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Abstract

NIVA has examined the effect of a water quality improvement agent consisting of treated corraline algae- and shell sand, chitosan and calcium ascorbate packed in a "tea bag". One bag should be sufficient to treat 2 litres of water. The bag is submerged in the water during stirring, and shall remain in the water for approx. 10 min. before it is removed and the water is ready for consumption.

The effect of the product was examined in 4 different untreated water qualities. The results show that the effect was dependent on the initial water quality. More alkalinity, minerals and essential elements such as K, Na, Ca, Mg and Cl were added to acidic water with low mineral content than to water with a higher pH and mineral content. The treatment with the water quality improvement agent generally resulted in a small increase in turbidity, colour and organic matter. However, this is not believed to have any practical consequences. With regard to the examined metals and trace elements, there were small or no differences between treated and untreated water. One exception was mercury, which was reduced. The water quality improvement agent proved to be efficient in the removal of free and combined chlorine from chlorinated drinking water. The results also indicate that the agent reduces the number of culturable *E. coli* in water.

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1. Drikkevann	1. Drinking water
2. Vannforbedring	2. Water quality improvement
3. Mineraler	3. Minerals
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Helge Liltved
Project manager

Henning Mohn
Research manager

Benthe Wathne
Head of research department

Testing of a Water Quality Improvement Agent produced by Health by Nature as

Preface

The water quality improvement agent was tested at NIVA regional office
in Grimstad, during the summer of 2001.

Grimstad, 27.09.2001

Helge Liltved

Contents

Summary

The water quality improvement agent that was tested in this examination consists of treated corraline algae- and shell sand, chitosan, and calcium ascorbate. It is packaged in a "tea bag". One bag should be sufficient to treat 2 litres of water. The bag is submerged in the water during stirring, and shall remain in the water for approx. 10 min. before it is removed and the water is ready for consumption. NIVA has examined the effect of the product in 4 different types of water.

The results of the examination reveal that the effect of the product depended on the initial water quality. More alkalinity, minerals and essential elements such as K, Na, Ca, Mg and Cl were added to acidic water with low mineral content than to water with a higher pH and mineral content. In water that had already been treated with reverse osmosis, the sum of K, Na, Ca, Mg and Cl increased from 1,33 mg/l to 5,35 mg/l. The alkalinity increased from 0,063 mmol/l to 0,113 mmol/l.

With regard to the examined metals and trace elements, there were small or no differences between treated and untreated water. One exception was mercury, which was reduced. The water quality improvement agent proved to be efficient in the removal of residual chlorine (free and combined) from chlorinated drinking water. The results also indicate that the agent reduces the number of culturable *E. coli* in water. Treatment with the water quality improvement agent generally resulted in a small increase in turbidity, colour, and organic matter, but this is not believed to have any practical consequences.

The product from Health by Nature is intended as a water quality improvement agent that shall not necessarily result in water that satisfies the values of the Drinking Water Regulation (Ministry of Health and Social Affairs, 1996). With regard to the values of the various water quality parameters in the applicable Drinking Water Regulation, it is important to be aware of the fact that many of these are given to secure that the disinfection of the water treatment plants have a good effect, and to prevent corrosion of the mains. Therefore, these boundary values are not as pertinent for a treatment that takes place at each individual consumer.

1. Introduction

The drinking water in Norway origins mainly surface water. There are 3 main problems connected to Norwegian surface water:

- <u>Corrosive water</u>. Surface water is often acidic, with low alkalinity and low dissolved mineral content. This makes the water corrosive toward materials in pipework and armature that are in contact with the water. This has consequences both for the economy and the water quality.
- <u>High content of humic substances</u>. In eastern Norway, on the south coast of Norway, and in the counties of mid-Norway (Trøndelag), the water often contains a great deal of humic substances, resulting in a yellowish brown colour. Humic substances, also referred to as natural organic matter (NOM), are a complex mixture of organic macromolecules, created through a slow microbial and chemical degradation and transition of plant residue. High content of humic substances may give the water smell and taste, reduce the effect of disinfection agents (ultraviolet radiation or chlorination), form raised levels of detrimental chlororganic compounds, and be substrate for microorganisms and, thus, cause increased fouling of the mains.
- <u>Microorganisms</u>. Surface water is vulnerable to microbiological contamination. Even when a spring is well sheltered from direct sewage exposure, agricultural drainage as well as human, animal, and bird activities in the drainage basin will represent possible pollution sources.

