- **Final Report**
    - Comprehensive summary of the main project results (max. 25 pages)

Description of main S & T results/foregrounds

I. Materials Characterization (SU, AIT, KTH)

All the chitosan materials, raw and modified (ECM), were characterized by using various structural analysis techniques such as UV-Vis, FT-IR, Fluorescence Spectroscopy, Thermal Gravimetric Analysis (TGA/DTA) etc.. Among all the tested materials, Chitosan 85/1000/A1 and Chitosan 85/2500/A1 were found to be more insoluble in water than the others due to their degree of polymerization and therefore more suitable for practical application. TGA of the materials showed a single degradation stage for these biopolymers. It begins at 46.16 °C with 7.86 % mass loss. The morphology of the chitosan biopolymers were studied by scanning electron microscopy (SEM) which proved their porous and fibril structures. Comprehensive information about the materials properties is given in the corresponding Deliverables.

II. Design of Enhanced Chitin-based Materials (FTMC)

Main purpose: Improvement of the adsorption rate and capacity of raw chitosan through chemical and physical modifications

The main reason of the limited use of chitosan as a solid sorbent in the water purification systems is its propensity to swelling and clogging. These phenomena cause increase in the pressure and lead finally to interruption of the filtration process. The other risk to apply chitosan as a sorbent in water purification systems is its biodegradability and the tendency to mould. In order to avoid these negative effects and find ways to regenerate and reuse this valuable biopolymer, chemical and physical modification was performed. Moreover, the new sorbent was manufactured in form of beads in order to facilitate its practical use.

It is known that crosslinking of chitosan can increase its mechanical and chemical stability. However, this procedure leads to: 1) a remarkable decrease in the sorption rates; 2) the filtering material is hard to biodegrade; 3) mould growth is not avoided. These problems are solved in the framework of this project by using physical treatment and introducing of additional functional groups, which enable to keep the main chitosan chemical properties and its mechanical stability. Functional groups which are compatible with the filtering of drinking water and can be introduced into the chitosan chain are f.e. succinate, malonate, tartrate, and citrate. **Citrate exposed itself as the most promising from the point of view of mechanical stability and biocidal effect.** Among the physical approaches such as compacting of chitosan powder, air bubbling through dissolved chitosan, electrochemical anodic and cathodic treatment, and freezing, the last one appeared to be most the promising.

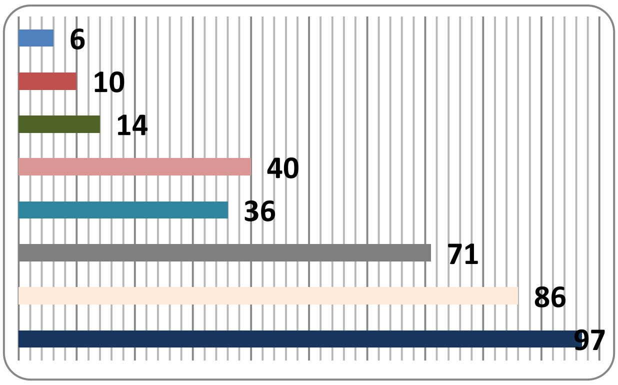


Fig. 1. Compressibility (in %) of chitosan beads produced under different conditions

Based on the experimental results was found that **freezing of the beads in ethanol enables to obtain a mechanically stable and more developed structure, which in turn increases the sorption rate**. **The best results were obtained by combination of modification with citrate and introduction of Fe (II) ions after freezing in ethanol for several days** (**Fig.1**). Citrate was introduced into the chitosan in two ways: a) after keeping in a freezer and drying, chitosan beads were treated with a NaCitrate+Citrtic acid solution at pH~6 and then heated for 24 h at 70°C; b) production of chitosan beads after its dissolution in citric acid and keeping for 5-7 h at 70°C; Fe (II) was introduced after drying the prepared beads.

III. Biocide introduction and durability assessment (FTMC + GTC)

The physical and chemical treatment influenced the biocidal activity of the modified chitin-based materials. The **main novelty** of this approach is that **a correlation between the biocidal activity of the modified chitosans and their capability to sorb dissolved oxygen** from the aqueous solution was found (**Fig.2**). Chitosan modified with citrate and Fe (II) is capable of sorption rather large amounts of dissolved oxygen (see curves 3 and 3’ in Fig.2); accordingly, this modification demonstrates the best antimicrobial activity. It could be assumed that the antimicrobial action of chitosan is based on its capability to sorb oxygen. Such a property of chitosan has not been described in literature till now.

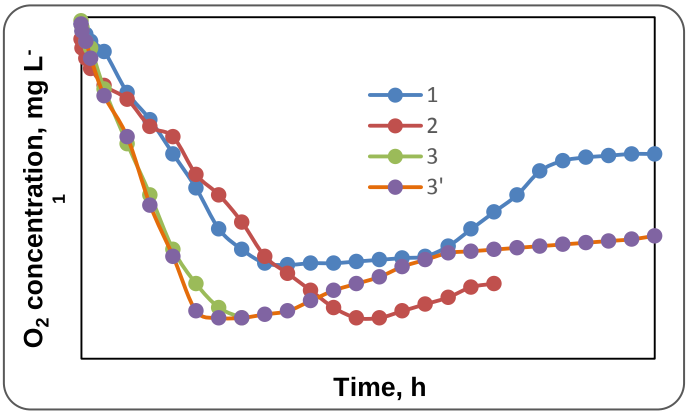


Fig.2. Sorption of dissolved oxygen from distilled water onto 1: raw chitosan; 2: chitosan modified with citrate; 3: chitosan modified with citrate & Fe (II); 3’: second treatment of modified chitosan with citrate & Fe (II) with a new portion of deionized water

Remarkably, additional amounts of oxygen could be adsorbed by bubbling air oxygen through a mixture of water and chitosan or by its electrochemical treatment. In these cases, the antimicrobial activity increases thousands times.

The antimicrobial effect of the raw and modified chitosan materials was investigated by GTC. For this purpose, two groups of microorganisms were selected: model microorganisms *Escherichia coli* and *Saccharomyces cerevisiae* as well as a larger group of microorganisms common in the natural environment and existing in drinking water (bacteria, yeasts and fungi): *Pseudomonas aeruginosa, Micrococcus sp., Rhodotorula mucilaginosa, Candida lipolytica, Aureobasidium pullulans, Penicillium chrysogenum* and *Aspergillus niger*. Experiments were performed using the zone inhibition method on microorganisms grown in Petri dishes and by evaluating viability of microbes after exposure them in water with chitosan materials (by viable counts on agar plates). The model microorganisms were exposed in distilled water, the other group of microorganisms – in tap water. Light microscopy was used for evaluation of growth of microorganisms.

Investigation of antimicrobial effect of the raw chitin-based materials

Five chitosans (90/30/A1, 90/200/A1, 85/400A2, 85/1000/A1, 85/2500/A1) and two chitin materials (snow crab and white shrimp), delivered by Biolog, were tested.

The investigation on the effect of raw chitosan and chitin materials against different microorganisms showed that **the raw materials demonstrated very little or even no effect on the microorganisms.** The effect was mostly seen on decreased growth rate (biostatic effect) of some cultures. In case of model microorganisms, the raw materials demonstrated weak activity against *E. coli* only. There was no evident effect on the viability of the bacteria in water. They also did not demonstrate clear activity against other group of microorganisms such as *Micrococcus sp.* and *Pseudomonas aeruginosa.* Chitosan 90/200/A1 slowed down growth of *Aureobasidium pullulans* (zone inhibition tests, fungistatic effect) and chitosans 85/400/A2, 85/2500/A1 slightly reduced the viability of *Candida lipolytica* when exposed in tap water. Chitosan materials did not reduce the number of the fungi *Aspergillus niger* and *Penicillium chrysogenum* in water, whereas microscopical examination of fungal germ tubes showed that the materials had slowed down their initial growth. Both the model microbes and the other group of microorganisms did not demonstrated sensitivity to chitin materials.

