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TRR: 02022-0090915441

Inštitut za fizikalno biologijo
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Determination of shelf life of fresh water in unused new Flaska glass bottle

FINAL REPORT

Reference according with offer No. P2107-1/11 dated 21/7/2011

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Ljubljana, 09.12.2011

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Aims of the study

In water samples from Flaska glass bottle we determined total nucleic acid concentration (DNA and RNA) using modern molecular techniques. Samples of water from Flaska bottle were taken periodically within 1 week interval. Based on measurement data we determined microbial growth dynamics and estimated viability of bacterial communites through days. In addition we defined standard microbiological parameters using traditional culturing techniques. From all the results obtained we assessed the shelf life of fresh water in new, unused Flaska bottle.

Methods

For the assessment of fresh water shelf life in new, unused Flaska bottle we observed bacterial growth in the water for 7 days. We used the same fresh water from bore hole (Dana company) for one step of washing and subsequent filling of the bottles. Bottles were incubated at room temperature in dark covered with aluminium foil. For DNA and RNA concentration measurements we filtered 1 L of water (two 0,5L-bottles) for each of three parallel measurements through 0,2 µm Millipore filter. After filtration filters were stored at -20 °C. We repeated that protocol everyday for 7 days. In parallel we performed traditional microbiological tests in which we determined the number of colony forming units (CFU) grown at 22 °C and 37 °C on nutrient agar (NA) plates.

Measurement of nucleic acid concentration

We isolated nucleic acids from samples (cells captured on filters) using SmartHelix[1, 2] method that was developed in our laboratory. We prepared nucleic acids for concentration measurements by using Quant-iTTM dsDNA HS Assay Kit and Quant-iTTM RNA Assay Kit (Invitrogen). Concentrations were measured with Qubit device (Invitrogen) in 2 parallels for each sample. Concentration of DNA gives information about total amount of microorganisms present, whereas the viability of microbial cells can be estimated from the RNA to DNA ratio.

Traditional microbiological tests

We distributed 100 µl of water sample equally on NA plate in 3 parallels per sample. Plates were incubated at 22 °C and 37 °C for 72 hours. After incubation the CFUs were counted and averaged over the 3 parallels. Finally concentration of microbial cells was calculated from known sample volume.

Results

Traditonal microbiological tests

Table 1: overall count of microorganisms at 22 °C and 37 °C

	22 °C					37 °C			
	1	2	A	CFU/ml	SD	1	2	A	CFU/ml
Day 1	0	1	0,5	**5**	7,1	0	1	0,5	**5**
Day 2	4	13	8,5	**85**	63,6	0	0	0	**0**
Day 3	22	21	21,5	**215**	7,1	0	1	0,5	**5**
Day 4	30	28	29	**290**	14,1	0	0	0	**0**

A- average value of duplicates

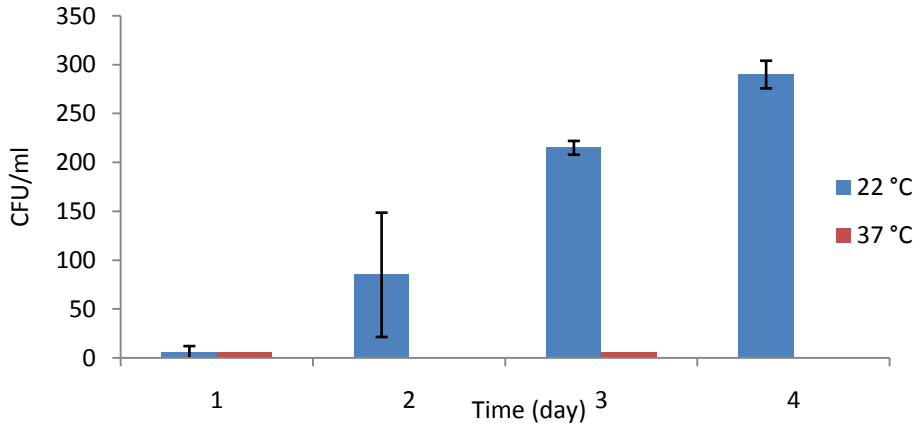

Figure 1: overall count of microorganisms at 22 °C and 37 °C

After day 4 we did not monitor the total number of microbial cells (CFUs) anymore because at that time it already exceeded the limit of 100 CFU/ml proposed by the national regulative for quality of potable water (annex 1).

