Bactericidal Properties of Neutral Anolyte in relation to Micro-organisms Sanitary-Significant for fishing industry

L.B Mukhina, N.V. Duznik, Ye.Yu.Dmitriyeva, E.B. Altshul

In the fishing industry one of them most urgent problems is search for efficient disinfectant agents manifesting low toxicity, minor allergenic action and easily removable from treated surfaces. Scope of new disinfectant preparations allowed for application in the food industry is rather limited.

Due to the fact that ANK Anolyte is not widely used in the fishing industry yet, the objective of this study is to:

- Test bactericidal properties of ANK with respect to sanitary-significant bacteria, both on pure cultures and in the presence of albuminous load imitating impurities of albuminous nature (blood, albuminous fish remnants, slime ,and so on);
- Define minimal active chlorine content in ANK, producing bactericidal effect on sanitary-significant bacteria.

Anolyte's bactericidal ability was studied on four sanitary-significant bacterial species. The following strains were used at test-micro-organisms: Enterebacter cloaceae, Staphylococus aureus 906, Bacillus cereus BKM 687, Pseudomonas fluorescens. Three-day old bacterial cultures were used, which were incubated on slant fish peptone agar (FPA). Five ml of fish-extract broth (FEB) were loop-inoculated and placed into a thermostat for 72 hours at 30 degrees C. (Bacillus cereus and Ps. Fluorescens) and 37 degrees C. (E. cloaceae and St. aureus). As test-surfaces, materials widely used in the fish processing industry were employed: sandstone plate, Dutch tile, Stainless steel and plastic.

Nine ml of saline solution were added to 1 ml of three-day old broth culture. Two ml of this suspension were applied to 7.5x7.5 cm2 squares and dried without heating (~2 hours). Treatment of the surface was accomplished through distributing ANK Anolyte by 2 ml portion for a square. After incubation at room temperature during 40 minutes, the wet treated surface was wiped with two sterile cotton wads. The wads were put into 20 ml saline solution containing 0.1 N hyposulphite and stirred well for 1-2 minutes. The dilutions were made and inoculated on FPA surface. In experiments with organic load instead of saline solution, 9 ml of FPA were added to 1 ml of bacterial suspension.

Efficiency of ANK bactericidal action was assessed comparing the number of viable cells in the experiment and in control. It was assumed that positive effect was to be accompanied by no less than 2log 10 reduction of the amount of viable cells in the experiment as compared to control.

Of coliform bacteria, representatives of genus Enterobacter are mostly found on fish and fish products. They are not only indicators of sewage liquid pollution, but also complicate a number of piscine diseases. That is why in the given investigation of ANK bactericidal action we used the strain Enterobacter cloaceae isolated from fish products. The efficiency of ANK bactericidal action on three-day old Enterobacter cloaceae culture is presented in Table 1.

Active chlorine in ANK, mg/l	Material	Number of viable cells E. cloaceae		
		Applied, CFU/ml of prospective washing sample	Washed without ANK treatment, CFU/ml	Washed after ANK treatment, CFU/ml
250	Sandstone plate	3.5x10log6	2.6x10log4 (no protein) 2.5x10log5 (protein)	4.2x10log2 1.6x10log5

Age of culture = 72 hours at 37 degrees C.
Results after 40 minutes exposure.

Table 1 shows that the number of E. Cloaceae cells considerably declines (by 2log109 at the stage of application and drying on the tested material (sandstone plate). Bactericidal effect of ANK without organic load is rather impressive, showing 2log 10 reduction of viable cells number. In the presence of organic load, ANK bactericidal effect (active chlorine concentration 250mg/l) is negligible, and amounts to 35% of initial bactericidal number. Therefore, it may be concluded that to successfully disinfect sandstone plate surfaces seeded with ECBG (E.coli bacteria group), ANK of 250mg/l and higher concentration of active chlorine must be used.

Representatives of genus Pseudomonas are most commonly met in water, on fish and on fish product. The most widespread species of this genus is Pseudomonas fluorencens. It was Pseudomonas fluorencens that was used for studying ANK bactericidal activity. The findings of the study aimed at assessing ANK effect on tree-day old culture Ps. Fluorencens in the presence of albuminous impurities are presented in Table 2.

Active chlorine in ANK, mg/l	Material	Number of viable cells Ps. Fluorencens		
		Applied, CFU/ml of prospective washing sample	Washed without ANK treatment, CFU/ml	Washed after ANK treatment, CFU/ml
350	Sandstone plate	3.2x10log8	2.4x10log6 (protein)	< 5 (protein)

Age of culture = 72 hours at 30 degrees C.
Results after 40 minutes exposure.

Table 2 indicates that in the process of application and drying on sandstone plate 2log10 reduction in the number of viable cells in bacterial suspension Ps. Fluorencens is observed. Bactericidal effect of ANK (active chlorine 350mg/l) is considerable and adds up to 6log 10 reduction of initial bactericidal number. Summing up, we can conclude that in case of Ps. Fluorencens contamination, and in the presence of albuminous impurities ANK with 350mg/l of active chlorine can be recommended for disinfecting of sandstone plate surfaces.

