Microbially available phosphorus in drinking water

Markku Lehtola

National Public Health Institute
Department of Environmental Health
Laboratory of Microbiology
P. O. Box 95
FIN-70701 Kuopio
Finland

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Natural and Environmental Sciences of the University of Kuopio for public examination in auditorium L22 of the Snellmania building, University of Kuopio, on 11th of October 2002, at 12 o´clock noon.

Author´s address: National Public Health Institute
Department of Environmental Health
P.O.Box 95
FIN-70701 Kuopio, Finland

Phone   +358-17-201371
Telefax   +358-17-201155
E-mail   Markku.Lehtola@ktl.fi

ABSTRACT

Drinking water microbial growth has many undesirable effects on water quality, evoking esthetic and technical problems and increasing health risk. Growth of microbes in drinking water is affected by various factors like disinfection, residence time in the distribution network, temperature, and microbial nutrients like organic carbon and phosphorus. Most of the bacteria in drinking water originate from biofilms in pipelines, so that also the pipe material and hydraulics can affect the microbial growth. Chlorination is one effective and widely used method for preventing microbial growth in drinking water.

In central Europe and USA organic carbon is usually the nutrient which is limiting for microbial growth. However, in Finland and Japan, phosphorus has proven to be the limiting nutrient for microbial growth in drinking water. Standard methods are not sensitive enough for analysing the low (<2 μg/l) concentrations of phosphorus present in drinking water. Therefore a new sensitive bioassay for the analysis of low concentrations of (detection limit is 0.08 μg/l P) microbially available phosphorus (MAP) was developed. The method is based on the growth of test bacteria, *Pseudomonas fluorescens,* in water sample. The maximum growth of *P. fluorescens* is converted to the content of MAP by a conversion factor of 3.73×10^8 CFU/μg P taken from the standard curve.

The effects of different water purification techniques on the microbial nutrients (assimilable organic carbon, $AOC_{potential}$ and MAP), microbial concentrations and microbial growth potential were studied in 25 Finnish waterworks and pilot scale experiments. Chemical coagulation, activated carbon filtration and infiltration in soil removed effectively microbial nutrients (MAP and $AOC_{potential}$), microbes and microbial growth potential in the water. Ozonation increased both $AOC_{potential}$ and MAP concentrations in water, which was also seen in increasing growth potential of microbes in ozonated water. Liming of water increased MAP and disinfection with chlorine increased $AOC_{potential}$. UV-disinfection did not increase the content of MAP, in fact $AOC_{potential}$ even slightly decreased.

In drinking waters produced from groundwater, the content of MAP and microbial growth potential were higher than in drinking waters produced from surface water this being probably attributable to the more effective water purification of surface waters. In phosphorus limited drinking waters MAP, in contrast to AOC, correlated with the growth potential of microbes in the water.

The effect of phosphorus on the formation of biofilms was studied in a pilot scale experiment. In phosphorus limited drinking water, supplementation with low levels of phosphate (1-5 μg/l P) led to an increase in the concentration of microbes present in the biofilms.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Talousveden mikrobikasvu heikentää veden laatua monin eri tavoin. Haitat voivat olla esteettisiä, teknisiä tai terveydellisiä. Talousveden mikrobikasvuun vaikuttavat useat tekijät kuten desinfiointi, veden viipymä vesijohtoverkostossa, lämpötila, sekä vedessä olevat mikrobiravinteet, kuten orgaaninen hiili ja fosfori. Suurin osa talousveden mikrobeista on peräisin putkiston sisäpinnoilla olevista biofilmeistä, joiden kasvuun vaikuttavat lisäksi myös putkimateriaali ja hydrauliset olosuhteet. Veden klooraus on tehokas ja yleinen tapa estää mikrobikasvua talousvedessä.

Ravinteista orgaaninen hiili on yleensä mikrobikasvua rajoittava tekijä Keski-Euroopassa ja Yhdysvalloissa. Suomessa ja Japanissa sitävastoin fosforin on havaittu rajoittavan talousveden mikrobikasvua. Standardimenetelmät eivät ole riittävän herkkiä analysoimaan alhaisia (<2 µg/l) fosforin pitoisuuksia vedessä. Työssä kehitetty uusi, herkkä (määritysraja 0.08 µg/l P) biologinen testi mikrobeille käyttökelpoisen fosforin (MAP) määrittämiseksi vedestä perustuu *Pseudomonas fluorescens* bakteerin kasvuun vesinäytteessä. Testibakteerin maksimaalinen kasvu vesinäytteessä voidaan laskea standardoinnissa saadulla muuntokertoimella 3.73×10^8 CFU/µg P vastaamaan veden mikrobeille käyttökelpoisen fosforin pitoisuutta.

Erilaisten vedenkäsittelytekniikoiden vaikutusta veden mikrobiravinne-(assimiloituva orgaaninen hiili, $AOC_{potential}$ ja MAP) ja mikrobipitoisuuksiin tutkittiin 25:llä suomalaisella vesilaitoksella, sekä pilot mittakaavan kokein. Kemiallinen saostus, aktiivihiilisuodatus sekä maahanimeytys vähensivät tehokkaasti veden mikrobiravinteita, mikrobeja, sekä mikrobien kasvukykyä. Veden otsonointi lisäsi sekä $AOC_{potential}$ että MAP pitoisuutta, mikä heijastui myös vedessä olevien mikrobien kasvuun. Kalkin lisääminen veteen lisäsi myös veden MAP pitoisuutta ja kloorid desinfiointi $AOC_{potential}$ -pitoisuutta. UV-desinfioinnilla ei ollut vaikutusta veden MAP-pitoisuuteen, mutta $AOC_{potential}$-pitoisuus laski hieman.

Pohjavedestä valmistetussa vedessä MAP-pitoisuudet ja mikrobien kasvukyky olivat korkeampia kuin pintavedestä valmistetussa vedessä. Tämä johtui pintavesien tehokkaammasta puhdistamisesta. Fosforirajoitteisissa vesissä veden MAP-pitoisuus korreloi mikrobien kasvukyvyn kanssa.

Fosforin vaikutusta biofilmien muodostumiseen tutkittiin pilot-mittakaavassa. Fosforirajoitteisessa vedessä jo pienen fosfaattilisäyksen (1-5 µg/l P) havaittiin nostavan biofilmien mikrobipitoisuutta.

ACKNOWLEDGEMENTS

ABBREVIATIONS

AOC	assimilable organic carbon
$AOC_{potential}$	assimilable organic carbon analysed with addition of inorganic nutrients
AODC	acridine orange direct counts
ARG	artificially recharged groundwater
ATP	adenosine triphosphate
ATCC	American Type Culture Collection
BAC	biologically activated carbon
BDOC	biodegradable organic carbon
CFU	colony forming unit
DNA	deoxyribonucleic acid
GAC	granular activated carbon
HGR	heterotrophic growth potential of bacteria
HPC	heterotrophic plate count
MAP	microbially available phosphorus
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone
PAC	polyaluminum chloride
PVC	polyvinyl chloride
RNA	ribonucleic acid
SFS	Finnish Standards Association
TOC	total dissolved organic carbon
UV	ultraviolet
UV_{254}	ultraviolet radiation at the wavelength of 254 nm
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following five original articles, which are referred to in the text by the Roman numerals

I   **Lehtola M. J.,** Miettinen I. T., Vartiainen T. and Martikainen P. J. 1999. A new sensitive bioassay for determination of microbially available phosphorus in water. Applied and Environmental Microbiology. 65(5):2032-2034.

II   **Lehtola M. J**., Miettinen I. T., Vartiainen T., Myllykangas T. and Martikainen P. J. 2001. Microbially available organic carbon, phosphorus and microbial growth in ozonated drinking water. Water Research. 35(7):1635-1640.

III   **Lehtola M. J**., Miettinen I. T., Vartiainen T., and Martikainen P. J. 2002. Changes in content of microbially available phosphorus, assimilable organic carbon and microbial growth potential during drinking water treatment processes. Water Research. 36(15):3681-3690.

IV   **Lehtola M. J**., Miettinen I. T., Vartiainen T., Rantakokko P., Hirvonen A. and Martikainen P. J. Impact of UV-disinfection on microbially available phosphorus, organic carbon, and microbial growth in drinking water. Water Research, in press.