Measurement of DNA and RNA concentrations

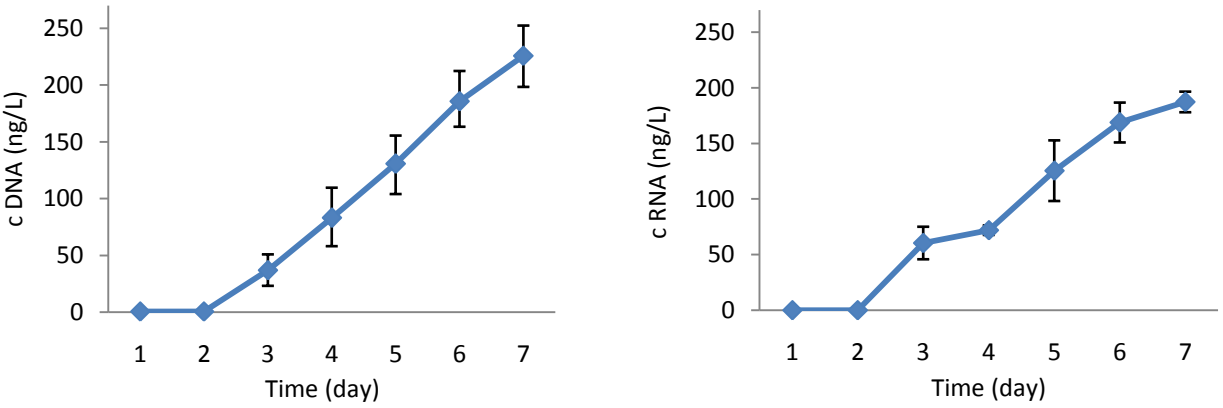

Figure 1: amount of DNA (left) and RNA (right) in water samples in ng/l by day

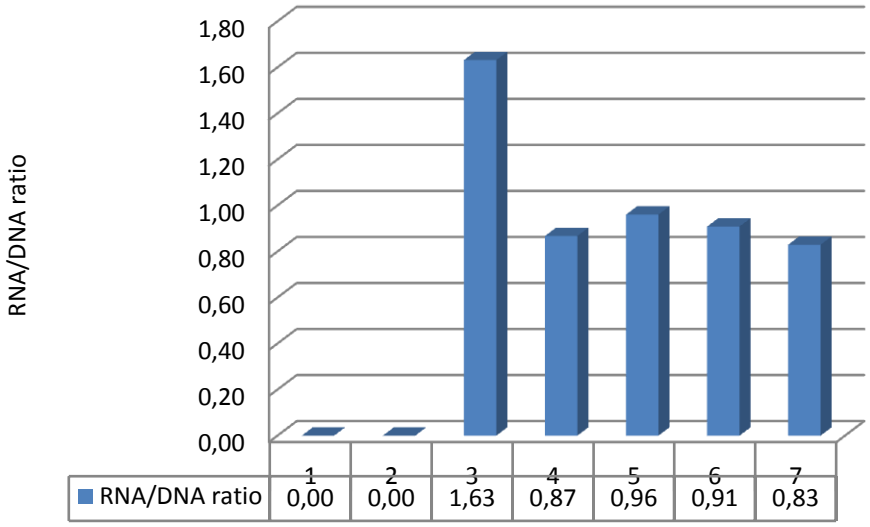

Figure 2: assessment of microbial activity in water samples based on the RNA/DNA ratio by day

Summary and conclusion

To estimate the shelf life of fresh water in new, unused Flaska bottles we daily analyzed different parameters for 7 days. By traditional culturing techniques we daily determined the total number of microbial cells in in the water (one of the key parameters according to the national regulative for quality of potable water- annex 1). Modern molecular techniques were used to daily measure DNA and RNA concentration and from that to determine the microbial activity in the water through days. Results in table 1 and the diagram (figure 1) show that after two days at 22 °C the total number of microbial cells already exceeds the limit of 100 CFU/ml according to national regulative. Concentrations of DNA and RNA shown on diagram (figure 2) are below the limit of detection in first two days and thereafter increases through days. The RNA/DNA ratio shows that activity of microbial cells was the highest on day 3 (figure 3).

On the basis of all parameters analyzed we concluded that the shelf life of fresh water in firstly used Flaska bottle is **2 days** because in third day the number of microbial cells exceeds the limit according to the national regulative. It was also shown that concentrations of DNA and RNA are below the limit of detection in first two days.

We speculate there are options regarding the amount of oxigen available to microorganisms that could be diminished by applying properly designed cap to provide tighter sealing.

REFERENCES:

1. LAPANJE, Aleš. *The procedure for concentrating cells and isolation of nucleic acids from the polymer matrix for the analysis of cells from the air: application number:P-20100233 dated 29/07/2010. Ljubljana: Institute of Intellectual Property, 2010.p.9.-Patent application.*

2. LAPANJE, Aleš. *Procedure and mix chemicals for the isolation of nucleic acids from complex samples: No. patent application.P-200700310 dated 28/11/2007: No. patent.SI 22679 A dated 30/6/2009. Ljubljana: Office of the Slovenian Intellectual Property, 2009.p.16.*

3. Directive for drinking water, Official letter No. 19/2004, dated 1/3/2004.

Annex

ANNEX 1: Microbiological parameters, requirements for water intended for packaging (Directive for drinking water, Official letter No. 19/2004, dated 1/3/2004)

Parameters	Acceptable values
Escherichia coli (E.coli)	0/250 ml
Enterococcus	0/250 ml
Pseudomonas aeroginosa	0/250 ml
No. of colonies (22 °C)	100/ml
No. of colonies (37 °C)	20/ml