By far, the majority of Norwegian inhabitants receive water that has undergone treatment. Many receive water that has only been disinfected, either through ultraviolet radiation or chlorination. Most people, however, receive water that has, in addition, undergone more extensive treatment, such as removal of humic substances and carbonatisation.

Chlorination is the dominant method of disinfecting drinking water. The disadvantages of chlorination are first and foremost connected to the formation of unwanted byproducts and, in some cases, smell and taste. The degree of byproduct formation as well as smell and taste depends of the water quality (content of organic matter and ammonium) and dose of chlorine.

Chlorine may be added to water in the shape of sodium hypochlorite or calcium hypochlorite (NaOCl or Ca(OCl)$_2$), or as chlorine gas (Cl$_2$). When chlorine is added to water, a hydrolysis takes place. OCl$^-$ ions are released, and a pH-dependent balance between hypochloric acid (HOCl) and the hypochlorite ion (OCl$^-$) is regulated. The sum of HOCl and OCl$^-$ is defined as *free chlorine*. Free chlorine quickly inactivates bacteria, and will oxidise a number of organic and inorganic compounds in water. When organic molecules are oxidised by chlorine, organic acids and aldehydes may form, as well as some low molecular chlororganic compounds such as trihalomethanes. Low concentrations of chlorinated phenols, methoxybenzene, indoles and acetonitrils may also form. Free chlorine and ammonium will react to chloramines that have an inactivating effect on bacteria, but with a weaker effect than HOCl and OCl$^-$. The sum of chloramines is defined as *combined chlorine*. The sum of free and combined chlorine is defined as *total chlorine*.

The water quality improvement agent that is tested in this study consists of treated corraline algae- and shell sand, chitosan and calcium ascorbate packaged in a "tea bag". One bag should be sufficient for treating 2 litres of water. The bag is submerged in the water during stirring, and shall remain in the water for approx.10 min. After this reaction period, the water is ready for consumption.

The treatment with the water quality improvement agent shall supply the water with alkalinity (raise the carbonate content), raise the content of calcium and minerals, and raise the pH to a stable level. In addition, it is possible that the chitosan in the product may bind some humic substances and metals

while any free and combined chlorine are removed. Previous reports have stated that the water quality improvement agent will remove coliform bacteria and mercury (Aanonsen 1999, Aanonsen 2001).

The three components in the product may affect the water quality in various ways:

Treated corraline algae- and shell-sand:
This mainly contains calciumcarbonate ($CaCO_3$), caustic lime (CaO) and various minerals and trace elements. Calciumcarbonate will dissolve in water, causing the concentration of calcium- (Ca^{2+}) and carbon ions (CO_3^{2-}) to increase. The solubility of calciumcarbonate is pH-dependent. More calcium and carbonate will dissolve at a low pH than at a higher pH. Various minerals and trace elements will also dissolve more or less, depending on the pH. Both calciumcarbonate and caustic lime will contribute to increasing the water's pH.

Chitosan:
Chitosan is a natural polymer made of shrimp- and crab shells that normally do not contain toxic compounds. Chitosan [2-Amino-2-deoxy-(1→4)-β-D-glycopyranan] consists of long, linear polymeric molecules composed of glycans. The molecular weight of the commercial substance is in the range 10 000 – 1 000 000 Dalton. Chitosan is soluble in weaker organic acids, as well as in dilute hydrochloric acid. The viscosity of a chitosan solution depends on the molecular weight.

In an acidic environment, the amino groups in chitosan will be protonised and become positively charged; thus, they will be able to react with negatively charged reactive groups on other molecules, such as carboxyl groups connected to humic substances molecules and particles (Liltved and Norgaard, 1999). In addition, chitosan has the ability to combine metals into complexes (Guibal *et al.*, 1999), and has been reported to have a bacteria impeding effect (Sudarshan *et. al.,* 1992).

Calcium ascorbate:
Calcium ascorbate will disintegrate in water and contribute to raising the calcium content as well as the organic matter content. Ascorbate will reduce oxidants such as chlorine in water.