V   **Lehtola M. J.,** Miettinen I. T., and Martikainen P. J. 2002. Biofilm formation in drinking water affected by low concentrations of phosphorus. Canadian Journal of Microbiology. 48(6):494-499.

TABLE OF CONTENTS

1   INTRODUCTION

Good drinking water should not be harmful for health or for the materials of distribution system (McFeters 1990; Council directive 1998). According to the EC directive, drinking water should fulfill the quality requirements at the consumers tap (Council directive 1998), i.e. it is not sufficient the water leaving the waterworks should be of high quality, that quality has to be maintained until the consumer opens his/her water tap. Undesired health effects can be caused by chemical or microbiological agents and many various viruses, bacteria and fungal can be found in some drinking waters (Gerba *et al.* 1975; Lippy and Waltrip 1984; Augoustinos *et al.* 1995; Lahti and Hiisvirta 1995; Zacheus and Martikainen 1995; Gavriel *et al.* 1998; Ford 1999; Kukkula *et al.* 1999; Miettinen *et al.* 2001a; Kramer *et al.* 2001).

There is no clear evidence for a relationship between the incidence of pathogenic bacteria or human diseases and heterotrophic plate counts in drinking water (Payment *et al*, 1993; Gavriel *et al.* 1998; Hunter 2002). Usually waterborne epidemics are caused by accidental contamination of drinking water e.g. by flooding, surface runoff or leakage of a wastewater pipeline (Miettinen *et al.* 2001a). Some heterotrophic bacteria, like *Pseudomonas sp, Aeromonas, Bacillus, Klebsiella* and *Acinetobacteria*, commonly found in drinking water may have virulence factors and thus must be viewed as potential health risks, particularly to immunocompromised consumers (Payment *et al*. 1994; Rusin *et al*. 1997a; Pavlov *et al*. 2001). However, Edberg and Allen found that even though some bacteria growing in drinking water have virulence factors, they are not associated with human disease (Edberg *et al*. 1996; Edberg and Allen, 2002). Pathogenic faecal microbes like enteric viruses, protozoan parasites, *Campylobacter sp., Enterohemorragic Esherichia coli* (EHEC), *Yersinia enterocolitica, Microsporidia, Helicobacter pylori, Salmonella ap.* and *Shigella sp.* from contamined raw water sources may gain access to the drinking water as a result of inadequate water treatment (Lippy and Walltrip 1984; Lahti and Hiisvirta 1995; Kukkula *et al.* 1999; Ford 1999; Percival and Walker 1999; Szewzyk *et al*. 2000; Miettinen *et al*. 2001a). These microbes probably do not multiply in the drinking water environment, but they may survive better in biofilms (Percival and Walker 1999, Storey and Ashbolt 2001). LeChevallier *et al.* (1991) found that a high organic carbon concentration was associated with higher concentrations of coliform bacteria in drinking water. On the other hand, during starvation, bacteria become highly resistant to disinfectants (Matin and Harakeh 1990).

The growth of native non-pathogenic microbes in distribution system can still have several undesired effects on the water quality; e.g. it can complicate bacteriological water quality monitoring, enhance the growth of opportunistic pathogenic bacteria, iron bacteria can precipitate iron and produce iron flocs, the growth of actinomycetes and fungi give an unpleasant taste and odor to the water and microbial growth can promote biocorrosion of pipes (LeChevallier and McFeters 1985; Van der Kooij 1990; Percival and Walker 1999). There are also some pathogens like *Legionella, Pseudomonas aeruginosa, Mycobacteria* and *Aeromonas*, which are able to grow in drinking water distribution systems (Rusin *et al*. 1997a and 1997b; Percival and Walker 1999; Szewzyk *et al*. 2000). Bacteria and fungi serve as food for protozoa and higher animals present in the distribution system (Van der Kooij, 1990; Sibille *et al*., 1998).

In Europe, there are some national guidelines for HPC in drinking water, e. g. in Germany the guidance value for HPC is 100 CFU/ml (Uhl *et al*. 2001). In USA the acceptable level for HPC is less than 500 CFU/ml, and in Canada the guideline for HPC is 500 CFU/ml (Robertson and Brooks 2002). In Australia, the guideline is 100 CFU/ml for disinfected supplies and 500 CFU/ml for undisinfected supplies (Robertson and Brooks 2002). In Finland, the guideline for HPC is in accordance with the European Council directive, which says that there should be no abnormal changes in HPC (22 °C) (Council directive 1998; Soveltamisopas talousvesiasetukseen 461/2000). The WHO guideline states that HPC is of little sanitary value, but a good indication of the efficiency of water treatment, thus the WHO recommendation is that the HPC concentration should be at the lowest level possible (WHO 1996; Robertson and Brooks 2002).

The bacterial counts analysed by plate counting method depends on the characteristics of the agar medium, incubation temperature and incubation time (Reasoner and Geldreich 1985; Block 2002; Reasoner 2002). Culturability of heterotrophic bacteria is affected also by environmental stress like nutrient starvation and presence of electron acceptors (Block 2002; Boualam *et al*. 2002).

The growth of microbes in drinking water is affected by various factors like residence time (Kerneis *et al.* 1995; Zhang and DiGiano, 2002), temperature (Nedwell 1999; Zhang and DiGiano 2002), disinfection (chapter 2.1.6) and nutrients (chapter 2.3). In biofilms, also the pipe

material and hydraulics can affect the microbial growth (chapter 2.2). Low temperatures may decrease the affinity of microbes for substrates (Nedwell 1999).

Water treatment processes remove part of the chemical compounds and microbes from raw water, in treated water in water distribution system there still remain some microbes as well as the essential nutrients to support microbial growth (LeChevallier 1990; Logsdon 1990; Van der Kooij 1992; Miettinen *et al*. 1996b and 1997a; Sathasivan *et al.* 1997; Percival and Walker 1999). In this thesis the associations between microbial nutrients, especially that of phosphorus, microbial concentrations and microbial growth in different Finnish drinking waters were studied. The effects of different water purification techniques on water chemistry and microbial growth were examined. The importance of phosphorus on biofilm microbial growth was also studied.

2 REVIEW OF THE LITERATURE

2.1 Drinking water treatment

Drinking water purification consists of various treatment processes which are deviced and adjusted individually depending on the raw water characteristics. However, all water treatment has some common processes e. g. chemical coagulation, ozonation, activated carbon filtration and disinfection. In this section, some of the most widely used drinking water treatment processes are shortly reviewed, and their effects on microbial nutrients and microbes are discussed.

2.1.1 Chemical coagulation

During chemical coagulation negatively charged particles are first neutralized. Neutral particles can then become attached to each other and form larger particles, and these can be separated from water by sedimentation or flotation and rapid sand filtration. Usually iron or aluminium salts are used for coagulation (Dennet *et al.* 1996; Jiang 2000; Hansen 2001). The effect of coagulation depends on pH, coagulant and its dose and the concentration and characteristics of the organic matter to be coagulated (Dennet *et al.* 1996; Exall and Vanloon 2000; Hansen 2001).

Coagulation is an efficient way of removing total organic carbon and biodegradable organic carbon (BDOC) (Singsabaugh *et al.* 1986; Dennet *et al.* 1996; Jiang 2000; Volk *et al.* 2000a). There is wide variation in the efficiency of removal of assimilable organic carbon (AOC) by chemical coagulation. Volk *et al*. (2000a) found poor removal of AOC with chemical coagulation, whereas some other studies have shown a significant reduction in AOC (Van der Kooij 1990; Charnock and Kjønnø 2000). Chemical coagulation effectively removes phosphorus from water (Nishijima *et al.* 1997). It enhances the removal of microbes in filtration (Jiang 2000) and is effective in removing also viruses from water (Gerba *et al.* 1975)

2.1.2 Ozonation

Ozonation is a commonly used technique for removing pathogenic microbes, taste and odor from water (Anselme *et al.* 1988; Langlais *et al.* 1991). Ozone can also coagulate natural water constituents and can thus be applied as a preoxidant in chemical coagulation (Glaze 1987).