Investigation of antimicrobial effect of modified chitosan materials and beads

The following types of modified chitosan materials were tested: modified with organic acids (citric, succinic and tartaric), citric acid + Fe(II), succinic acid + Fe(II), tartaric acid + Fe(II) as well as with cathodic (K), anodic (A) and reverse (A→K→A and K→A→K) modifications. The chitosan 85/1000/A1 was tested also in form of beads with different modifications, after loading and regeneration (non-modified, after sorption of humic acids, after sorption of humic acids and H_2O_2 regeneration, regenerated after Cu(II) sorption, regenerated after citrate sorption), modified with citric acid +Fe(II) and the latter after sorption of humic acids. Composite chitosan beads containing alginates were examined as well: alginate-chitosan (food grade) + acetic acid, alginate-chitosan + acetic acid and alginate-chitosan + citric acid. The results showed that the **most effective antimicrobial properties were demonstrated by chitosan materials modified with organic acids + Fe (II)**. *E. coli* was sensitive to a wide spectrum of the tested modified materials. Investigation of the wider group of microorganisms showed that the highest effect in water was observed by the chitosans 85/1000/A1 and 90/30/A1 both modified with succinic acid + Fe. **Chitosan 85/1000 modified with citric acid + Fe, which was chosen for manufacturing of sorbent beads, showed an antimicrobial effect on viability against *E. coli, Micrococcus sp., R. mucilaginosa* and *S. cerevisiae.***

Investigation of the modified and regenerated beads demonstrated that model organisms in water were sensitive to chitosan 85/1000/A1+citric acid+Fe, to the latter with humic acids and also to the regenerated 85/1000/A1 after Cu sorption and after citrate sorption.

In conclusion, the **modified chitosan materials showed higher antimicrobial effects than the raw materials. The most effective antimicrobial properties were demonstrated by the materials modified with organic acids and Fe. Regenerated chitosan beads (after copper and citrate sorption) showed also antimicrobial activity**. The most sensitive microorganisms towards chitosan materials are: bacteria *E. coli* and *Miocrococcus sp.*, whereas the most resistant are the fungi *A. niger* and *P. chrysogenum*.

IV. Pilot production of ECM beads by Biolog

The first attempt for production of larger quantity of beads (several litres) by Biolog (Germany) was based on the information from the lab-scale production of ECM beads (FTMC, Lithuania). Further, the lab-recipe of FTMC was changed by Biolog especially concerning of both chemicals citric acid and NaOH; moreover, the duration time for washing by water as well as for freezing and drying were also adapted to the Biolog's capacities and equipment availability. All these changes were made to be possible to produce on pilot scale beads needed for the practical application at real conditions. The pilot bead's production recipe is described below:

Step 1: The first production step includes dissolution of chitosan flakes into hot water solution of 7% citric acid, followed by filtration using 100 µm Deltafilter (Fig 3).

Fig. 3. ***Dissolution of Chitosan into citric acid solution***

Step 2: In the second production step, the chitosan-citric acid solution is dropped into 30% NaOH solution in which beads are formed; production time 24 h (Fig.4). The NaOH-concentration decreases to 10%, therefore, additional NaOH flakes have to be added therein to increase its concentration up to the initial value of 30%.

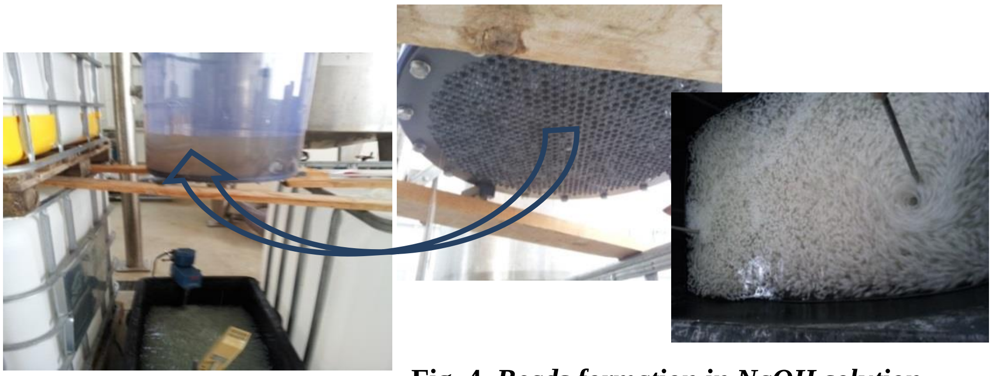


Fig. 4. ***Beads formation in NaOH solution***

Step 3: Washing of the beads with water and ethanol (Fig.5)

Fig. 5. ***Washing of chitosan-beads***

Step 4: Freezing in ethanol for 4 days and subsequent defreezing (Fig.6)

Fig. 6. ***Beads freezing in ethanol***

Step 5: Treatment with iron (Fe 2+) sulphate solution at pH~2 for 2h (and 24 h): Fig.7

Fig. 7. ***Beads modification with iron sulphate solution***

Step 6: Drying of the modified beads on air
During the air drying, the beads have to be mixed/moved from time to time to avoid agglomeration as shown on the right picture (Fig.8).

Fig. 8. ***Beads drying on air***

At the end, based on the described procedure, 2.4 L dried beads were produced from 50 L 2.5% chitosan gel.

V. Determination of Selectivity & Capacity of ECM

*Removal of **heavy metals** (SU)*

For removal of metals ions, the chitosan beads made of chitosan 85/1000 and modified by citrate, frozen in ethanol, treated by and Fe (II) are recommended.

Batch experiments to determine the sorption capacity of the modified (by citrate and Fe(II)) Chitosan 85/1000 in form of beads showed that: a) capacity towards Cu is 1,37 mmol/g or 342 mg/g (conc. 10 mg/L); b) Cu-removal increases with the solid /liquid ratio; c) contact time - 180 min (equilibrium is reached); d) pH values close to the natural values are appropriate; at pH ~2-3, the ECM material converts into a gel form; e) sorption of Cu(II) decreases with increasing temperature - temperature suggested 25 °C ; f) mean energy of adsorption (*E*) of 7.071 kJ/mol revealed that the physical sorption is predominant. g) monolayer coverage of sorbent surface through physical adsorption of metal ions from water occurs.

The sorption properties of the ECM were studied in column experiments by using on-line solid phase extraction (SPE) with five steps: pre-conditioning with ultra-pure water (pH 5.6); loading of metal ions (breakthrough curve) with nitrate salts of Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Pb(II), and Cd(II) ions); washing of unsorbed metal ions with ultra-pure water; stripping with a complex mixed solution (pH ~10) as stripping agent; post-conditioning with ultra-pure water. For analysis of the adsorption isotherms, Freundlich, Langmuir, Dubinin-Radushkevich (D-R) and Scatchard plot models were used. The result obtained from D-R isotherm showed that E-values calculated for each metal ion are lower than 8 kJ mol^{-1}. These values indicate **physical interactions between metal and chitosan in most cases**. For **Zn (II),** the E value indicates that **the interactions are rather chemical than physical.**

In case of **multi-component system, the sorption capacity of ECM made of Chitosan 85/1000 towards different heavy metals is similar to that of single metal systems:**

Cd(II) > Pb(II) > Zn(II) > Cu(II) > Ni(II) > Co(II) > Mn(II)

*Removal of **organic pollutants** (AIT)*

Chitosan-Material preferred: Chitosan 85/1000/A1

Humic Acids: Both kind of beads unmodified and modified with iron sulphate can be successfully applied for purification of water containing humic acids; About **80% of DOC** and **70-75%** of the **coloured substances** are removed by both materials (at C°=30 mg/L)

Pesticides**:** Treated water contained atrazine, bromacil, metolachlor, propazine, simazine at initial concentration of **100 µg/L**; results depend on the kind of pesticide; about **20% removal** at the given concentration

Hormones*:* mixture of estrone, estradiol, estriol, ethinylestradiol (**100 µg/L)** was treated; Up to **20% hormones** were removed; **Best results** are achieved for the synthetic hormone ***Ethynylestradiol***

Pharmaceuticals: mixture of erythromycin, diclofenac, clarithromycin, ibuprofen, carbamazepine, trimethoprim, sulfamethoxazole (100 µg/L) was treated; **Removal between 20% and 87%** depending on the drug composition; selectivity follows the order: **Erytromycin>Diclofenac=Sulfamethoxazole= Trimethopim> Carbamazepin> Clarithromycin**

*Removal of **Fluoride and Nitrate** (AIT):* Up to **10% of 25 mg/L F^-** and **20% of 50 mg/L NO_3^-** can be removed by ECM beads.

*Removal of **Arsenic** (KTH)*

Waters containing **100 µg/L** of Arsenic were treated using the column design presented on Fig.9 and different kind of beads made of Chitosan 85/1000/A1. About **85% As (V) was removed** under stable flow rate of 3 ml/min. It was found that the sorption efficiency decreases with increasing in pH and water flow rate.