2. Material and Methods

In the test that was carried out, bags with the water quality improvement agent supplied by the client were used. The bags contained 2,5% chitosan and 2,75% calcium ascorbate. The remaining amount consisted of treated corraline algae- and shell sand.

One bag was used for 2 litres of water in a clean plastic can. After the bag had been submerged in the water, a lid was put on. The can was shaken and turned for approx. 10 seconds. Subsequently, it stood for 10 minutes before the bag was removed and water was taken out for analysis. A control test was run parallel with the test in which the water quality improvement agent was added. This control test was treated exactly in the same way, only without adding the water quality improvement agent. The test and the control test were analysed with regard to the same parameters. In all the tests, the water temperature was 8-10 ºC.

2.1 Water types

4 different types of water with different quality were tested. Table 1 shows the quality of the various water types. The guide value in accordance with the Drinking Water Regulation (Ministry of Health and Social Affairs, 1996) is also shown. It must be mentioned that the guide values are predominantly directed towards municipal and private water treatment plants. The values for pH, alkalinity and calcium content, for example, are primarily set in order to avoid corrosion of the mains between the water treatment plant and the consumer. This problem is out of the question with regard to the use of the water quality improvement agent, as this is applied by the consumers at the end of the pipes.

The following 4 types of water were used:

- *Water that had been treated with reverse osmosis in advance (RO-water).*
  This was used to study the effect of the water quality improvement agent in water that initially had a low content of minerals, salts, and organic carbon (TOC).

- *Water tapped from the mains of the laboratory at NIVA in Grimstad (Lake Rore water source).*
  The water originated from Lake Rore, and had undergone the water treatment at the water treatment plant in Grimstad. There, CO_2 was added to the water before it was filtered through crushed marble. Subsequently, sodium hypochlorite was added for disinfection. The water treatment caused the water to have a high content of calcium, high alkalinity and a high pH. It also contained low values of total chlorine. The content of humic substances, measured as total organic carbon (TOC) and the colour were relatively high, as the water treatment in Grimstad did not include humic substances removal.

- *Well water from Vegårshei.*
  The water had a low colour and moderate values for dissolved minerals and salts. A relatively high turbidity value indicates that it contained some particles.

- *Water from the Olav spring.*
  The water had poor quality with very high turbidity and high contents of organic matter.

Table 1. The quality of the various types of water that were tested in comparison with the guiding values of the Drinking Water Regulation.

Parameter	RO-water	Water from Lake Rore	Well water	The Olav spring	Guide value
pH	6,63	7,82	6,92	6,30	7,5<pH<8,5
Conductivity, mS/m	0,50	10,28	4,06	6,10	
Turbidity, FNU	0,34	0,81	0,66	1,75	0,4
Colour, non-filtered mgPt/l	1,55	29,0	5,03	39,5	
Colour, filtered mgPt/l	<1	27,5	<1	7,35	1
UV abs. per cm	<0,010	0,148	0,015	0,076	
Alkalinity, mmol/l	0,063	0,756	0,204	0,108	0,6-1,0
TOC, mg/l	<0,10	3,8	0,82	15,1	3
Calcium, mg/l	0,21	15,8	3,59	1,76	15-25

2.2 Sampling

Chemical sampling
Tests were sent to NIVA in Oslo for chemical analysis. They were analysed according to accredited standards. In order to test the effect of the water quality improvement agent on mercury, water from Lake Rore with and without the addition of mercury was used. 15 µl 1% $Hg(Cl)_2$-solution was added to 2 l of water.

Microbiological sampling
Water from Lake Rore and well water were used to test the effect of the water quality improvement agent on *Escherichia coli* in water. A stem of *E. coli* was cultivated on an agarplate. Part of a colony was picked from the plate and dissolved in large amounts of water. Then, volumes of 1 litre were removed and the water quality improvement agent was added. In addition, volumes of 1 litre were removed to which no water quality improvement agent was added (control tests). Tests for bacteriological analysis (100 ml) were taken out for membrane filtration. The filters were placed on agar for colonies to be counted after 24 hours. The bacteriological tests were analysed at AnalyCen A/S in Arendal.

Measuring the effect on chlorine
Water from Lake Rore with sodium hypochlorite solution (Merck) was used to measure the effect of the water quality improvement agent on free chlorine and total chlorine. Testing was done with low and high quantities of chlorine. In the tests with low quantities of chlorine, 10 µl chlorine solution was added to 2 l of water from Lake Rore, whereas 70 µl was added to 2 l of water from Lake Rore in the tests with high quantities of chlorine. Tests were analysed at the time of start-up and after 10 min. contact with the water quality improvement agent.