Ozone is an unstable gas, which has to be generated on-site in the waterworks. Usually ozone is generated in water treatment by the cold plasma discharge method, where ozone is produced from the decomposition of diatomic oxygen (Glaze 1987; Langlais *et al.* 1991). Ozone is a strong oxidant, which degrades effectively natural organic matter (Glaze 1987; Kainulainen *et al.* 1994; Karpel *et al.* 1996; Miettinen *et al.* 1998). Ozonation reduce the formation of disinfection by-products in postchlorination (Tuhkanen *et al*. 1994; Kainulainen *et al*. 1995). Reactions with natural organic matter increase the content of easily available organic carbon in water, and may thus enhance microbial growth in the distribution network (van der Kooij *et al.* 1982; Van der Kooij and Hijnen 1984; Miettinen *et al.* 1998; Escobar *et al*. 2001; Escobar and Randall 2001). Ozonation can lead to the formation of ozonation by-products, of particular concern is carcinogenic bromate which is produced when water containing bromide is ozonated (Fielding and Hutchison 1995; Myllykangas *et al*. 2000).

Ozone is an efficient disinfectant. The disinfection mechanism is based on the reaction with the double bonds in fatty acids of bacterial cell walls and cell membranes and the protein capsid of viruses (Singer 1990). One disadvantage in ozone disinfection is its unstability in water, ozone decomposes rapidly back to oxygen. At pH 8 the half life of ozone is less than one hour, which is too short to ensure efficient disinfection throughout the distribution systems (Glaze 1987; Singer 1990).

2.1.3 Activated carbon filtration

Activated carbon removes contaminants from water by adsorption (Culp and Culp 1974; LeChevallier and McFeters 1990). Its high surface area is the key to efficient adsorption. Granular activated carbon (GAC) has a surface area in the range 500-1400 m^2/g (Culp and Culp 1974). GAC filters are effective in removing humic substances from water (Servais *et al.* 1991; Klavins *et al.* 2000).

Often GAC filtration is used after water ozonation (Boere 1992; Hu *et al*. 1999). Bacteria colonize GAC beds, and GAC filters have always some biological activity (BAC, biologically activated carbon) which enhances the removal of organic compounds (DeLaat *et al.* 1985; LeChevallier and McFeters 1990; Singer 1990; Kainulainen *et al*. 1995; Nishijima *et al.* 1997). As a result of the microbial activity in BAC filter, the efficacy of the activated carbon filtration is affected by temperature (Servais *et al.* 1992), pH and content the of dissolved oxygen

(Scholtz and Martin 1997) and phosphorus (Nishijima *et al.* 1997; Scholtz and Martin 1997). However, Vahala *et al*. (1998a) found no enhanced removal of organic matter with BAC by addition of phosphorus, although the concentration of bacteria in the filter effluent increased significantly. BAC/GAC filters are important after ozonation because they remove biodegradable organic carbon compounds produced during ozonation (Van der Kooij *et al.* 1989; LeChevallier *et al.* 1992; Servais *et al.* 1992; Pietari 1996; Ribas *et al*. 1997; Hu *et al.* 1999; Vahala *et al*. 1998b and 1999).

The growth of microbes in BAC filters is high, causing some release of bacterial biomass into the outflow (Van der Kooij *et al.* 1989; LeChevallier and McFeters 1990. Servais *et al.* 1991; Pietari 1995; Vahala *et al*. 1998a), often attached to the carbon particles (Camper *et al.* 1986; LeChevallier and McFeters 1990)

2.1.4   pH-adjustment

Treated surface waters are acidic, in Finland ground waters are also often acidic (Hatva 1989; Kivimäki 1992). To prevent the corrosion of pipes, the water pH should be elevated, and aggressive CO_2 binded (carbonate CO_3^-, bicarbonate HCO_3^-) before distribution into the network (Kajosaari 1981). In Finland, water pH is increased by $Ca(OH)_2$, NaOH, CaO, Na_2CO_3 or $NaAlO_2$ (Kivimäki 1992; Raassina and Suokas 2001).

Lime rock ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) filtration as a final stage treatment is becoming more common in small waterworks in Finland (Raassina and Suokas 2001). This treatment increases pH, hardness and alkalinity, and there is no risk for overdosing (Jacks and Frycklund 1996; Sallanko and Lakso 2000; Raassina and Suokas 2001). Alkalizing wet filtration of ground water can remove iron, manganese, organic matter and microbially available phosphorus (Sallanko and Lakso 2000).

2.1.5   Artificial recharge of ground water

Ground water has many advantages over surface waters in drinking water production. It needs less treatment, it has usually of better quality and is protected against pollutants (Hatva 1996). Also the temperature varies less in ground water than in surface waters. In 1996, 56 % of

drinking water in Finland was ground water, of which 9 % was artificially recharged and 9 % bank filtrated (Hatva 1996).

Artificially recharging of ground water can be divided into two main categories. In the direct methods, the yield of aquifer is increased by spreading surface water in permeable soil deposits (basin recharge, sprinkling, pit recharge). In indirect methods, the yield of the aquifer is increased by lowering the water level in wells in order to allow water flow from the nearby surface water source into the aquifer (bank filtration) (Hatva 1996). Soil can improve water quality in many ways. For example mechanical straining, sedimentation, adsorption and biochemical/bacterial activities (Huisman and Olsthoorn 1983; Juhna 1999). The quality of artificially recharged water depends on the quality (dissolved and particulate substances) of filtrated water, the microbiology of water and filter, pore structure of the filter, surface structure of the solid matrix, residence time of water in the filter and algae growth in the infiltration zone and environmental conditions (Huisman and Olsthoorn 1983; Literathy and Laszlo 1996; Schmidt 1996; Juhna 1999).

There are several studies demonstrating how artificial recharging of ground water can affect the quality of water. It removes organic matter (Roberts and Valocchi 1981; Farooq *et al.* 1994; Miettinen *et al.* 1996a; Juhna 1999), bacteria (Eighmy *et al.* 1992; Farooq *et al*. 1994; Miettinen *et al.* 1996a;), viruses (Peters *et al.* 1998; Schijven *et al*. 2000), and some pollutants (Schwarzenbach *et al.* 1983; Stuyfzand and Kooiman 1996; Zullei-Seibert 1996). However, in some conditions the number of bacteria can increase during the artificial recharge of ground water (Eighmy *et al*. 1992; Albrechtsen *et al.* 1998). The concentration of AOC decreases during artificial recharge of ground water (Miettinen *et al.* 1996a and 2001b; Albrechtsen *et al.* 1998; Kivimäki *et al.* 1998; Kuehn and Mueller 2000).

2.1.6   Disinfection

Disinfection of drinking water is required to destroy pathogenic organisms causing waterborne diseases. Waterworks disinfect water by ozonation (chapter 2.1.2), chlorine agents or UV-radiation (LeChevallier 1999).

Chlorine agents can be added to water as free chlorine, chlorine dioxide or via chloramines. Free chlorine destroys bacteria mainly by reactions with their enzymes (White 1986), while chloramine reacts with nucleic acids, tryptophan and sulfur-containing amino acids (LeChevallier *et al*. 1988). The factors affecting the disinfection efficiency are: the chemical nature of disinfectant, the concentration of disinfectant, the length of the contact time, the temperature, the type and concentration of organisms and the pH (Wolfe *et al*. 1985; White 1986). Various chlorine compounds act differently in the distribution system. Chloramines are much better than free chlorine in termes of residual stability, biofilm control and the tendency to form unwanted byproducts (LeChevallier *et al*. 1988 and 1990; Trussel 1998; Nissinen *et al*. 2002). Addition of chloramines may enhance ammonia oxidation by nirtifying bacteria and increase the content of nitrite and nitrate in drinking water (Odell *et al.* 1996; Wilczak *et al.* 1996).

One major disadvantage of using chlorine as the disinfectant is the formation of harmful by-products (Boorman *et al*. 1999). In 1974, the formation of trihalomethanes (THMs) in chlorination was first reported (Bellar *et al.* 1974; Rook 1974). Chlorination increases the mutagenicity, mainly via the formation of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (called MX), especially if the water contains a high amount of organic matter (Vartiainen and Liimatainen 1986; Vartiainen *et al.* 1988; Smeds *et al*. 1997). Chlorine also degrades organic matter and increases the content of easily available organic carbon for bacteria (AOC) (Van der Kooij 1990; Miettinen *et al.* 1998; Charnock and Kjønnø 2000; Lehtola *et al*. 2001; Okabe *et al.* 2001). In spite of its negative effects, chlorination of drinking water is considered to be the most effective public health measure ever instituted (Bull *et al.* 1995; Boorman *et al*. 1999).

