Assessment of sustainable antimicrobial polymers with regard to their applicability in the food chain

Yvonne Ilg [a*], Martin Kreyenschmidt [b], Reinhard Lorenz [b], Jana Zerbe [a], Judith Kreyenschmidt [b]

[a] *Cold Chain Management Group, Institute of Animal Science, University of Bonn, Germany (yvonne.ilg@uni-bonn.de)*
[b] *Institute for Construction- and Functional Materials, University of Applied Science, Steinfurt, Germany*

ABSTRACT

The objective of this study was the investigation of sustainable antimicrobial (SAM) surfaces in terms of their ability to reduce surface bacteria in contact with food.
The antimicrobial activities of SAM polymers were analyzed by adapting the test method JIS Z 2801 (2000) for the special application in contact with perishable foods. Hence, the influence of temperature, time, bacterial strain, and food residuals on the level of antimicrobial activity was investigated. In order to compare the extent of activity, surfaces containing silver sulfadiazine were used.
Investigations showed a high antimicrobial capability of SAM polymers. The high extent of antimicrobial activity of these materials becomes obvious by comparing it with the rate of activity of surfaces containing silver sulfadiazine. At incubation temperature of 35°C, the antimicrobial properties of the surfaces containing silver increases with time, whereas at 3°C, even after 48 h only a low reduction of *Staphylococcus aureus* of 0.5 log cfu/ml was reached. In contrast to this, SAM polymers are able to reduce the surface count at both temperatures below the detection limit as early as after 2 h of incubation and against a range of organisms. Additionally high levels of antimicrobial activity were detected against a range of other pathogen and spoilage organisms, also in the presence of food residues.
The results show the high biocidal potential of SAM polymers. Thus, the material shows great potential for a wide range of applications, e.g. in the in perishable food industry. However, before introducing SAM polymers to these areas, further developments and research of the physical and chemical properties are necessary.

Keywords: sustainable antimicrobial (SAM) polymers, food, microbiocides, hygienic conditions, JIS

INTRODUCTION

Sustainable antimicrobial (SAM) polymers provide a new class of functional surfaces [2]. According to Buranasompob [1] these polymers are intrinsic antimicrobial thus do not migrate out of the surface. Thölmann et al. [3] revealed that the antimicrobial activity of these polymers is based on their helical structure and their high concentration of functional amino groups as well as their three dimensional structure (see figure 1).

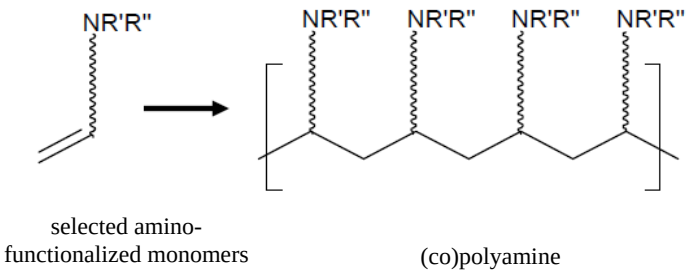

Figure 1. Principle chemical structure of SAM polymers [3].

Hewitt et al. [6] found by multi-parameter flow cytometry, that the mechanism of action is based on a progressive change in the cell physiology within the organism. They described that the exposure of the polymer directly affected the reproductive ability of the cells. The extent of this effect after a defined exposure time is specific to species. The authors discuss two different possible modes of actions, firstly the chemical reaction between functional groups at the surface and water molecules can lead to a protonation of the functional amino groups leading to a local decrease in pH-value. Secondly, electrostatic interactions can

lead to a positive charge of the SAM polymer surface [6], this cause depolarization of the cytoplasmic membrane, thus an increased permeability and finally the cell death [1].
Investigations on the toxicity of one exemplified SAM polymer prototype towards mammal cells indicate that the material is safe. The acute oral ($LD\ 50_{rat}$) toxicity in rats is > 2000 mg/kg. Furthermore, the tested polymer prototype does not cause any irritation on skin but causes irritation to the eyes [1, 3].
Previously conducted studies provide good antimicrobial properties against a wide range of microorganisms, such as spoilage and pathogen bacteria, moulds, yeasts, and different algae [1, 4]. However, until now there are no scientific data with regard to the antibacterial activity for the specific conditions encountered in the perishable food supply chain, such as low temperatures, prevalent microorganisms or the presence of food components.
Thus the objective of this study was to investigate the sustainable antimicrobial surfaces in terms of their ability to reduce surface bacteria in contact with food.