Free and combined chlorine were measured by means of reagent diethyl-p-phenyldiamine (DPD) and a spectrophotometer (Hach DR 2000). Free chlorine immediately reacts with DPD, and a red colour develops. The intensity of colour is proportionate with the amount of free chlorine in the water. The intensity of colour was measured at a wavelength of 530 nm.

For measuring the total chlorine, a DPD-reagent with iodide was used. Combined chlorine in the solution oxidises iodide to iodine. Together, iodine and free chlorine will react with DPD to the red colour complex that is proportional with the concentration of total chlorine. In order to determine the amount of combined chlorine, the measured amount of free chlorine was subtracted from the measured amount of total chlorine.

3. Results and discussion

3.1 Effects with regard to the general water quality

The effect of the water quality improvement agent with regard to general water quality is shown in table 2. It is evident that the effect is different in the various water types. The effect will be greatest in water with low mineral and salt contents as well as a relatively low pH-value. This becomes especially evident when comparing RO-water, which is low on ions (low content of dissolved minerals and salts) and which has a pH-value under 7, with water from Lake Rore, which is rich in ions and has a high pH. In RO-water, the pH, conductivity and alkalinity increased, whereas the pH-value and the alkalinity decreased somewhat in water from Lake Rore. The conductivity showed a small increase. The conductivity is a measure of the water's ability to conduct electric current. A high conductivity indicates a high content of minerals and salts.

The turbidity of the water is a measure of the content of small suspended particles. The particles may be both organic and inorganic in origin. The turbidity values were generally somewhat higher in treated water than in untreated water. This increase may be caused by colloids and fine particles from the corraline algae- and shell sand, as well as in the chitosan that is used. The demand for low turbidity value in the Drinking Water Regulation is made mainly in order to secure effective disinfection. Thus, a small increase in turbidity in connection with the treatment that takes place after disinfection is of little practical importance.

After the water quality improvement agent was added, both the non-filtered and filtered colour as well as organic carbon (TOC) increased. An increase in organic matter was expected due to the presence of the organic compound ascorbate, which is one of the components in the water quality improvement agent. With the exception of water from Lake Rore, which had a high colour from the start, they were all far below the largest permitted concentration for filtered colour, which is 20 mgPt/l. The high colour indicates a high content of humic substances compounds. Humic substances are formed through natural decomposition of organic compounds, mainly plant residue, in and around the water source.

Table 2. Concentrations in untreated water (without the water quality improvement agent) and concentrations in water treated with the water quality improvement agent. The effect has been tested in 4 different types of water.

	RO-water		Water from Lake Rore		Well water		The Olav spring	
	without	with	without	with	without	with	without	with
pH	6,63	6,87	7,82	7,72	6,92	6,96	6,30	6,47
Conductivity, mS/m	0,50	2,00	10,28	10,71	4,06	5,02	6,10	7,43
Alkalinity, mmol/l	0,063	0,113	0,756	0,725	0,204	0,229	0,108	0,147
Turbidity, FNU	0,34	0,89	0,81	1,00	0,66	0,93	1,75	2,15
Colour, non-filt. mgPt/l	1,55	3,48	29,0	29,8	5,03	7,35	39,5	29,4
Colour, filt. mgPt/l	<1	1,94	27,5	27,9	<1	1,55	7,35	7,74
UV abs. per cm	<0,010	0,010	0,148	0,145	0,015	0,019	0,076	0,077
TOC, mg/l	<0,10	3,8	3,8	6,4	0,82	4,2	15,1	19,6

3.2 Effects with regard to essential elements

Some elements having important functions in the human body are K, Na, Ca, Mg and Cl. They all contribute to maintaining important functions such as the ion balance in tissues and organs, as well as nerve functions. In addition, calcium is included in the formation of teeth and bones.

Table 3 contains the analytical findings for these elements shown in RO-water and water from Lake Rore without the use of the water quality improvement agent, and using the water quality improvement agent. The calcium values for well water and water from the Olav spring are also included.