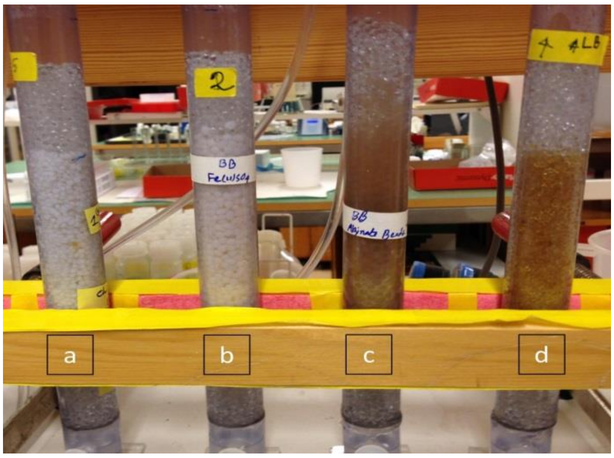


Fig. 9 Column design (KTH)

a) ***chitosan beads preserved in Ethanol***

b) chitosan beads modified with Fe(II)SO4

c) chitosan-alginate beads

d) chitosan beads treated with Fe(III)

*Removal of **Uranium & Thorium** (KTH)*

Waters containing **100 µg/L** of Uranium or Thorium were treated using modified beads made of Chitosan 85/1000/A1. **98% U** and **99% Th was removed** under stable flow rate of 3 ml/min at pH 6. Sorption efficiency decreases with increasing in pH and flow rate.

VI. Field tests in Austria (Ludwig-Wassertechnik GmbH)

Tests on mechanical stability

The first tests performed by Ludwig in Austria were aimed at determination of the mechanical stability of the modified beads on site in open ended system using tap water (tests conditions: 4 bar initial pressure and 3000 L/h water flow rate). The test facility used is shown in Fig.10

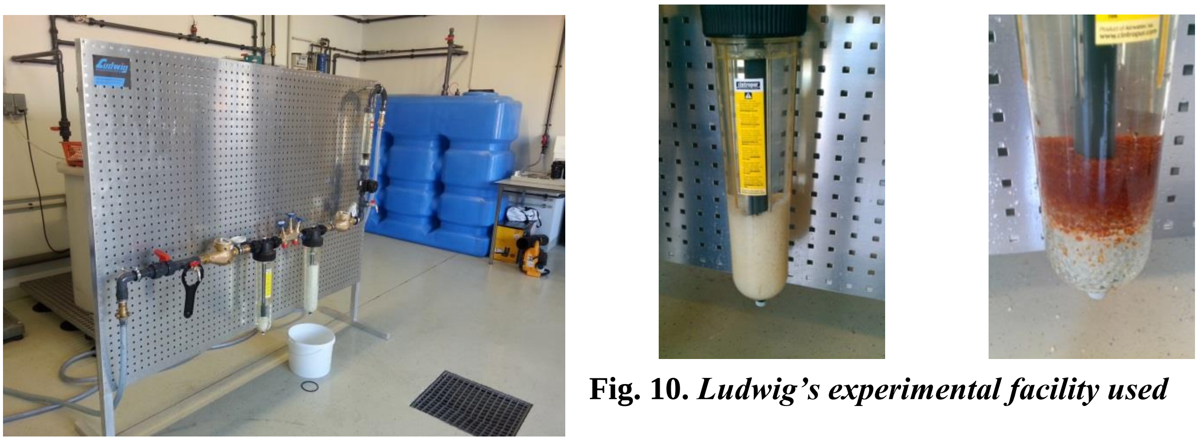


Fig. 10. ***Ludwig's experimental facility used***

The first fine tuning of the filtration process by using ECM beads to the practical conditions included small-scale experiments with household filters at the premises of Ludwig-Wassertechnik in Upper Austria.

The five different materials in form of beads were tested at the same conditions; all made of Chitosan 85/1000:

(1) Biolog (Lot.40200702, $FeSO_4$, 15 L wet, beads with tail)
(2) Biolog (Lot. 40200707, $FeSO_4$, 30 L wet beads without tail)
(3) AIT/Biolog (Biolog beads 1., additionally modified by AIT with $FeSO_4$ (pH=3) for 5 days, wet)
(4) AIT/Biolog, (Lot. 40200664, $FeSO_4$ 0.5 L, dried, additionally modified by AIT with $FeSO_4$ (pH=3) for 20 h; dried (1 day), soaked in water + swelled (1 day)
(5) FTMC (beads frozen in EtOH, modified with 6 mg/g Fe, swelled in water (1 day)

Results on the mechanical stability of the modified beads

It has been confirmed that:

- The modification of the chitosan materials by using iron positively impacts the mechanical stability of the beads in the filter
- Drying of the beads is absolutely necessary to enhance the bead’s mechanical stability under the increased pressure at the real conditions

Because of mechanical stability problems with the beads No1 and No2, which were firstly delivered by Biolog, both charges are mixed together (60 L wet mixed beads from Lots. 702, 707, and 664) and modified additionally on site to facilitate the first field tests performed by Ludwig-Wassertechnik on different places in Upper Austria

Fig. 11. ***Additional modification of Biolog’s beads at Ludwig-Wassertechnik***

The pre-swelled chitosan beads were filled into a small filter containing a supporting layer of glass balls on the bottom, as shown in the picture (see Fig.11). Then, tap water was starting to flow down through the new filter system. During the first 15 minutes, the water flow decreased from 2000 L/h to 1500 L/h due to the additional swelling of the beads. After that it remained constant and stable during the next four hours. These beads were used for the tests performed by Ludwig-Wassertechnik on different places in Upper Austria.

Field tests in Austria

First field tests are conducted by Ludwig-Wassertechnik in Upper Austria, in the region of Wasen (see the map on Fig.12; the blue marked area consists of clay, silt and sand). The purpose of these tests was to find out if the modified beads remain mechanically stable at real conditions and how the concentration of iron, ammonium and TOC in the drinking water will be affected after passing it through the new filter system. The water flow rate in the small filter system was changed in the range from 500 L/h to 100 L/h to determine the effect of the residence time on the sorption efficiency.

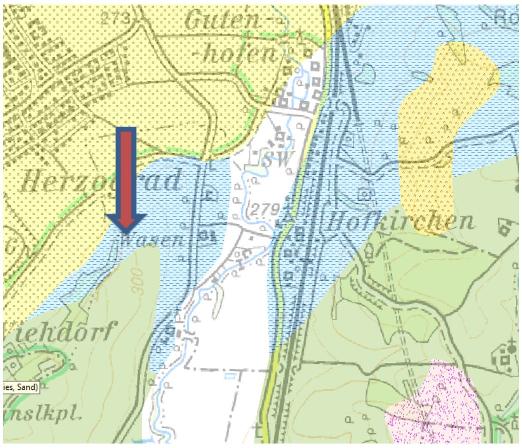


Fig.12 ***Feld tests in Upper Austria / St. Valentin - Wasen, Ground water well***

In Wasen, St. Valentin, Ludwig used $FeSO_4$ modified Chitosan beads (3 Charges mix) with $FeSO_4$, done by Ludwig and AIT, 2.5L Beads. Wasen is a household of 4 persons, with a private drilled well (about 15-20 m deep). The water has been pumped via their "normal delivery system" out of the tap into the container with the Chitosan-beads. Raw water samples were taken before, and treated water samples after passage through the Chitosan container.

Die main results from these drinking water treatment tests are presented in Table 1.