MATERIALS & METHODS

The antimicrobial activity of SAM polymer prototypes was analyzed by adapting the test method JIS Z 2801 (2000) for the special application in contact with perishable foods. Details of the microbiological method have been published by our working group [5, 7], therefore, only a brief description is provided here. The test method is based on a comparison of surface counts on reference sheets and on samples after a defined incubation period at defined temperature conditions by classical cultivation method. The influence of temperature, time, bacterial strain, and food residuals on the level of antimicrobial activity has been investigated by varying these parameters in the experiments.
For testing the general antimicrobial activity of SAM polymer samples against a range of organisms, at minimum six reference sheets and three SAM polymer sheets were inoculated with 0.4 ml of the respective bacterial solution (*Staphylococcus aureus ssp. aureus* (DSM no. 799), *Listeria monocytogenes* (DSM no. 19094), *Salmonella enterica ssp. enterica serovar typhimurium* (DSM no. 19587), *Escherichia coli* (DSM no. 1576), *Pseudomonas fluorescence* (DSM no. 304), *Bacillus cereus* (DSM no. 4321), *Aeromonas caviae* (DSM no. 30025) *and Klebsiella pneumoniae ssp. pneumonia* (DSM no. 13883), each in saline solution in a concentration of around 5,4 $\log_{10}$ cfu/ml). The influence of food components was investigated by adding different food components to the inocula. In all investigations, both types of sheets, SAM samples and references, were inoculated with the same inoculum, respectively. All sheets were covered with a foil to prevent evaporation of inoculum. Three reference sheets (depending on the test setup with pure inoculum or an inoculum containing a food component) were directly washed out with 10 ml soybean-casein digest broth with lecithin polysorbate (Roth, Karlsruhe, D) to determine the starting concentration.
The test sheets and the remaining reference sheets were washed out after 2 to 24 h incubation at 3°C or 35°C and humidity > 90 %.
The colony forming units (cfu) was raised using the pour plate method with plate count agar (Roth, Karlsruhe, D) followed by strain specific incubation temperature and time (30-37°C for 48-72 hours). Results were expressed as the number of colony forming units per millilitre.
Antibacterial activity was calculated by subtracting the arithmetic means of the logarithmic values of viable counts on coated materials from the arithmetic means on untreated materials after inoculation and incubation:

$$\log_{10}\text{ Reduction} = \log_{10}(T_{x,Re} / T_{x,Pr})$$

where $T_{x,Re}$ = arithmetic mean of bacterial concentration on reference material x hours after inoculation,
$T_{x,Pr}$ = arithmetic mean of bacterial concentration on coated material x hours after inoculation.

According to the JIS Z 2801:2000, the calculated reduction value of antimicrobial activity against the two exemplified test organisms *S. aureus* or *E. coli* had to be ≥ 2.0 $\log_{10}$ cfu/ml after 24 h of incubation at 35°C for materials to be regarded as antibacterial.

As a comparison material for the extent of antimicrobial activity, prevalently used surfaces containing silver zeolithe were applied. The value of antimicrobial activity of these materials was determined similarly as described for SAM polymer surfaces.

RESULTS & DISCUSSION

The surface count on reference materials and samples at various time intervals are shown in figure 2 (left: SAM polymers; right: materials containing silver). It is notable that on both materials, the degree of

antimicrobial activity increases with increasing time. On SAM polymer surfaces, the increasing reduction rate is predominantly caused by the increasing surface count on reference surfaces, already after 2 h of incubation at 35°C, the surface count of *S. aureus* on SAM polymer surfaces is nearly decreased to the detection limit. Whereas, on surfaces containing silver, no clear reduction of surface count is visible until 8 h of incubation. Thus, the SAM polymer surfaces act much quicker antimicrobial at 35°C compared to surfaces containing silver.
The differences in the degree of antimicrobial activity between SAM polymer materials and materials containing silver are even more significant at incubation temperature of 3°C. At this temperature SAM polymer prototypes reduces the bacterial counts of *S. aureus* about 3.7 $\log_{10}$ cfu/ml after 3 h of incubation, whereas silver containing surfaces show a very low reduction of 0.2 $\log_{10}$ cfu/ml. After 24 h of incubation at 3°C, the reduction level increases on SAM polymer surfaces up to 6.2 $\log_{10}$ cfu/ml, but on surfaces containing silver a reduction level of only 0.5 $\log_{10}$ cfu/ml is reached. These results indicate that SAM polymers are also highly antimicrobial active at the low temperature conditions; which is common for the perishable food chain.