UV-radiation (λ=253.7 nm) is effective in destroying bacteria and viruses in water or wastewater (Anghern 1984; Wolfe 1990; Oppenheimer *et al.* 1997; Parrotta and Bekdash 1998; Clancy *et al.* 2000). The destructive effect of UV-radiation is based on DNA and RNA damage such as thymine dimer formation, hydrate formation in the DNA, denaturation of the DNA double strand and polymerization between nucleic acids and proteins. (Anghern 1984; Wolfe 1990; Parrotta and Bekdash 1998). The absorption maximum of DNA and RNA 255-265 nm is near the wavelengths emitted from mercury low pressure lamps (253.7 nm). Most microorganisms are inactivated by relatively low UV_{254} dosages – usually in the range of 2-6

mWs/cm^2, though viruses tend to be more resistant to UV$_{254}$ radiation than bacteria (Wolfe 1990). Bacteria posses photoreactivation mechanisms at wavelengths of 300-500 nm to repair damaged DNA (Harris *et al.* 1987; Wolfe 1990). Some water quality parameters like iron, manganese and organic matter can affect UV-disinfection (Parrotta and Bekdash 1998). Guidelines for UV-disinfection require UV$_{254}$ radiation doses of 16-38 mWs/cm^2 throughout the water disinfection chamber (Parrotta and Bekdash 1998).

UV$_{254}$ radiation degrades organic matter (Armstrong *et al.* 1966; Corin *et al.* 1996; Kulovaara *et al.* 1996), with the simultaneous release of phosphate (Armstrong *et al.* 1966; RonVaz *et al.* 1992; Vähätalo and Salonen 1996). There is no evidence of the formation of undesirable by-products (mutagenicity) or any increase in the content of biodegradable organic carbon after UV-disinfection (Wolfe 1990; Kruithof *et al.* 1992; Shaw *et al.* 2000a).

Disadvantage of UV-disinfection is similar to ozonation, the lack of residual activity in the distribution system (Wolfe 1990). Some residuals inhibiting bacterial growth after UV$_{254}$-irradiation can occur, probably attributable to hydroxyl radicals produced in the photochemical reactions when UV reacts with water organic matter (Gjessing and Källqvist 1991; Lund and Hongve 1994). Kruithof *et al.* (1992) suggested that UV-disinfection is especially suitable for drinking waters with low nutrient concentrations (AOC) to prevent microbial regrowth in the distribution networks.

2.2   Biofilms in the distribution network

The deterioration of water microbiological quality in the distribution system is one of the main problems faced in drinking water production (Laurent *et al.* 1993). Microbial cells can become firmly attached to almost any surface in an aquatic environment (Characklis and Marshall 1990). In drinking water distribution networks, bacteria in biofilms represent the most important part of the bacterial biomass (Laurent *et al.* 1993; Zacheus *et al.* 2001) and detachment of bacteria from biofilms accounts for most planktonic cells present in the water (Van der Wende *et al.* 1989). Other problems caused by the occurrence of biofilms are: bacteria are part of the food web and support the growth of higher organisms, they may generate turbidity, taste and odors, high counts of heterotrophic bacteria interfere with the detection of coliforms, microbes cause biocorrosion and they increase frictional resistances in the distribution system (Block 1992;

Critchley *et al.* 2001). Biofilms can also promote the survival and growth of pathogenic bacteria and enhance the survival of viruses and parasites (LeChevallier *et al.* 1987; Keevil 1989; Buswell *et al.* 1998; Ford 1999; Percival and Walker 1999; Storey and Ashbolt 2001) and increase the disinfection resistance of bacteria (LeChevallier *et al.* 1987 and 1990; Percival and Walker 1999; Gilbert *et al*. 2002). In biofilms, there is an accelerated transfer of genetic material by conjugation (Angles *et al*. 1993; Hausner and Wuertz 1999; Ghico 2001). This may cause conjugational spread of virulence factors, antibiotic resistance and enhanced environmental survival capabilities of the bacteria (Watnick and Koltner 2000; Ghico 2001).

Many factors can influence the formation of biofilms on the surfaces of the pipeline e.g. microbial nutrients, pipe materials, disinfectants, bacteria from water and the hydraulic regime (Block 1992; Mathieu *et al.* 1994; Van der Kooij *et al.* 1995; Camper *et al.* 1996; Niquette *et al*. 2000; Zacheus *et al*. 2000). Biofilms are dynamic, there is continual attachment and detachment of microbes, their death and regrowth (Block 1992; O´Toole *et al.* 2000; Watnick and Kolter 2000). After attachment to a surface, bacteria often undergo adaptation to life in a biofilm. For example, there is an increase in synthesis of exopolysaccharides (O´Toole *et al.* 2000; Watnick and Kolter 2000). In drinking water distribution networks in general, organic carbon is the limiting nutrient for microbial growth in biofilms (Block 1992; Block *et al*. 1993; Laurent *et al.* 1993; Van der Kooij *et al.* 1995; Chandy and Angles 2001; Appenzeller *et al*. 2001).

Some countries use phosphate based anticorrosion chemicals in their distribution systems. There is no evidence that these chemicals increase microbial numbers (Abernathy and Camper, 1998; Chandy and Angles 2001; Rompré *et al.* 2000; Volk *et al*. 2000b; Appenzeller *et al.* 2001). Phosphates can have a positive impact by controlling corrosion (e.g. lead and iron) (Abernathy and Camper 1998; Rompré *et al.* 2000; Appenzeller *et al.* 2001 and 2002). The efficiency of phosphates for biofilm control is based on neutralization of positively charged corrosion products, such as goethite (α-FeOOH), to negatively charged $FePO_4$ and thus lowering the adhesion of bacteria to pipe surfaces (Abernathy and Camper 1998; Appenzeller *et al*. 2002).

It is difficult to prevent the formation of biofilms by disinfection with chlorine since it requires a residual concentration >1-2 mg/l (LeChevallier *et al*. 1987; Van der Wende *et al.* 1989; Block 1992). Inactivation of fixed bacteria in biofilms needs even an higher concentration of chlorine (> 3 mg/l) (LeChevallier *et al.* 1990; Paquin *et al.* 1992). The inactivation efficiency depends on

the composition of the pipe material (LeChevallier *et al.* 1990), the disinfection agent (chloramine vs. chlorine) (LeChevallier *et al.* 1988; Block 1992), temperature and water velocity (Characklis 1990).

2.3   Nutrients in drinking water and microbial growth

2.3.1   Carbon

Organisms with the exception of photoautotrophs and chemoautotrophs need organic compounds both for their carbon and energy sources. Organic compounds are partially assimilated into the cell material and partially oxidised to provide energy (Schlegel 1997). The polysaccharides, cellulose and starch, are commonly found organic compounds in the biosphere. Glucose and other sugars are the preferred nutrients for most heterotrophic microorganisms (Schlegel 1997).

Bacteria can utilize a wide range of substrates, but some substrates are more readily usable than others. According to Van der Kooij *et al.* (1982a), most amino acids and many carboxylic acids and carbohyhrates are utilized by *Pseudomonas fluorescens* in preference to aromatic compounds.

In drinking water, all of the organic carbon is not available for microbial growth. Usually only a small part of total organic carbon is easily utilized by microbes (Van der Kooiij 1982b). There are some analytical methods available to determine this biodegradable portion of organic carbon.

Analytics

There are two approaches to analyse the microbial usability of aquatic natural organic matter: determination of assimilable (available) organic carbon (AOC) or biodegradable organic carbon (BDOC). AOC is that part of organic matter that can be converted to cell mass and expressed as a carbon concentration by means of a conversion factor, BDOC is the part of organic carbon which can be mineralized by heterotrophic microbes (Servais *et al*. 1987; Huck 1990).