As the values indicate, the water quality improvement agent will contribute to increae the content of all the elements. In RO-water with an initially low content of the elements, the increase was considerable for calcium, chloride, sodium, as well as the sum of the elements. Smaller increases were observed for potassium and magnesium. The increase was smaller in water from Lake Rore, where the element content was initially higher.

Table 3. Concentrations in untreated water (without the water quality improvement agent) and concentrations in water treated with the water quality improvement agent.

	RO-water		Water from Lake Rore		Well water		The Olav spring	
	without	with	without	with	without	with	without	with
Potassium, K, mg/l	0,58	0,60	0,33	0,35				
Calcium, Ca, mg/l	0,21	2,07	15,8	16,9	3,59	5,00	1,76	3,35
Chloride, Cl, mg/l	0,2	1,2	5,9	6,7				
Magnesium, Mg, mg/l	0,11	0,19	0,52	0,55				
Sodium, Na, mg/l	0,23	1,29	3,92	4,76				
Sum of elements, mg/l	1,33	5,35	26,47	29,26				

3.3 Effects with regard to metals and trace elements

In RO-water and water from Lake Rore, a number of metals and trace elements were analysed in addition to the essential elements. Furthermore, some representatives of the lanthanide series (Ce, Nd, Pr) were analysed (table 4). The analytical values are compared with the largest permitted concentrations as described in the Drinking Water Regulation.

As expected, the RO-water initially contained low contents of metals and trace elements. As shown, small increases in the content of Al, Fe, Mn and the lanthanide series were observed, whereas the concentration of Cu, Cr, Ni, Pb, V and Cd remained constant or approximately constant. The Mo content was reduced.

There was initially a higher content of metals and trace elements in water from Lake Rore. There were small or no changes between the water without the water quality improvement agent and the water that had been treated with the water quality improvement agent. In the case of Ni and Zi, small increases were observed. As shown, only Al came close to the largest permitted concentration in the Drinking Water Regulation. The water quality improvement agent did not contribute to reducing this. The Fe-content was not reduced significantly either, despite the fact that chitosan has proven to be able to bind several metals. In experiments with coagulation of humic substances with chitosan, efficient removal of Fe has been demonstrated. It looks as though the chitosan's potential for complex linkage of metals

does not come into its own in the product. This may be the result of lacking possibilities for contact between metal and chitosan.

The level of mercury (Hg) was very low in water from Lake Rore (3,5 ng/l) (one ng is 1/1000th µg). A small decrease was recorded in treated water. In order to examine the effect of the water quality improvement agent at higher concentrations, mercury was added to a concentration of 87,5 µg/l. After the treatment, this was reduced to 65,5 µg/l. This indicates that the product reduces the content of mercury in water. Previous tests carried out with the water treatment product show a reduction of mercury from 0,51 µg/l to <0,01 µg/l (Aanonsen, 2001).

Table 4. Concentrations in untreated water (without the water quality improvement agent) and concentrations in water treated with the water quality improvement agent. The effect on metals and trace elements in RO-water and water from Lake Rore has been examined.

	RO-water		Water from Lake Rore		Greatest permitted conc.
	without	with	without	with	
Metals and trace elements					
Aluminium, Al, µg/l	<2	9	181	186	200
Iron, Fe, µg/l	<5	18	98	95	200
Copper, Cu, µg/l	0,95	1,1	12	11	300
Chromium, Cr, µg/l	<0,1	<0,1	0,2	0,3	50
Nickel, Ni, µg/l	<0,2	<0,2	0,4	0,7	50
Lead, Pb, µg/l	0,06	0,06	0,35	0,38	20
Zinc, Zn, µg/l	3,9	4,0	9,7	12	300
Vanadium, V, µg/l	<0,1	<0,1	0,2	0,2	-
Molybdenum, Mo, µg/l	0,4	<0,1	<0,1	0,2	-
Cadmium, Cd, µg/l	<0,003	0,005	0,072	0,075	5
Manganese, Mn, µg/l	0,19	1,2	14	14	50
Mercury, Hg, µg/l			0,0035	0,0030	0,5
The lanthanide series					
Cerium, Ce, µg/l	<0,003	0,046	0,072	0,075	
Neodymium, Nd, µg/l	0,003	0,028	0,53	0,51	
Praseodymium, Pr, µg/l	0,006	0,009	0,15	0,15	

3.4 Effects with regard to free and combined chlorine

Attempts were made to determine the effect of the water quality improvement agent in water that was chlorinated with low (table 5) and high (table 6) doses of chlorine. The concentrations of free chlorine were measured to 0,15 mg/l and 1,15 mg/l, respectively at the time of start-up. As indicated by the figures in the tables, both free and combined chlorine were reduced to zero after 10 minutes of contact with the water quality improvement agent. In the control tests where the water was chlorinated, but no water quality improvement agent was added, free and combined chlorine was still present after 10 minutes.