Table 1. ***Concentration changes in the effluent with time and water flow rate***

Time min	Flow rate L/h	pH	λ µS/cm	**NH_4** mg/L	**B** mg/L	**Fe** mg/L	**Ca** mg/L	**Mg** mg/L	**Mn** mg/L	**Zn** mg/L	**TOC**
0		7,69	1036	2,18	1,5	1,41	35,2	9,66	0,024	0,12	1,579
15	500	7,35	984	2,3	1,67	1,39	31,3	8,74	0,021	0,064	1,505
30	400	7,33	999	2,44	1,58	0,893	33,7	9,41	0,022	0,054	1,406
60	450	7,48	1003	2,44	1,61	1,02	33,7	9,35	0,022	0,053	1,459
75	200	7,42	1015	2,5	1,63	1,02	33,7	9,36	0,022	0,051	1,455
90	200	7,42	1003	2,36	1,64	1,22	33	9,27	0,022	<0,05	1,441
105	200	7,41	1015	2,3	1,66	1,15	33,4	9,29	0,022	<0,05	1,446
200	100	7,49	1017	2,4	1,63	0,439	33,8	9,34	0,023	<0,05	1,527
215	100	7,35	1013	2,4	1,63	0,764	34	9,39	0,022	<0,05	1,50
245	100	7,40	1018	2,4	1,65	0,636	34,1	9,42	0,022	<0,05	1,54

Conclusions

1. Values of pH, conductivity, Ca and Mg were not affected by the water treatment with the modified chitosan beads
2. It seems that additional functional groups have to be attached to the bead's surface to get a special selectivity towards removal of B & ammonia from drinking water (Limit values in Austria for Bor = 1 mg/L; for NH4=0,5 mg/L; for Mn= 0,05 mg/L).

3. Fe and Zn can be removed (Limit values for Fe=0,2 mg/L and Zn=5 mg/L) especially when the water flow rate is low; for higher removal more material is needed
4. Zn can be removed below the analytical proof limit of 0.05 mg/L
5. TOC is not increased during the treatment what means that there is no leaching of organic matter from the beads

The second place at which field tests were performed by Ludwig-Wassertechnik is Zupfing (see Fig.13).

Fig.13. ***Zupfing in Upper Austria***

The green marked area is an Ottnang-Formation (coarse clay, silt, sand).

Mixed beads (volume 7.5 L), delivered by Biolog and additionally modified by Ludwig were pre-swollen in the 20 L blue filter tank. Pictures from the field experiment in Zupfing are shown below.

Fig. 13 ***Field tests in Zupfing, Upper Austria***

The drilled well was about 30-40 meters deep and the water was pumped into the basin. Raw water samples were taken after the basin, and treated water samples after passage through the $FeSO_4$ modified Chitosan container. The results from the tests in Zupfing/Austria (drinking water flow rate was 500 L/h) are presented in the next Table 2:

Table 2. ***Concentration changes with time at down & up-flow of the treated water***

	Time min	Flow rate L/h	pH	λ µS/cm	**NH_4** mg/L	**NH4 removal %**	**Ca mg/L**	**K mg/L**	**Na mg/L**
	0		8,9	432	2,81		1,2	2,0	100
Downflow	15	500	7,09	410	2,28	18,9	1,4	1,6	89
	30	500	7,04	419	2,62	7	1,4	1,7	90
	45	500	7,15	426	2,58	8	1,4	1,7	93
	60	500	7,11	428	2,40	14,59	1,3	1,8	95
	160	500	7,45	439	2,71	3.5	1,1	1,9	98
	175	500	7,37	437	2,68	4.6	1,1	1,8	98
Upflow	190	500	7,55	438	2,56	8,9	1,2	1,9	100
	205	500	7,55	438	2,60	7,5	1,2	1,8	98

<u>Conclusions</u>:

1. Ammonium is removed to some extent, however, not substantially; higher bed volume (more material, low flow rate) can increase the removal efficiency concerning Ammonia in drinking water
2. Mineral content and balance is not affected
3. The filtration system works without any problems in both possible directions, in down flow and in up flow (important for the regeneration step).

The third place in which field tests are carried out by Ludwig-Wassertechnik is situated in **Andrichfurth (Upper Austria)**

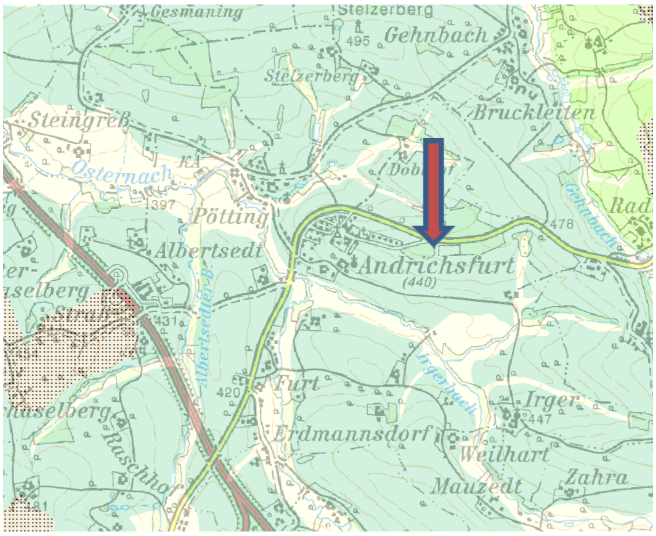


Fig. 14. ***Andrichsfurt – Upper Austria***

Two field tests were performed in Upper Austria/ Andrichsfurth.
The turquoise marked area consists of coarse clay, silt, and sand.

Beads used (7.5L) are additionally modified by Ludwig. Flow rate was changed from 500 to 1000 L/h; down-flow in the filter.

The results are presented in Tables 3.

Table 3. *Concentration changes in the treated water with time (down-flow)*

Time min	Flow rate L/h	pH	λ [μS/cm]	Fe mg/L	Ca [mg/L]	Mg [mg/L]	K [mg/L]	Na [mg/L]	TOC [mg/l]
0	500	6,8	640	7,54	110	22	1,5	5,3	1,5
15	500	6,83	661	6,35	110	23	1,6	4,6	1,4
30	500	6,93	669	5,81	110	23	1,5	4,4	1,4
45	1000	6,98	672	5,21	110	23	1,5	4,2	1,5
135	500	7,19	682	3,28	110	23	1,5	4,5	1,4
150	500	7,03	682	3,30	120	23	1,5	4,5	1,4
165	500	7,09	684	3,14	120	24	1,5	4,6	1,4
185	1000	7,23	686	2,48	120	24	1,4	4,5	1,4
200	1000	7,24	690	2,59	120	24	1,4	4,6	1,4

***Conclusions*:**

1. Fe removal using the modified beads is possible; ratio material quantity/flow rate has to be optimised
2. No changes in TOC are available what means there is no leaching of organics from the Chitosan beads**.**
3. No changes in the mineral balances of the treated drinking water

VII. Field test in Turkey (Denizsu)

Based on the experiences gained in the small-scale tests, a general process schema was designed for the application of ECM beads for water purification as shown below in Fig.15.

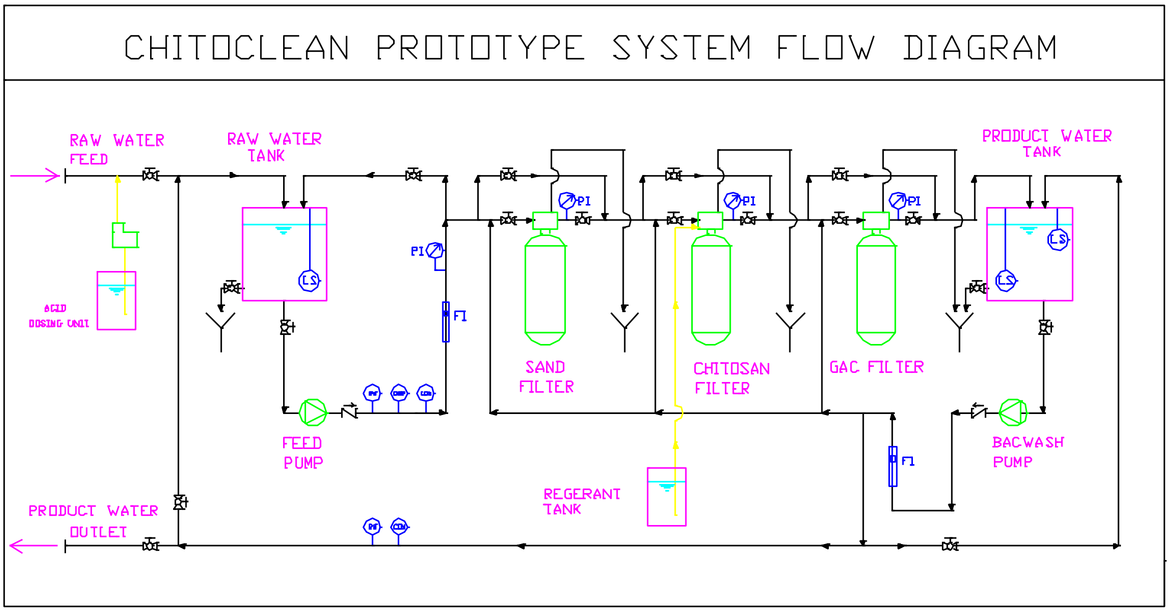


Fig.15 ***Schematic flow diagram of the pilot water treatment plant with ECM beads***

The water treatment system, developed and applied by Denizsu, contains three filter tanks: the new synthetized chitosan-based sorbent beads are loaded into one pressurised filter tank (called *Chitosan Filter*). Upstream of the chitosan-beads containing filter, a multimedia sand filter is installed for the removal of suspended solid particles. Downstream of the chitosan-beads filter, a Granular Activated Carbon (GAC) filter is introduced into the set-up to enable the polishing of the effluent coming from the chitosan-beads filter. This last process could be necessary to guarantee a consistent product water quality but it can be by-passed if it is not needed. The chitosan-beads tank is connected in a parallel manner in order to allow a continuous flexible operation mode of the pilot plant (see Fig.16).