The first method for AOC assay was published in 1982 by Van der Kooij *et al.* (1982b). After that, many studies were published to improve the method (Huck 1990). At present, the AOC test is the proposed standard method according to Standard Methods (1995). The test is based on the growth of a bacterial inoculum in a water sample and AOC is calculated using empirical yield values. The bacterial strains used in the test are *Pseudomonas fluorescens* strain P-17 and *Spirillum* strain NOX (Standard Methods 1995). In regions where there is a high content of organic matter and limitation of phosphorus, some modification of the test is required. Miettinen *et al.* (1999) modified the test by adding inorganic nutrients to the water sample to ensure that only organic carbon would limit the bacterial growth in the water, and suggested the term $AOC_{potential}$ should be used.

Servais *et al.* presented the first method for BDOC in 1987. In that method organic carbon was mineralized by the natural microbial community, and BDOC was measured as the difference in content of dissolved organic carbon (DOC) before and after (>10 days) incubation of the inoculated water sample (Servais *et al.* 1987). DOC is nowadays usually analysed with combustion-infrared method after water filtration through a 0.45 μm filter (Standard Methods 1995). Later methods were developed where the water sample was filtered through a column where the microbes were attached via a biofilm to the support matrix (e.g. glass, sand), and the difference in DOC between inlet and outlet was analysed (Lucena *et al.* 1990; Frias *et al.* 1992).

Only a small part of total organic carbon is easily utilized by microbes (Van der Kooij *et al.* 1982a and b). LeChevallier *et al.* (1991) proposed that to limit the growth of coliform bacteria in drinking water, AOC levels should be below 50 μg/l. Van der Kooij (1990) reported that if one wishes to limit aftergrowth, the AOC concentration should be less than 10 μg/l. With respect to the BDOC, it has been proposed the guideline value of 0.15 mg/l for biologically stable water (Servais *et al.* 1995). Because only two bacterial strains are used in AOC analysis, the concentration of AOC is lower than content of carbon obtained in the BDOC method (Frias *et al.* 1995; Standard Methods 1995).

2.3.2 Phosphorus

Phosphorus occurs in nature only in the form of chemical compounds, either as inorganic orthophosphate (HPO_4^{2-}, $H_2PO_4^-$) or in organic compounds. Total phosphorus can be subdivided

into particulate phosphorus and soluble phosphorus. Furthermore, soluble phosphorus can be divided into soluble reactive phosphorus and soluble unreactive phosphorus (Holtan *et al.* 1988).

Particulate phosphorus consists of adsorbed, exchangeable phosphorus, organic phosphorus, precipitates, reaction products with Ca^{2+}, Fe^{2+}, Al^{3+} and other cations as well as crystalline minerals and amorphous phosphorus. The soluble form of phosphorus is normally though to consist of orthophosphate, inorganic polyphosphates and dissolved organic phosphorus (Holtan *et al.* 1988). The distribution of different species of orthophosphate (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, or PO_4^{3-}) is pH-dependent (Holtan *et al*. 1988). In a humus-rich environment, phosphorus becomes associated with higher molecular weight humic materials, especially in the presence of iron or manganese (Jones *et al.* 1988; Shaw *et al*. 2000b; Hens and Merckx 2002). A large part of the identified organic phosphorus fraction is represented by inositol phosphates, phospholipids, nucleic acids, organic acids and phosphate esters (Stevenson 1982). Organic phosphorus can be hydrolysed to inorganic forms through chemical and/or biological reactions (Holtan *et al.* 1988), or by reactions driven by UV-radiation (Armstrong *et al.* 1966; Ron Vaz *et al.* 1992; Vähätalo and Salonen 1996; Tranvik 1998). Phosphorus combined to biological material (bacteria, phytoplankton) can comprise a large fraction of the total phosphorus in lake water (Jones 1997).

Phosphorus is an essential nutrient for microbes since it is one of the macronutrients which are present in all cells (Schlegel 1997). When the phosphorus concentration is sufficient, bacteria like *E. coli*, use a low affinity P_i transport system known as Pit system. In phosphorus deficiency this system is inefficient and the Pho regulon genes turn on, inducing alkaline phosphatase activity (Ammerman 2002). Pho regulon is also a code for proteins that facilitate phosphorus assimilation in phosphorus deficient conditions, including the high affinity Pst transport system for phosphate (Ammerman 2002).

Bacteria need phosphorus for the biosynthesis of nucleic acids, lipopolysaccharides and phospholipids (Jones 1997; Schlegel 1997). Phosphate is a vital component of the intracellular energy- transferring ATP system (Jones 1997; Schlegel 1997). The main fraction of phopsphorus in bacterial cell is DNA + RNA + lipids, constituting approximately 60 % of the total cell phosphorus, other fractions are cytoplasmic phosphate (organic and inorganic) and polyphoshate (Vadstein 2000). This is apparent in the optimum C:N:P ratio for bacterial growth which is 100:10:1 (Van der Kooij 1982b; Zhang and DiGiano 2002). According to Anderson

and Domsch (1980) the ratio of C:P in bacterial cells is 17. Similarly, Gächter and Meyer (1993) reported the C:P ratio in bacteria to be 20. In an environment with a high phosphorus availability, the C:P ratio can be as low as 5, corresponding to a phosphorus content of 10 % of the dry weight (Gächter and Meyer 1993). In the study of Hochstädter (2000), the C:P ratio in bacteria in a lake varied between 50-130 being highest in phosphorus limiting conditions during the summer. With phosphorus limitation, the phosphorus content in the bacteria depends also on the specific growth rate (Vadstein 2000). In a lake ecosystem, there is evidence that bacteria may also act as a sink of the available phosphorus and thus also heterotrophic bacteria can be important consumers of inorganic phosphorus (Vadtstein 2000). Various bacterial strains have different maximum specific phosphorus uptake rates, affinities and half saturation constants (k_m) for phosphorus, the k_m values can vary between 0.39 and 7.6 µg/l P (median 3.0 µg/l P) in different bacterial strains (Vadstein 2000).

Heterotrophic bacteria can store polyphosphates in granules, which serve as a storage site of phosphorus to be used via the polyphosphate kinase for synthesis of nucleic acids and phospholipids during external phosphorus limitation (Schlegel 1997; Vadstein 2000). Polyphosphates are accumulated when phosphate is present but microbial growth is terminated by a growth limiting factor or the presence of a growth inhibitory agent (Schlegel 1997). Under aerobic conditions, bacteria can store polyphosphates also as an energy reserve for later use. Stored polyphosphate is transformed to ATP and the use of ATP leads to phosphate release (Waara *et al.* 1993).

In terms of its nutrient status, soluble inorganic phosphate is considered to be entirely biologically available (Chapelle 1992; Jones 1997). All living organisms posses the enzyme, alkaline phosphatase, to convert organic phosphorus to inorganic phosphorus, but only microbes and fungi can excrete the enzyme outside of their cells (exoenzymes), for remineralization and dissolving of organic phosphates (Jones 1997). Also the hydrophobicity or hydrophilicity of the phosphorus compounds can affect the availability of phosphorus for microbial use (Lemke *et al*. 1995). Acid phosphatases are active in the internal cell metabolism (Jansson *et al*. 1988). The synthesis of external alkaline phosphatases is often repressed at high phosphate concentrations (Jansson *et al.* 1988). The synthesis of external alkaline phosphatases has been used as a phosphorus deficiency indicator (Jansson *et al*. 1988).

Analytics

Chemical phosphorus analysis has two steps: 1) conversion of phosphorus compounds to dissolved orthophosphate, and 2) colorimetric determination of the dissolved orthophosphate. According to the Finnish standards, phosphorus is analysed by the ascorbic acid method, where ammonium molybdate and potassium antimonyl tartrate react in an acid medium with orthophosphate to form phosphomolybdic acid, which then reacts with ascorbic acid forming the colored compound molybdenum blue, which can be analysed with a spectrophotometer (Standard Methods 1995; SFS 3026, 1986). In analysing total phosphorus, all phosphorus compounds are digested with peroxodisulphate to orthophosphate (SFS 3026, 1986). With these colorimetric standard methods, the detection limit for phosphorus in water is 2 and 10 μg/l P (SFS 3026, 1986; Standard Methods 1995, respectively). When analysing orthophosphate, there are some problems encountered in the analytics. Baldwin (1998) and Stainton (1980) showed that part of organic or colloidal phosphorus compounds is hydrolysed/displaced during the analysis and there can be an overestimation on the concentration of orthophosphate.