The results show that the water quality improvement agent is efficient in terms of removing residual chlorine from chlorinated drinking water. A reduction reaction between chlorine and organic compounds present in the water quality imporvement agent, primarely ascorbate and chitosan, that

forms chloride ions, may explain this. When chlorine and the water quality improvement agent only are in contact for 10 minutes, problematic levels of chlorinated organic compounds (trihalomethanes) are not likely to form, as such formation is reported to require more time (Casey and Chua, 1997). In a single test in which the water quality improvement agent (without chitosan) was added to water containing chloroform, without residual chlorine, a small reduction in the chloroform concentration from 18 til 15 µg/l was shown (Lernstål, 1998).

Table 5. The effect of the water quality improvement agent in water from Lake Rore, chlorinated with a low dose of chlorine at 8°C.

	At time of start-up		After 10 minutes	
	Free chlorine, mg/l	Combined chlorine, mg/l	Free chlorine, mg/l	Combined chlorine, mg/l
Water from Lake Rore with	0,15	0,04	0,00	0,00
Water from Lake Rore without	0,15	0,04	0,04	0,14

Table 6. The effect of the water quality improvement agent in water from Lake Rore, chlorinated with high doses of chlorine at 8°C.

	At time of start-up		After 10 minutes	
	Free chlorine, mg/l	Combined chlorine, mg/l	Free chlorine, mg/l	Combined chlorine, mg/l
Water from Lake Rore with	1,15	0,05	0,00	0,00
Water from Lake Rore without	1,15	0,05	0,86	0,11

3.5 **The effect with regard to** *Escherichia coli*

The effect of the water quality improvement agent on the *E. coli* bacterium is shown in table 7. As indicated, 2 parallel test series were made with water from Lake Rore and well water with and without the addition of the agent. The figures for water from Lake Rore show that the viability of the bacteria is reduced, even without the water quality improvement agent. Culturable *E. coli* were reduced from 47 and 22 per 100 ml after 10 min., to zero in both tests without the water quality improvement agent after 60 min. Low concentrations of combined chlorine in the water may explain the reduction. It was not possible to detect bacteria in any of the tests where the water quality improvement agent was added.

After 10 min. in well water without the water quality improvement agent (control test), the bacterial count was 1880 and 1110 per 100 ml, whereas the number of bacteria in the tests with the water quality improvement agent was 1300 and 600 per 100 ml, respectively. This represents an average reduction of 36% in relation to the control test. After 60 min., the bacterial count in the well water without the water quality improvement agent was 1170 and 1060 per 100 ml, whereas in the well water with the water quality improvement agent, it was 80 and 0 per 100 ml. That means that the bacterial count in the tests with the agent was on average 96% lower than in the tests without the agent.

Table 7. The effect of the water quality improvement agent on the *E. coli* bacterium in water from Lake Rore and well water (parallel tests).

	After 10 minutes		After 60 minutes	
	Test 1	Test 2	Test 1	Test 2
Water from Lake Rore without the agent	47	22	0	0
Water from Lake Rore with the agent	0	0	0	0
Well water without the agent	1880	1110	1170	1060
Well water with the agent	1300	600	80	0

The results indicate that the water quality improvement agent reduces the amount of culturable *E. coli* in water, and coincide with a previous study that also shows that the water quality improvement agent reduces the bacterial count (Aanonsen, 1999). This effect has two possible explanations:

1. Bacteria are adsorbed to chitosan and/or other components in the bags containing the water quality improvement agent, causing the bacterial count in the aqueous phase to be reduced.
2. One or more components in the bags containing the water quality improvement agent have an inactivating effect on bacteria, causing them to lose their ability to grow on the agar plates. According to the literature, chitosan may inhibit the growth of various types of microorganisms (Sudarshan and associates, 1992).

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