Process specification used:

- chitosan-beads tank size → 30 id, 72 inch cylindrical height
- filtration rate → 600 L/h
- backwash rate → 900 L/h
- bed depth → approx. 1 MT (%60 of height, %40 free board)
- bed volume of tank → 40 L
- contact time → 6-7 min
- chitosan-beads quantity → approx. 15 L
- supporting material (sand)→ 15 L
- 75% of the tank was filled with sand + chitosan-beads

Fig.16 ***Pilot plant with three filter units (Denizsu, Konya)***

Physical and operational properties of ECM beads made of Chitosan 85:

- particle size → typical bead size - 0.5-1.5 mm, sphercal beads
- uniformity of beads → typical uniformity coefficient 1.1-1.2
- optimal operating pH → 7.0-8.0;
- optimal temperature range → 10-15 ^{0}C,
- bed expansıon rate → low
- the beads can be frequently re-used as sorbent material

Results from the field tests in Konya, Turkey

Pilot tests were performed with the purpose to evaluate the efficiency of the heavy metal removal from drinking water by using newly synthetized chitosan-based beads. Waters from two drinking water sources in the vicinity of the industrial area of Konya, Turkey, are treated. One of them was ground water (used as drinking water in one industrial area) and the other was lake water (used as drinking water for animals and/or for cleaning surfaces in a village and in a university campus area). Water from a drilled well, located outside Konya city, 200 m depth, was used for the experiment. Additionally, “artificial” ground water (ground water with an addition of some metal ions) is also treated and the results are compared. All three kinds of drinking waters are passed through the treatment filter system without any additional pre-conditioning. The main physical parameters of the three drinking waters are shown in the Table 4.

Table 4. Physical parameters of the three water sources before (feed) and after treatment (product)

Type of water used		**pH**	**Turbidity (NTU)**	**Conductivity (µS/cm)**	**Hardness (^{o}fH)**
Ground water	**Feed water**	7.74	1.38	1331	67
	Product water	7.63	0.39	1323	66
Lake water	**Feed water**	8.07	3.0	335	17
	Product water	7.89	0.37	353	17
Artificial ground water	**Feed water**	7.66	3.1	1425	75
	Product water	7.66	0.58	1448	74

In the Table 5., the results of the pilot tests in Konya are summarised:

Table 5. ***Removal of metal ions by modified chitosan-beads / Field tests in Konya***

Type of water	**metal ions present**	**metal concentration in feed water (mg/L)**	**metal concentration in product water (mg/L)**	**% Removal**
Ground water	**As**	0.0672	0.0264	60.68
	Cr	0.0107	0.0057	46.65
	Cd	0.0016	0.0003	82.26
	V	0.3692	0.1620	56.11
	Mo	0.1365	0.0556	59.28
	Pd	0.0082	0.0042	48.08
	Pt	0.0038	0.0012	69.55
Lake water	**As**	0.0133	0.0000	99.91
	Sr	0.0914	0.0001	99.92
	V	0.4054	0.0004	99.89
	Mo	0.0322	0.0000	99.92
	U	0.0004	0.0000	99.92
	Pd	0.0017	0.0000	99.85
	Sb	0.0142	0.0000	99.93
	Pt	0.0004	0.0000	99.94
“Artificial” ground water	**As**	0.0087	0.0038	55.71
	V	0.0375	0.0250	33.44
	Se	0.0028	0.0005	83.35
	Mo	0.0140	0.0101	27.94
	Pd	0.0011	0.0006	48.43
	Sb	0.0042	0.0023	45.12

The main achievements are as follows:

1. The use of chitosan-based beads as sorbents does not influence negatively the mineral balance in the treated drinking waters; it remains constant as there is no any significant change in the concentration of Calcium and Sodium ions after the treatment of the different types of water as shown in the Figures 17 below. This is one of the most important advantages of the new sorbent material because keeping the mineral balance during the treatment represents a major problem mostly encountered with other materials applied for the same purpose.

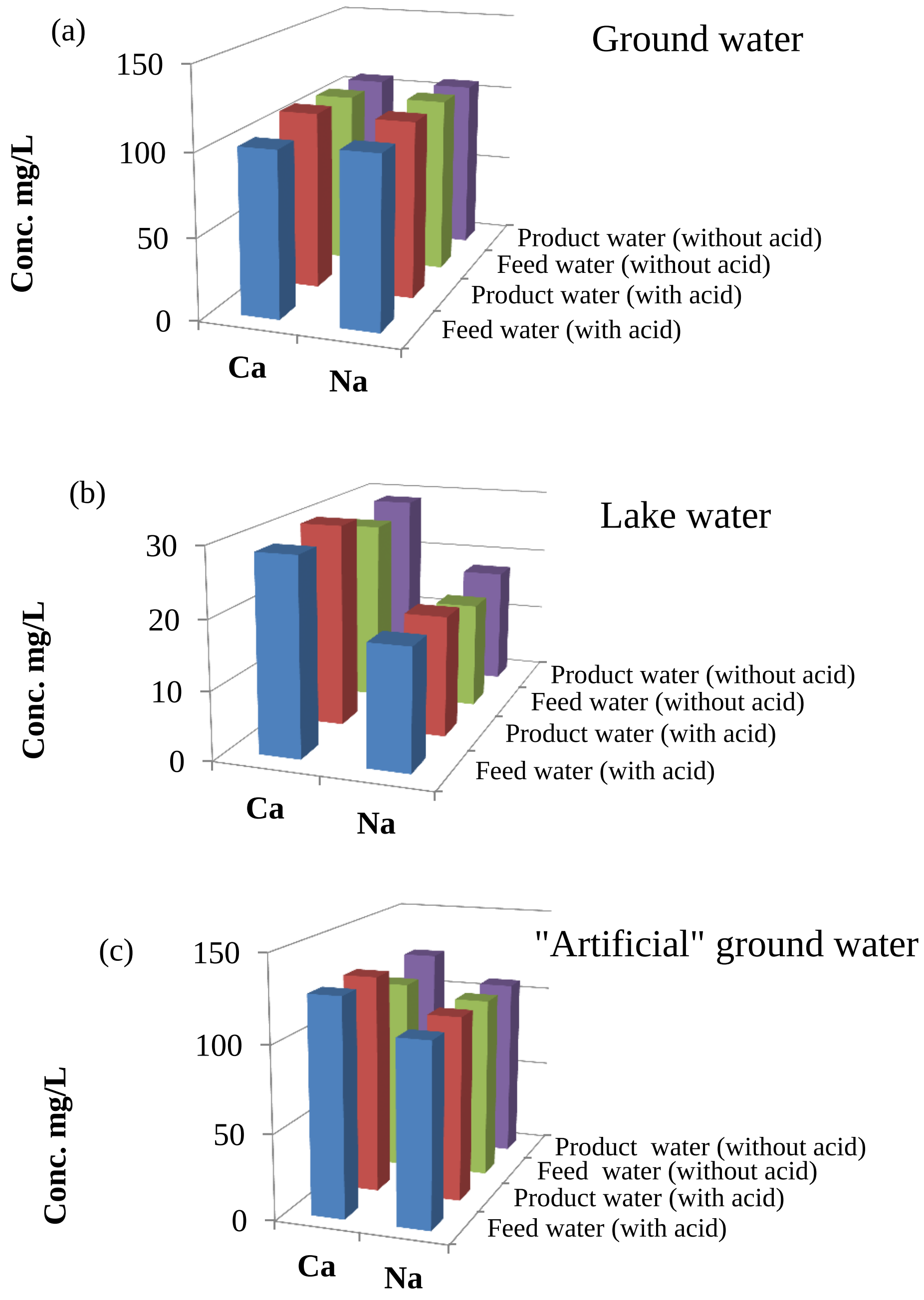


Fig.17 ***Concentration of sodium and calcium ions before and after treatment of real drinking waters with chitosan-based beads: (a) ground water; (b) lake water; (c) "artificial" ground water***

2. Chitosan-based beads are found to be highly efficient for the removal of a variety of heavy metal ions such as As, Cr, Cd, V, Mo, Pb and Pt from ground and lake waters, as shown in Figure 18 below. The feed concentration is given in Table 5.

