline in culturability of about 0.4 to 0.9 log units of the respective bacteria. Only when hydrogen peroxide was added continuously over a time period of 24 h, an appreciable reduction in numbers of culturable target bacteria could be achieved. *E. coli* showed a

decrease of 4.4 log units after the continuous addition of hydrogen peroxide solution of 12 g/l; at higher concentrations *E. coli* was no longer detected at all by cultivation. Numbers of culturable *E. faecalis*, however, only declined 3.2 log units at most when H₂O₂ (up to 34 g/l) was added continuously. In the presence of ochre, hydrogen peroxide was rapidly decomposed catalytically. Interestingly, the presence of bacteria (*P. aeruginosa*) also caused a decrease of the H₂O₂ concentration; presumably by the production of high amounts of hydrogen peroxide scavenging enzymes which are constitutionally present in that species.

In conclusion, ochre can function as a habitat for hygienically relevant bacteria from which the waterphase in contact with the ochre is contaminated over a longer period of time. Once a well has been contaminated by hygienically relevant bacteria and ochre incrustations are present, the contaminants could not be removed completely during well rehabilitation. An eradication of bacteria of faecal origin and pathogens, attached to ochre, seems not to be achievable just by the addition of a hydrogen peroxide solution (up to 3 % (v/v) application concentration, corresponding to about 34 g/l) into the well.