There also are some other sensitive analytical methods for analysing phosphorus from water. The magnesium-induced coprecipitation procedure (MAGIC) can analyse phosphorus concentrations down to 31 ng/l P (Karl and Tien 1992). A fast method for analysing phosphate was developed utilizing capillary electrophoresis, but the sensitivity of this method is rather low 10 μg/l P (Pantsar-Kallio and Manninen 1995). For organic phosphorus, there is a method where organically combined phosphorus is converted to orthophosphate by UV-radiation in an excess of dissolved oxygen, the detection limit for this method is 0.64 μg/l P (Ron Vaz *et al.* 1992). There also are bioassays to analyse algal-available phosphorus (see review by Ekholm 1998).

2.3.3   Other nutrients

In addition to phosphorus, the other essential macronutrients for cells are hydrogen, oxygen, nitrogen, sulphur, sodium, potassium, calcium, magnesium and iron (Schlegel 1997). These nutrients generally do not limit heterotrophic microbial growth in drinking waters (Miettinen *et al.* 1996b and 1997a). Ammonia may enhance the growth of chemolithotrophic ammonia oxidizing and nitrite oxidizing bacteria (Odell *et al.* 1996; Wilczak *et al.* 1996).

2.3.4 Nutrient limitations of bacterial growth in drinking water

Since the first article was published to determine the concentration of easily assimilable organic carbon (Van der Kooij *et al*. 1982b), there has been much research undertaken on the effects of nutrients on the microbial growth in drinking water. Genereally speaking, microbial growth in drinking water is limited by assimilable organic carbon (AOC) or biodegradable organic carbon (BDOC) (Van der Kooij *et al.* 1982b, LeChevallier 1990; Joret *et al.* 1991; Mathieu *et al.* 1992; Van der Kooij 1992; Servais *et al.* 1995, Prévost *et al*. 1998; Niquette *et al*. 2001). This is due to the low content of AOC compared to phosphorus; e.g. in an American study the C:P ratio in drinking water was found to be in the range 100:250 to 100:43, while the typical ratio for optimal microbial activity is 100:1 (Zhang and DiGiano 2002). Fransolet *et al.* (1988) found that bicarbonate/sodium could limit microbial growth in some oligotrophic waters.

In some areas, the correlation between AOC and heterotrophic growth response is weak (Noble *et al.* 1996; Zhang and DiGiano 2002) or there is no correlation at all (Gibbs *et al.* 1993; Miettinen *et al.* 1997b). Kerneis *et al.* (1995) found no correlation between BDOC and growth of heterotrophic microbes in a drinking water distribution network.

In lakes and rivers, microbial growth is many times limited by phosphorus (Haas *et al.* 1988; Coveney and Wetzel 1992; Mohammed *et al.* 1998; Hochstadter 2000; Hudson *et al.* 2000; Vadstein 2000). In 1996 it was found that in Finland microbial growth in drinking water is limited by the availability of phosphorus (Miettinen *et al.* 1996b and 1997a). Subsequently similar results were published also from Japan (Sathasivan *et al.* 1997; Sathasivan and Ohgaki 1999). In recent studies some indirect evidence for nutrient limitation other than organic carbon has come from Norway, where inorganic nutrient addition resulted in a higher $AOC_{potential}$ content than when the AOC was analysed without nutrient addition (Charnok and Kjønnø 2000). This means that nutrients other than organic carbon limited microbial growth. Also, in Latvia, in the Riga water distribution system, phosphorus limits microbial growth in the distribution system where drinking water is produced from surface water (Juhna and Nikolajeva 2000). In Berlin, it was found that no polyphosphate granules occurred in *Aquabacterium* spp. growing in drinking water, but in pure cultures grown in artificial medium, polyphosphate granules were presented. This was considered to indicate a regulatory role of phosphorus in drinking water (Szewzyk *et al.* 2000).

In regions where microbial growth in drinking water is limited by phosphorus, very low amounts of phosphorus greatly increased the microbial growth. A major increase was achieved with additions of 1-5 µg/l PO_4-P in the phosphorus limited drinking waters (Miettinen *et al.* 1997a; Sathasivan *et al.* 1997). Sathasivan and Ohgaki (1999) reported that phosphorus could become a limiting nutrient at concentrations of 1 to 3 µg/l.

3 AIMS OF THE STUDY

In this thesis the effect of nutrients, especially that of phosphorus on microbial growth in Finnish drinking waters was studied. The specific aims were:

1. To develop a test for analysing microbially available phosphorus in water (I).

2. To study the effects of different water purification techniques on the contents of microbial nutrients (organic carbon and phosphorus), microbial concentrations and growth potential (II, III, IV).

3. To study the relationship between MAP and microbial growth in drinking waters (II, III).

4. To study the effect of phosphorus availability on the formation of biofilms in drinking water (V).

4 MATERIALS AND METHODS

4.1 Water samples and experiments

4.1.1 Bioassay for microbially available phosphorus (MAP) (I)

Standardization

Phosphorus standards were made in deionized water (Millipore, UK). Six milliliters of inorganic nutrient solution was added to the 94 ml of water to ensure that only phosphorus among the inorganic nutrients was limiting growth. Addition of inorganic salts meant that the the deionized water had the same electric conductivity level (c.a. 100 μS/cm) as in drinking water in general. The salt solution consisted of $(NH_4)_2NO_3$, $MgSO_4$ x 7 H_2O, $CaCl_2$ x 2 H_2O, KCl and NaCl (Merck, Darmnstadt, Germany). After addition of the salt solution, the standard water had 15 mg/l N, 0.6 mg/l Mg, 1.6 mg/l Ca, 3.2 mg/l K, 2.4 mg/l Na and 8.9 mg/l Cl. For the carbon source, sodium acetate (CH_3COONa) was added to a final concentration of 2 mg/l C.

Standards were made by adding different amounts of phosphorus (Na_2HPO_4, Merck) to the above mentioned standard water. The concentration of phosphorus ranged from 0.05 to 10 μg/l PO_4-P. After addition of inorganic nutrients and carbon, the standards were pasteurized at +60°C in a water bath for 35 min. After cooling, the samples were inoculated with *Pseudomonas fluorescens* P17 biotype 7.2 (ATCC 49642) (appr. 1000 CFU/ml). Strain P17 was tested for phosphatase activity by a fluorometric method with 4-methylumbelliferylphosphate-Na_2 salt (Fluka, Buchs, Switzerland) as the substrate (the method is described in Miettinen *et al.* 1996a).

Inoculated samples were incubated at +15 °C to obtain the maximum cell numbers. The bacterial cells in sample water were enumerated daily by spread plating on R2A-agar (Reasoner and Geldreich 1985). The plates were incubated at 22 °C for three days before colony counting. Standardization was repeated with four standard series with different phosphorus concentrations. Every standard set contained 3-6 different concentrations of phosphorus and a blank sample.

Analysis of water samples

Inorganic nutrients (except phosphorus) and organic carbon (see above standardization procedure) were added to the samples to ensure that inorganic nutrients or organic carbon did not restrict microbial growth. The final concentrations of added nutrients in the samples were 250 μg/l N, 53 μg/l K, 10 μg/l Mg, 27 μg/l Ca, 40 μg/l Na and 149 μg/l Cl. Sodium acetate was added as above. Residual chlorine in water samples (100 ml) was removed by adding 50 μl 0.02 M sodium thiosulphate. After addition of nutrients and thiosulphate, samples were pasteurized and finally inoculated with *Pseudomonas fluorescens* P17.

Water samples were incubated at +15 °C. The growth of bacteria in water samples was enumerated every day during 4-8 days from the inoculation by spread plating on R2A agar (Reasoner and Geldreich 1985), plates were incubated for 3 days at +22±2 °C before counting. The maximum plate counts of *Pseudomonas fluorescens* P17 were transformed to the amount of microbially available phosphorus with a conversion factor taken from the calibration curve of the standardization.