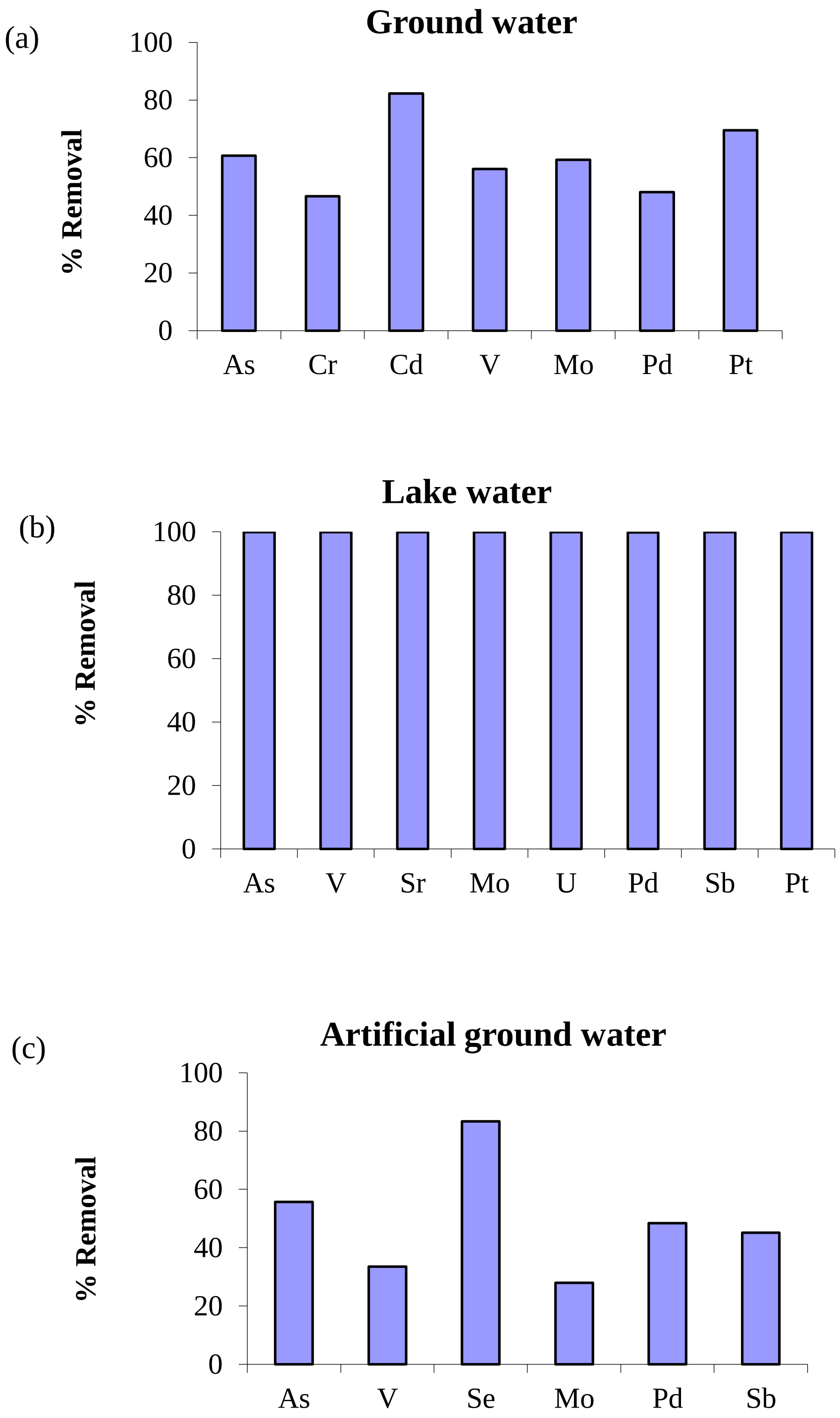


Fig.18 ***Removal of heavy metal ions by using chitosan-based beads: (a) ground water (b) lake water; (c) "artificial" ground water***

3. There is no leaching of the chitosan-based beads into the treated water - no contamination by organics containing in the sorbent material

Conclusion: The modified chitosan-based beads are highly efficient in the removal of heavy metal ions from water; they are appropriate as a sorbent/filter of polluted drinking waters as the ionic balance as well as the other physical parameters is found to be within the permissible limits for drinking water.

VIII. Regeneration/disposal of spent ECM beads

Regeneration of loaded chitin-based beads in small-scale (FTMC)

The regeneration mode depends on the nature of pollutants adsorbed by chitosan and the facilities available. Two regeneration methods – electrochemical treatment and eluation with alkaline and acidic solutions – were investigated as it is used in the ion exchanger regeneration. When heavy metals are sorbed onto chitosan, they are easily removable through cathodic treatment in acidic solutions by using a conductive electrode and an insoluble anode. Pollutants are precipitated as free metals or insoluble metal hydroxides or oxides. When a mixture of heavy metals and dissociable organics is sorbed, chitosan is also regenerated electrochemically using two camera cells (see the figure 19). In this case, metals are precipitated onto the cathode and organics destroyed to CO_2, NO_2, SO_2, Cl_2 etc.

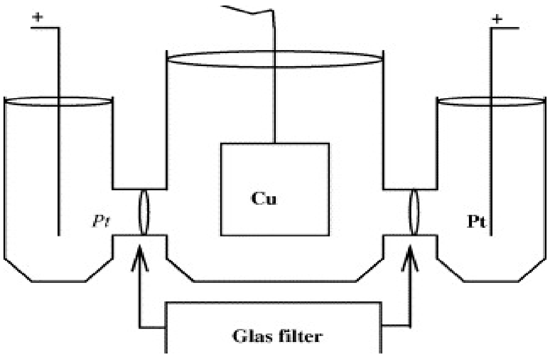


Fig.19 ***Schema of electrochemical cell for regeneration of loaded chitosan beads***

Application of the **electrochemical regeneration method** is questionable if organics of complex composition is adsorbed, for example humic acids. Prolonged anodic treatment causes only a partial destruction of humic acids. It has to be taken into account that humic acids, especially the strong bonded onto chitosan, do not negatively influence the sorption rates. After electrochemical regeneration, chitosan which contains some quantity of humic acids can be successfully apply for further removal of heavy metals from drinking water. The main disadvantage of electrochemical method is the necessity to install some new equipment. **Such a regeneration approach could be useful in companies where electrochemical facilities have already been used.**

Another regeneration method of spent chitosan is **eluation with acidic and alkaline solutions**. Experiments showed that such treatment enables to remove completely a large number of pollutants sorbed from drinking water such as heavy metal ions, metal oxianions, pesticides, organic acids and, to a rather high degree, humic acids. However, in this case large volumes of acidic and alkaline eluates emerge. These eluates require an additional treatment. The simplest way to treat the eluates is to use **metallic iron**, which is available as a scrap in different factories of metal finishing industry and is a rather cheap waste. Metallic iron enables to remove practically all pollutants from the eluates. **The formed precipitate,**

containing different iron hydroxocompounds and the removed pollutants, is compact, stable in the environment and can be landfilled.

Regeneration of loaded beads from the field experiments (MKDS)

Regeneration of beads from the field experiments in Austria

Two batches of loaded beads were obtained from the field experiments performed in Austria by Ludwig-Wassertechnik GmbH. Samples of the loaded beads where dried at 95 ºC overnight, acid digested and analysed with ICP-AES spectrometer. Results are given in the Table 6.