Staphylococcus aureus. As a test strain, Staphylococcus aureus, strain 906 was chosen, which is advised by normative documents for assessing disinfectants‘ efficiency. The effect of ANK bactericidal action against St. aureus, strain 906, on different surfaces is given in Table 3.

Active chlorine in ANK, mg/l	Material	Number of viable cells St. aureus		
		Applied, CFU/ml of prospective washing sample	Washed without ANK treatment, CFU/ml	Washed after ANK treatment, CFU/ml
250	Sandstone plate	3.3x10log7	1.4x10log7 (no protein) 1.8x10log7 (protein)	4.2x10log2 7.5x10log6
370	Plastic	2.4x10log7	3.1x10log5 (protein)	3.5x10log1
370	Stainless steel	3.6x10log7	1.6x10log6 (protein)	<10
500	Stainless steel	2.2xlog7	1.4x10log4 (protein)	3.6x10log2

Age of culture = 72 hours at 30 degrees C.
Results after 40 minutes exposure.

The presented data reveal that the quantity of viable cells of St. aureus bacteria suspension diminishes as soon as the culture is applied on the tested material and dried on its surface: on sandstone plate – twofold, on plastic – by 2lof10 CFU/ml, on stainless steel –by 1-3log19 CFU/ml.

Bactericidal activity of ANK (250mg/l) in experiments without albuminous load on a sandstone plate surfaces results in 2log10 reduction of bacterial quantity. In the presence of albuminous load ANK bactericidal effect naturally decreases. On a sandstone plate surfaces

the initial amount of bacteria after treatment with ANK (250 mg/l) shows 1log10 reduction, that being a proof of poor effect of the given concentration of active chlorine. When ANK concentration is 400-500 mg/l, a 4log10 reduction of the initial quantity of bacteria on plastic is observed, and on stainless steel 2-5log10 reduction. Thus, the conclusion follows that in the process of ANK application for disinfecting of working surfaces and equipment, which may be heavily contaminated with St. aureus, it is advisable to use ANK with 400-500mg/l active chlorine concentration.

As a test object of endospore-forming bacteria Bacillus cereus BKM 687 was chosen, a pathogen of toxic food infections, a facultative-anaerobic bacillus. The work was performed with a mixture of vegetative cells and spores – 32% (three-day old culture incubated at 37 degrees C.). Efficiency of ANK bactericidal action in relation to B. Cereus BKM 687 on different surfaces is presented in Table 4.

Active chlorine in ANK, mg/l	Material	Number of viable cells B. cereus BKM 687		
		Applied, CFU/ml of prospective washing sample	Washed without ANK treatment, CFU/ml	Washed after ANK treatment, CFU/ml
250	Sandstone plate	2.8x10log6	3.0x10log5 (no protein) 4.2x10log5 (protein)	1.0x10log2 1.0x10log4
400	Dutch tile	1.4x10log6	2.6x10log5 (protein)	1.7x10log2
400	Stainless steel	8.4x10log5	2.5x10log3 (protein)	2.2x10log3
450	Stainless steel	2.2x10log6	1.8x10log3 (protein)	1.3x10log3

Age of culture = 72 hours at 37 degrees C.
Results after 40 minutes exposure.

The above results demonstrate that the quantity of viable cells and spores of B. cereus suspension begins to decline immediately after application and drying on tested materials: on sandstone plate – by 1log10 CFU/ml, on Dutch tile – by 2log10 CFU/ml, and on stainless steel – by 2-3log10 CFU/ml.

ANK (250mg/l) bactericidal effect with no organic load on sandstone plate is considerable and is followed by 3log10 reduction of bacteria quantity in the experiment. In the presence of organic load ANK bactericidal efficiency falls. So, initial number of bacteria after ANK treatment on sandstone plate decreases by 40 times, and on Dutch tile, by 10 times. On stainless steel ANK bactericidal effect is minimal: maximum initial active chlorine concentration of 400-500mg/l results in 10-25% decrease of bacterial number.
Therefore, it can be concluded that for disinfecting of working surfaces and equipment possible seeded with vegetative cells and bacillus spores ANK of the following active chlorine concentrations can be recommended:

- To treat sandstone plate and Dutch tile 250...400mg/l
- To disinfect stainless steel it is advised to use other more efficient disinfectant agents.

It can be stated that ANK of 250...500mg/l concentration is highly efficient in disinfecting of working surfaces (sandstone plate, ceramic tile, stainless steel, plastic) from ECBG, Pseudomonas, Staphylococcus aureus, vegetative cells of sporulating bacteria, and can be successfully used for this purpose in the fishing industry. Disinfecting of working surfaces by ANK (400-500mg/l) in case of contamination with bacterial spores is low-efficient without preliminary treatment of the working surface with detergents for removal albuminous soiling.

Michel van Schaik,
Aquastel