4.1.2   Effects of ozonation (II)

In substudy II, water samples were taken from five Finnish surface water works which utilized ozonation (Table 1). The waterworks used ozonation after chemical coagulation (intermediate ozonation), and waterworks S9 used pre-ozonation of raw water. Water samples were taken before and after ozonation. One experiment was done by ozonating chemically purified water from the Kuopio waterworks in a laboratory scale ozonator (1.5 mg/l O_3) (described in Myllykangas *et al.* 2000). Waterworks S3, S8 and S12 used river water as raw water and S5, S9 and S10 lakewater. The ozonation doses used in the waterworks varied between 1.0-1.98 mg/l O_3.

4.1.3   Water purification techniques (III)

In substudy III, water samples were taken from 21 waterworks in Finland (Table 1). The samples originated from different purification stages and raw waters. Six surface waterworks (S1, S2, S5, S6, S9 and S10) used lake water as their raw water and five waterworks (S3, S4, S7

S8 and S11) used river water. All waterworks processing artificially recharged ground water (ARG) used lake water as their raw water, three waterworks infiltrated water through soil and one applied bank filtration (A3). The waterworks using ground water usually only adjusted pH without any other treatment. In some ground waterworks (G5 and G6), iron removal was required.

4.1.4   Effects of UV-disinfection (IV)

In substudy IV, samples were taken before and after UV-disinfection from three Finnish waterworks (Table 1). Waterworks G6 and G7 used ground water as the raw water, UV-disinfection doses were 15 mWs/cm^2 and 40-50 mWs/cm^2, respectively. Waterworks S13 produced drinking water from surface water, the UV_{254} dose was 25 mWs/cm^2. Laboratory scale UV-irradiation experiments were carried out with three different drinking waters taken from Finnish waterworks. Sample G7 taken before UV-disinfection, was irradiated in the laboratory. Samples A5 and S5 were taken from waterworks using lake water as the raw water.

UV_{254}-irradiation was carried out in the laboratory with 10 parallel Philips UV 15 W low pressure mercury vapor lamps. Water samples (500 ml) were placed in circular glass bowls under the lamps. Samples were irradiated for 5, 22 and 54 seconds, i.e. the UV_{254}-doses were 46, 204 and 501 mWs/cm^2 at the surface of the water sample. Water samples were mixed effectively during the irradiation to ensure dose uniformity. Based on the transmittance of 253.7 nm light in the water samples used in laboratory experiments, we estimated the dose of UV_{254} in the bottom of glass bowl. Based on these results, the actual UV_{254}-irradiation doses in sample G7 were 34-46 mWs/cm^2 (bottom-surface of water in glass bowl), 152-204 mWs/cm^2 and 373-501 mWs/cm^2, in sample A5 the doses were 21-46 mWs/cm^2, 95-204 mWs/cm^2 and 232-501 mWs/cm^2, and in sample S5 the doses were 23-46 mWs/cm^2, 101-204 mWs/cm^2 and 247-501 mWs/cm^2. Due to the tapered shape of the glass bowl, the higher values were considered to be more close to the real doses than the lower values.

Table 1. Sample codes and water treatments in studied water works. Water works applying ozonation (O_3) had also granulated activated carbon filtration (GAC).

	Water treatment		

Groundwaters

Sample code	substudy	treatment	pH-adjustment	disinfectant
G1	I, III	none	lime	none
G2	I, III	none	lime	ClO_2
G3	I, III	none	none	none
G4	III	none	NaOH	none
G5	III	air + lime	lime + H_2CO_3	NaOCl
G6	I, III, IV	SF	limestone filtr.	UV
G7	IV	rapid sand filtr.	limestone filtr.	NaOCl + UV

Artificially recharged groundwaters

	substudy	filtration	pH-adjustment	disinfectant
A1	I, III	infiltration on soil[1]	limestone filtr.	none
A2	I, III	infiltration on soil	lime	none
A3	III	bank filtration	none	none
A4	III	infiltration on soil	lime	NaOCl
A5	IV	bank+slow sand filtr	NaOH	NaOCl

Surface waters

	substudy	coagulant	oxidant	pH-adjustment	disinfectant
S1	I, III	alum	ClO_2	lime	NaOCl
S2	III	alum	O_3[2]	lime	NH_2Cl
S3	I, II, III	alum	O_3	lime	NH_2Cl
S4	III	Fe (III) salt (+SF)	none	lime	$NH_2Cl + Cl_2$
S5[3]	I, II, III, IV, V	alum	none	lime	NaOCl
S6	I, III	alum	none	lime	NaOCl
S7	III	Fe(III) salt (+GAC)	none	NaOH	NH_2Cl
S8	II, III	PAC	O_3[4]	lime	NaOCl
S9[5]	II, III	PAC	O_3[6]	lime	NaOCl + NH_4Cl_2
S10[7]	II, III	alum	O_3	lime	NH_2Cl
S11	III	PAC	O_3[8]	lime	NH_2Cl
S12	II	Fe(III) salt	O_3	lime	NaOCl + NH_4Cl_2
S13	IV	PAC	none	Na_2CO_3	UV+NaOCl

Symbols: PAC, polyaluminum chloride; SF, slow sand filtration

1) After infiltration also slow sand filtration
2) No GAC after ozonation
3) Raw water was first bank filtrated (waterworks A4)
4) After ozonation slow sand filtration
5) Purified water was mixed with unpurified ground water (appr. ½)
6) Water was ozonated before coagulation
7) Same waterworks as S2, after GAC was applied
8) Also pre-ozonation was used before coagulation

4.1.5 Biofilm formation as affected by phosphorus availability (V)

Biofilm development was studied with polyvinyl chloride (PVC) slides, with water for the experiment being taken from waterworks S5 before disinfection. Microbial growth of the water was limited by phosphorus availability. The slides (surface area 15.9 cm^2) were placed into PVC chambers at room temperature (21 °C). The PVC chambers were covered with aluminium foil. All materials contacted with water were treated (20 h) with sodium hypochlorite solution of 10 mg/l Cl$_2$ and rinsed with sterile distilled water before use. Water was pumped with total (feeding water + phosphorus solution, 16:1) flow velocity of 1 ml/min to the chambers. Phosphorus solution was pumped through the 0.2 μm filter. The water volume of chambers was 302±4 cm^3 corresponding to a water retention time of 5 h in the chamber. The growth of biofilms was monitored 12 times during the 72 day experiment.

For the analyses, slides were first rinsed slightly with sterile water, then put into a sterile 100 ml flask and 10 ml of sterile water was added. The flasks were sonicated (40 kHz) in a water bath for 5 minutes (Finnsonic mO3, Finland), the extracts were analysed for microbial occurrence. At every sampling time, two slides from two parallel columns were taken for analyses.

4.2   Chemical and microbiological analyses

4.2.1   Glassware

All glassware (Pasteur pipettes, tubes, Erlenmeyer flasks with glass-stoppers) and plastic pipette tips were first washed with phosphate free detergent (Deconex, Borer Chemie AG, Switzerland), then immersed in 2 % HCl solution for 2 hours and then rinsed with deionized water (Millipore, UK). Finally, clean glassware was heated for 8 hours at +250°C.

4.2.2 Organic carbon

Total organic carbon (II-V)

Total non-purgeable organic carbon (TOC) was analysed by a high temperature combustion method with a Shimadzu 5000 TOC analyser (Kyoto, Japan). Water was acidified and purged before analysis.

Assimilable organic carbon (I-V)

Assimilable organic carbon (AOC) was analysed by a modification (Miettinen *et al.* 1999) of the Van der Kooij method (1982b). The determination of the AOC concentration was based on the maximum growth of *Pseudomonas fluorescens* P17 (ATCC 49642) and *Spirillum* sp. strain NOX (ATCC 49643) in the water sample. The modification included addition of inorganic nutrients to ensure that only the AOC content restricted microbial growth, i.e. AOC was measured as $AOC_{potential}$ (Miettinen *et al.* 1999). With *P. fluorescens* the growth corresponded to acetate equivalents and with *Spirillum* NOX to oxalate equivalents. In water samples containing chlorine, residual chlorine was removed by the addition of 50 µl 0.02 M (for 100 ml) sodium thiosulphate.