Table 6. ***Metals loaded on the beads (Ludwig's field experiments)***

Analyte	Concentration mg/g Batch 1	Concentration mg/g Batch 2
Mg	1.16	0.97
Fe	13.1	14.7
Cu	0.08	0.20
Zn	0.10	0.03
Pb	0.04	<0.01
Ca	27.7	24.5

Regeneration experiment was carried out with the loaded beads from Batch 1. Beads were treated with a 2% H_2SO_4 solution; flow rate was 4.6 ml/min what corresponds to a linear flow rate of 1.3 cm/min. After acidifying, a washing step with distilled water followed. Regenerated and washed beads were dried at 95 ºC overnight, acid digested and analysed with ICP-AES spectrometer. Results are given in Table 7.

Table 7. ***Stripping of metals loaded on the beads (Ludwig's experiments)***

	Batch I		
metal	concentration mg/g		% removal
	loaded state	after regeneration	
Mg	1,16	0,02	98,2
Fe	13,1	0,52	96,0
Cu	0,08	0,04	50,0
Zn	0,1	0,03	70,0
Pb	0,04	0,04	0
Ca	27,7	5,70	79,4

The results presented in the Table 7 show that the applied regeneration procedure is very successful in case of removal of Mg, Fe, Ca and Zn from the loaded material. Cu can be also stripped to some extent; For Pb removal, H_2SO_4 is not appropriate, another acid such as HCl has to be preferred.

Regeneration of beads produced by FTMC

Beads were dried at 60 ºC for a 24 h and then re-swelled in distilled water for another 24 h (Fig.20a). Sorbent was loaded with water containing copper 5 mg/l, zinc 5 mg/l, chromium 0.15 mg/l, imidacloprid 0.5 µg/l. 9 L of such water was filtered through the chitoclean beads in down flow mode; samples of water were taken and analyzed by ICP-AES and HPLC-MS/MS at 1, 3, 5, 7, 9 liters. The loaded beads can be seen on Fig.20 b.

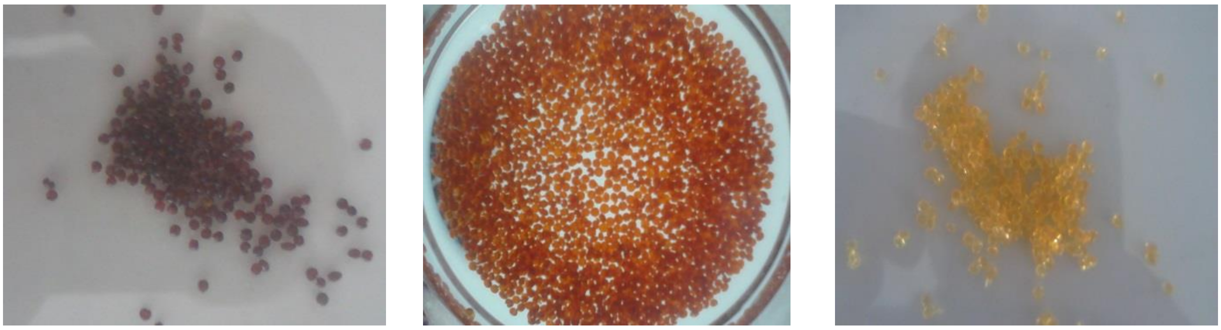


Fig.20 . ***FTMC beads: a) unloaded; b) loaded with metals and pesticides; c) regenerated***

Based on the analysis, following results concerning loading of the beads were received (Table 8):

Table 8. ***Loading profile as a function of time***

Water sample	**Ca** mg/l	**Mg** mg/l	**Fe** mg/l	**Cu** mg/l	**Zn** mg/l	**Cr** mg/l
Initial	72.0	18.7	<0.02	5.16	7.40	0.2
1 l	71.0	18.2	<0.02	<0.02	7.26	<0.02
3 l	72.3	18.6	<0.02	<0.02	7.20	<0.02
5 l	74.1	18.8	<0.02	<0.02	7.32	<0.02
7 l	74.3	19.0	<0.02	0.023	7.31	<0.02
9 l	73.1	18.9	<0.02	0.038	7.27	<0.02

Regeneration of loaded beads from FTMC

Loaded sorbent was regenerated according to the proposed protocol with 20 g/l H_2SO_4 solution, followed by washing until neutral effluents, and then again by washing. Washing was performed with distilled water solutions. Effluents were collected together until after the base treatment pH of wash water reached 9. Samples of Chitoclean beads after every stage of the process was taken, dried at 95 ºC, digested and analyzed by ICP-AES. Chitoclean beads and regeneration process effluents analysis data are given in Table 9.

Table 9. ***Regeneration results***

Sample	Ca mg/g	Mg mg/g	Fe mg/g	Cu mg/g	Zn mg/g	Cr mg/g
Chitoclean initial	0.03	<0.01	11.9	0.02	<0.01	<0.01
Chitoclean loaded	0.03	<0.01	12.1	2.85	0.21	0.03
Chitoclean regenerated	0.02	<0.01	0.17	<0.01	<0.01	<0.01
Regeneration wastes (combined)	0.36 mg/l	0.06 mg/l	205 mg/l	42.1 mg/l	3.23 mg/l	0.15 mg/l

Conclusions

- Treatment of drinking water with Chitoclean sorbent beads leads to effective removal of some transitional metals such as Cu and Cr
- Essential elements of the 2nd group were preserved
- Regeneration method as suggested by FTMC effectively removes pollutants (as long as their sulphates are soluble) but also stripes some modifier (Fe).

Decomposition/Disposal of spent chitin-based sorbents (GTC)

Experiments were performed on biodegrability of chitosan materials and ECM beads loaded with different pollutants in laboratory and field tests. A broad spectrum of microorganisms (over 200) was screened: fungi, yeasts and bacteria were taken from the laboratory collection, isolated from soil and directly from chitosan materials. To test ability to degrade chitosan, microorganisms were grown on nutritive agar plates with 1 % chitosan as a sole carbon source. The following microorganisms, further used for degradation of beads, were selected for the mixture Complex 1: *Geotrichum candidum, Geotrichum fermentans, Paecilomyces parvum* 0807, *Candida lipolytica, Streptomyces sp.* 275, *Actinomyces sp.* 1C and *Pseudomonas sp.*

Biodegradation experiments were performed by two methods: by cultivating microorganisms in aqueous media containing chitosan materials as a sole C source and by exposing the loaded materials in soil. Biodegrability of the chitosan samples was evaluated according to their weight loss after the experiments. Additionally, when cultures were grown in media, assessment of microorganism counts was performed periodically in order to evaluate the growth process of the cultures (for ECM beads).

Soil experiments were performed under laboratory conditions in glass vessels containing soil, where chitosan materials, placed in non-degradable bags, were buried. To evaluate microbial response towards chitosan materials in soil, respiration of natural soil microbiota was measured and calculated as cumulative CO_2 (for ECM beads). Light microscopy and SEM were used to observe growth of microorganisms on the materials.

In the first stage, biodegradation experiments of 5 different types of raw non-modified Chitosans (90/30/A1, 90/200/A1, 85/400A2, 85/1000/A1 and 85/2500/A1) were performed. Microorganisms used for degradation are: *Actinomyces sp.3, Streptomyces* 346, and *Verticillium sp*. Two biopreparations Septic-Gobbler and Compost-Gobbler (provided by MKDS) were also tested for their biodegradation potential. The results showed that the **highest degradation was observed in cases of the raw materials 85/2500/A1 and 85/1000/A1.** After 4 months experiment in liquid media, weight loss of the materials (added at 1g/100 ml concentration) was about 80%. The material 90/30/A1 was degraded at the lowest extent - up to 20%. High degradation ability was demonstrated by *Actinomycetes* and both biopreparations.

In the second stage, biodegradation of chitosan beads loaded with pollutants and then regenerated was performed. Beads from chitosan 85/1000 modified with citric acid + Fe with different loads were tested: non-loaded, loaded with copper at 4.7 and 9.8mg/g concentration (SU) and unloaded (FTMC) as well as loaded with humic acids 20 and 40 mg/g (AIT, used for soil experiments). Chitosan beads of 4 different lots (BIOLOG) loaded with pesticides (AIT) and unloaded from the pollutants (FTMC) were also investigated. Pesticides sorbed on beads were atrazin, bromacil, metolachlor, propazin, simazin. The composed mixture of screened microorganisms Complex 1 and two biopreparations Septic-Gobbler and Compost-Gobbler were used.