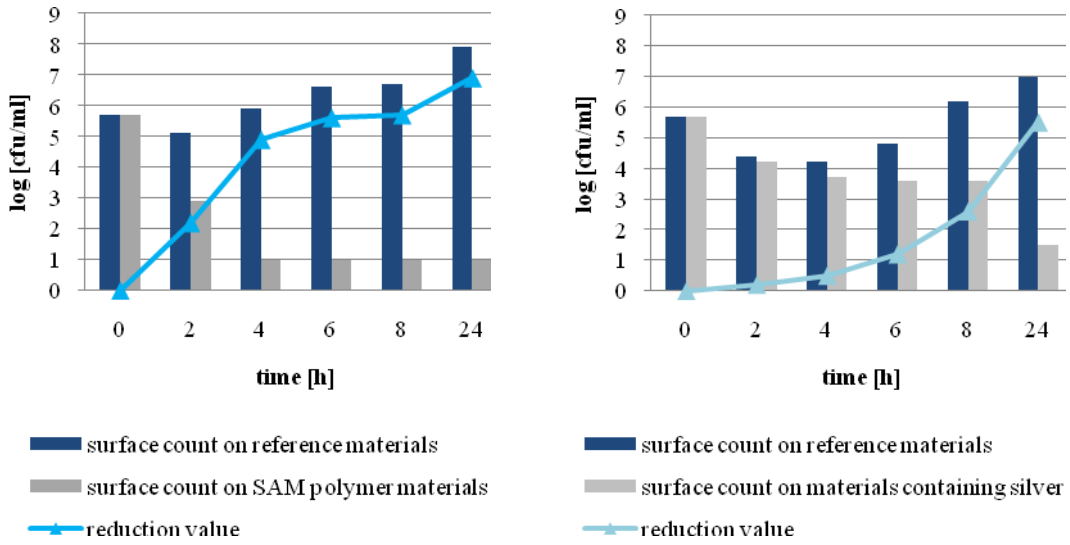

Figure 2. Reduction values in $\log_{10}$ [cfu/ml]) of *S. aureus* on SAM polymer surfaces (left) and surfaces containing silver (right) after incubation at 35°C at various time intervals.

This high level of antimicrobial activity of SAM polymers is also apparent against several other pathogens and spoilage bacteria (see figure 3). Within 2 h of incubation at 35°C, reduction levels between 1.8 and 4.8 $\log_{10}$ cfu/ml are reached against *S. aureus, K. pneumoniae, A. caviae, L. monocytogenes, S. enterica, B. cereus and E. coli*. Also while varying the inoculum, the surface count on SAM polymer surfaces is close to the detection limit of 1.0 $\log_{10}$ cfu/ml after 2 h of incubation in nearly all investigations. The exceptions were the two experiments tested with the Gram-negative, rod-shaped Enterobacteriaceae *K. pneumoniae* and *S. enterica*, where the surface counts on SAM-polymer surfaces were well above the detection limit. Nevertheless, the reduction values against these organisms are still 3.0 and 1.8 $\log_{10}$ cfu/ml after 2 h incubation, respectively.

Moreover the presence of food components affects the value of antimicrobial activity of SAM polymers in a subtly way compared to that surfaces containing silver. The investigations are still in process to determine the potential influencing parameters causing a reduced antimicrobial activity. However, as yet the tested components of the carbohydrate, fat and protein group did not reduce the degree of antimicrobial activity of the SAM polymer prototypes at all. Especially for use as packaging material, the unhindered antimicrobial activity in the presence of food components is of vital importance. Additional investigations on the interactions of SAM polymers and food component are still under research.

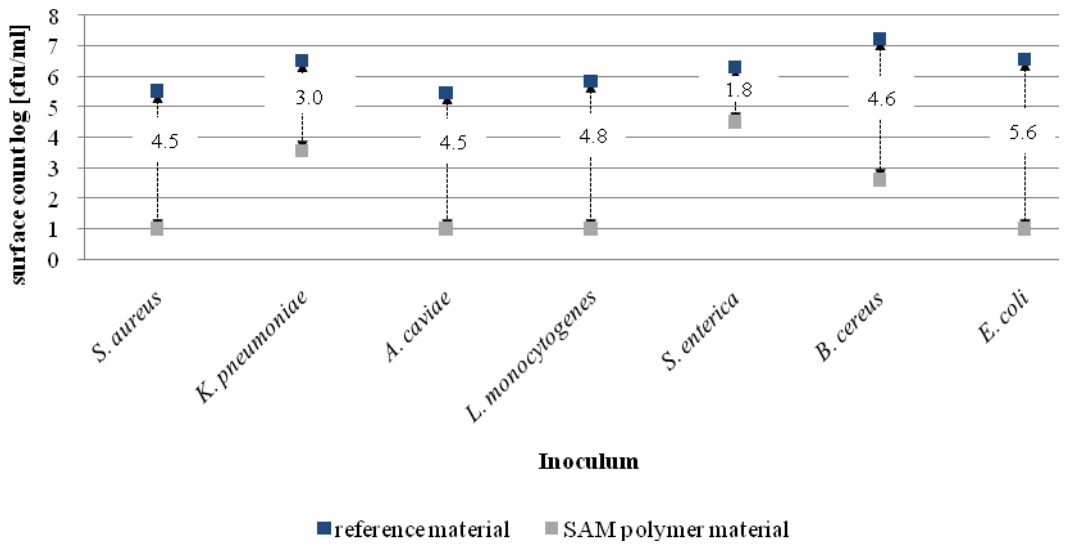

Figure 3. Reduction values (---, in $\log_{10}$ [cfu/ml]) of different bacteria on SAM polymer surfaces after 2 h of incubation at 35°C.

CONCLUSION

The results show the high reduction potential of SAM polymers, despite low temperature conditions, against a broad range of spoilage and pathogenic bacteria and in the presence of food residues.
On the other hand, the mammal toxicity of a tested SAM polymer prototype is quite low [1]. Thus the material shows great potential for a wide range of applications in perishable food industry, e.g. as food packaging materials or other food contact materials. However, before implementing SAM polymers to these areas, further developments and research of the physical and chemical properties are necessary.

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