The results showed that when cultivating microorganisms in liquid medium with beads, counts of the all tested microbial mixtures were higher than those grown without addition of the sorbents (in sugar-free medium). This indicates that the **microorganisms assimilated chitosan beads.** Beads with copper loads also were used by microbes but slower. The highest extent of degradation of the sorbents (added at 0.1 g/50 ml concentration) in the medium after 12 weeks was observed in cases with the beads unloaded from copper and non-loaded beads (MKDS) – 90-100%. Complex 1 showed good results in decomposing beads loaded with copper – weight loss reached up to 80%. This shows rather **high resistance of the microorganisms to the metal allowing them to grow on copper-polluted sorbents** (see the pictures on Fig.21). Microorganisms from Septic Gobbler and Compost Gobbler were more susceptible towards copper, and utilization of the loaded materials reached up to 38%.

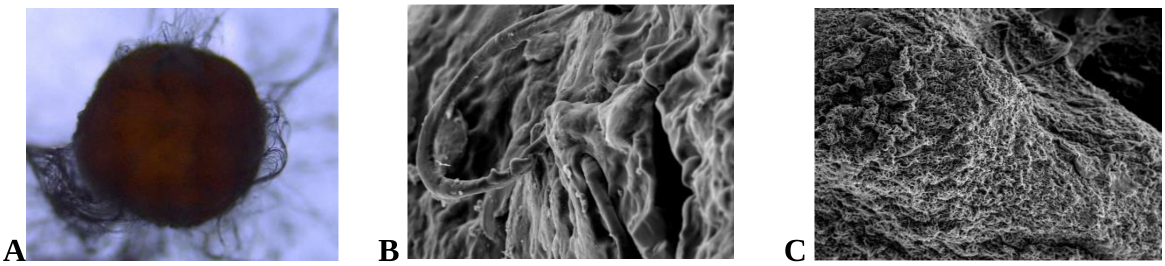
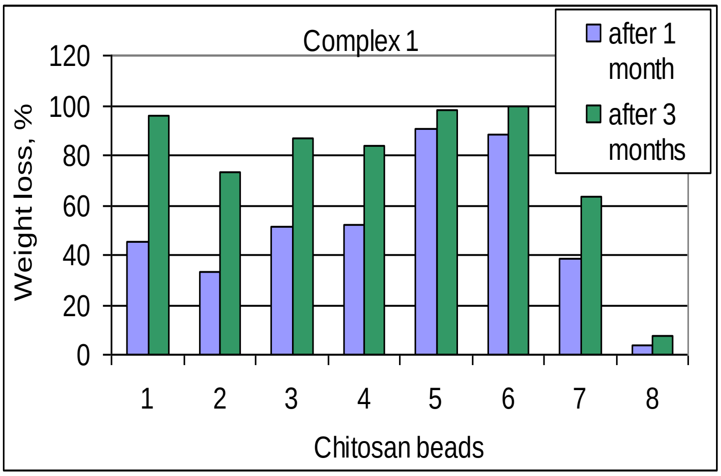


Fig.21 A - growth of Complex 1 microorganisms on chitosan beads loaded with copper (4.7 mg/g) after 1 month (light microscopy). Growth of Complex 1 on beads after 3 months :
B- fungal hyphus growing through the material of the bead loaded with copper (4.7 mg/g) SEM, x700;
C- tight net of microorganisms on a non loaded bead, SEM, x 300.

In soil experiments, the beads degradation (added 0,2 g in soil) proceeded similarly as that in the medium. The unloaded beads were decomposed completely or about 90% (Fig.22). Slower process of degradation of the sorbents loaded with copper was observed. Beads with sorbed humic acids at concentration of 40 mg/g greatly disturbed degradation of the sorbents – weight loss reached only up to 8 %, whereas the beads with lower concentration of humic acids (20 mg/g) were decomposed to 60-80%. Soil respiration measurements demonstrated that unloaded from copper and non-loaded materials stimulated metabolic activity of microorganims and it was higher than that in soil without chitosan beads, showing availability of the beads and indicating the proceeding degradation process. The copper-loaded beads had no negative effect on soil microorganisms (the values were similar to the control). Sorbents with humic acids (40mg/g) slowed down the microbial activity in soil indicating suppressed processes of soil microbiota.



1- non-loaded beads (FTMC)
2 - non-loaded beads (BIOLOG)
3- beads No1 loaded with copper 4,7 mg/g
4- beads No 2 loaded with copper 9,8 mg/g
5 - unloaded No 3 beads
6 - unloaded No 4 beads
7 - beads loaded with humic acids 20 mg/g
8 - beads loaded with humic acids 40 mg/g

Fig.22 Degradation of ECM beads in soil by Complex 1 microorganisms after 1 and 3 months

Good growth of microorganisms occurred in the medium with both unloaded beads and those loaded with pesticides. In most cases, unloaded materials supported slightly more abundant growth of microbes. Degradation extent of the loaded/unloaded beads (added at 0.1 g/50 ml concentration) after 12 weeks was high – weight loss of the materials GP008, GPP010, GPP011 loaded and unloaded was 90-100% (see Fig. 23). Only materials loaded/unloaded GPP009 were decomposed at lower extent.

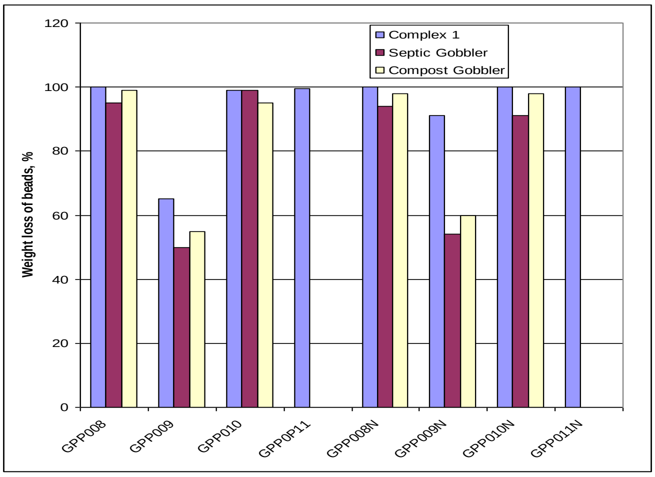


Fig. 23 Weight loss of beads (loaded with pesticides and unloaded) after 12-week incubation in the aqueous medium

Similarly, in soil weigh loss of the beads loaded with pesticides after 12 weeks was 54-100%. Slower degradation of loaded than of unloaded beads was observed in cases of GO008 and GP009. The use of the mixtures of microorganisms in most cases enhanced the decomposition process in comparison with the control (the natural microbiota in soil). Respiration experiments demonstrated that the loaded beads with pesticides at the given concentrations did not show negative influence on metabolic activity of microorganisms in soil - evolved CO_2 amounts were higher than those in control soil showing successful degradation process. Higher concentrations of pesticides could be expected to have a more significant influence on degradation course and complete decomposition would greatly depend on persistence of pesticides towards microbial degradation. Alternatively, **unloading of the used sorbents from harmful pollutants would possibly allow reusing chitosan material and could give more options for disposal safe from pollution of the environment.**

In conclusion, **loaded beads were significantly degraded both in aqueous media and in soil** showing that **different ways of microbial degradation are possible.** The sorbents **unloaded from copper were easier decomposed than those loaded with copper**. **Weight loss of the beads loaded with pesticides at the given concentrations was significant or complete, and in some cases did not differ from that of unloaded sorbents. Degradability of beads considerably depends on the concentration of sorbed pollutants** – this was showed with degradation of beads loaded with humic acids: the increased concentration of humic acids significantly reduced level of degradation of the material and disturbed microbial activity in soil. Degradation course also depends on microorganisms involved in the degradation. **The extent of decomposition was higher when screened microbes or biopreparations were added**.

IX. Mineral balances

All experiments, in laboratory or on field, clearly show that water hardness is preserved after treatment with both Chitosan and Citric Acid-$FeSO_4$-modified Chitosan. This indicates that a large number of macro and micro nutrients are preserved, since hard water contains many macro as well as micro nutrients, which all are important for the human health. More information on the evaluation of the health aspect is included in the corresponding deliverable.

The developed sorbent made of modified chitosan to a great degree complies the main requirements for sorbents, such as stability, selectivity, adsorption capacity, regenerability, and can be applied in the praxis for removal of contaminants of different chemical compositions from drinking water.