Organic acids (IV)

The organic acids were measured using Dionex ion chromatography (IC) (USA). The measurements were conducted with series 4000 I instrument with the Ionpac AG11-HC Guard Column (4*50 mm), and Ionpac AS11-HC Analytical Column (4*250 mm). Anion Trap Column (ATC-1) was used for eluent clean up. The size of the injection loop was 392-µl. An on-guard H^+ cartridge was installed on-line between the autosampler and the sample loop. The self-regenerating suppressor was ASRS-ULTRA (4-mm). As a preservative, 25 mg/l benzalkonium chloride was added to the samples. The IC run program consisted of equilibration (1 mM NaOH for 9 min), injection, isocratic analysis (1 mM NaOH for 8 min), and three gradient phases (from 1 to 15 mM NaOH during the following 10 min, from 15 to 30 mM NaOH during the next 10 min, and from 30 to 60 mM NaOH during the last 10 min). The eluent flow rate was 1.5 ml/min. During the equilibration, the sample was loaded to the sample loop with a flow of 1 ml/min.

Molecular weight fractions (IV)

The molecular weight fractions of organic matter in UV-experiments were determined with a high performance size exclusion chromatography (HPSEC) system, which consisted of a Waters 996 photodiode array detector (USA), Waters 600E system controller (USA) and Waters 717 autosampler (USA). Samples were prefiltered with 0.22 µm Millipore filters before analyses. Molecular weight fractions were separated with a TSK Gel SW guard column and TSK Gel G3000SW analytical column (Tosohaas, Japan). The eluent was 0.01 M, pH 7 sodium acetate. The absorbance of the fractions was detected at 254 nm. The peak area of the various fractions was used in the analysis of the results.

4.2.3 Phosphorus (I-V)

Total phosphorus (total P) was analysed by an ascorbic acid method according to the Finnish standard (SFS, 3026) at 880 nm wavelength using 1 (IV), 4 (II, III) or 5 (V) cm light path with Philips PU8700 (England) (II, III), Ultrospec 3000 Pro (IV) (England) and Shimadzu UV-1601 (V) (Australia) spectrophotometer

Microbially available phosphorus (MAP) concentrations were analysed by bioassay (I) (see 4.1.1)

4.2.4 Heterotrophic plate counts (I-V)

Heterotrophic plate counts (HPC) were analysed by the spread plating method on R2A-agar (Difco, USA) (Reasoner and Geldreich 1985). R2A-agar plates were incubated for 7 days at 22 °C before the colony forming units (CFU) were counted.

4.2.5 Total number of bacteria (III, V)

Total numbers of bacteria were analysed by an acridine orange direct counting method based on the method of Hobbie *et al.* (1977). Bacteria were filtered on a black 0.22 µm Nuclepore membrane filter and stained with 0.01 % acridine orange dilution. Bacteria were counted with

an Olympus BH-2 epifluorescence microscope (Olympus Optical co., Tokyo, Japan) using an eyepiece micrometer (Graticules Ltd., Tonbridge, U.K).

4.2.6 Growth potential of bacteria (II-IV)

The growth potential of native heterotrophic microbes in water was analysed with and without phosphorus addition by incubating the samples (100 ml) at 15 °C in the dark. Bacterial growth was followed for three weeks by spread plating every second or third day on R2A-agar plates (Difco) (Reasoner and Geldreich 1985). Phosphorus was added as Na_2HPO_4 (Merck) to obtain an extra concentration of 20 µg/l PO_4-P in water. R2A-agar plates were incubated for 7 days at 22 °C before colony forming units (CFU) were counted. In the results, maximum microbial numbers obtained during the water incubation are reported. In substudy II, all water samples were inoculated with one natural microbial community taken from drinking water S5.

4.2.7 Adenosine triphosphate, ATP (V)

In the biofilm experiments, 200 µl of the sonicated extract was mixed with 100 µl ATP releasing reagent (1620150 Labsystems, Finland), 200 µl ATP monitoring reagent 5000 (1243-247 Bio Orbit, Finland) and 500 µl buffer (0.1 M Tris acetate, 2 mM EDTA) (1243-227 Bio Orbit, Finland). Light production in millivolts after addition of the reagents was measured with an Bio Orbit 1251 luminometer (Finland). The measured light output was converted to ATP concentration by using conversion factor determined from the standards (ATP standard, 1243-201 Bio Orbit, Finland).

4.3 Statistical analyses

Statistical differences in nonparametric data were tested by Wilcoxon signed ranks test. The effect of phosphorus in biofilms was tested with one-way analysis of variances and Tukey´s multiple comparison test. Analyses were performed with SPSS for Windows program. Pearson correlations were calculated with Microsoft Excel 97 and SPSS for Windows programs.

5   RESULTS

5.1   Bioassay for analysing microbially available phosphorus (I)

There was a linear relationship between maximum cell count of *P. fluorescens* P17 and the phosphorus concentration in the range 0.05 to 10 µg/l PO_4-P (Fig. 1). The maximum cell count was generally reached after an incubation time of 4-8 days. Above 10 µg/l PO_4-P, there was no longer any linear relationship between the phosphorus concentration and bacterial growth. The detection limit of the bioassay was 0.08 µg/l PO_4-P (Fig. 2), which was determined by the mathematical technique of Hubaux and Vos (1970). The yield factor was derived from the slope of the line when cell growth was plotted against PO_4-P concentration. The equation indicated that 1 µg PO_4-P corresponded to 3.73 x 10^8 CFU of *P. fluorescens*.
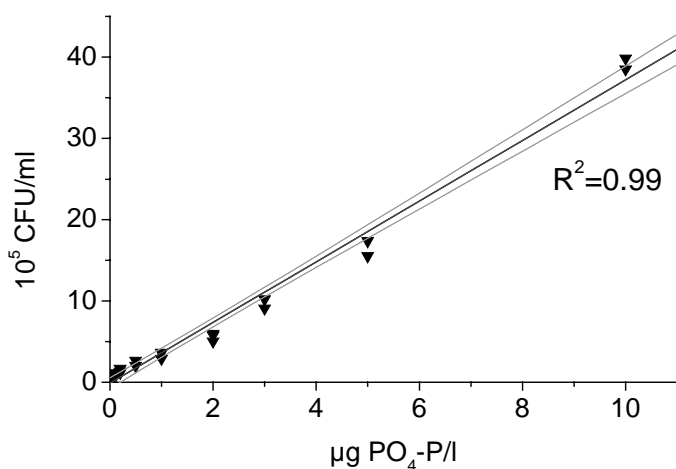
Figure 1. Relationship between the growth of *P. fluorescens* and phosphorus concentration in MAP standardization. The regression equation is y = 373 200x-10000 (I).
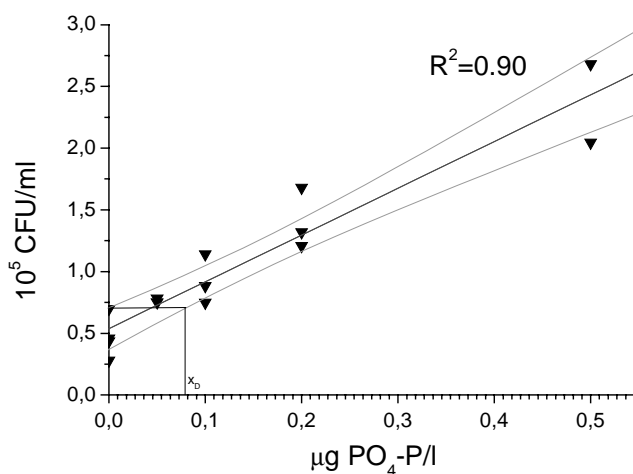
Figure 2. Determining the detection limit with 95 % confidence limits for the MAP bioassay. Detection limit for the method is 0.08 µg/l PO_4-P (I).

5.2 Effects of different water treatment techniques on microbial nutrients and microbial growth in drinking water

Summary of the effects of different water treatment techniques on drinking water nutrients and microbial growth is presented in table 2 (page 45).

5.2.1 Chemical coagulation (III)

Chemical coagulation removed 52 % of the TOC (p=0.003), 73 % of the $AOC_{potential}$ (in two waterworks $AOC_{potential}$ increased in coagulation), 84 % of the total phosphorus (p=0.010) and 97 % of the MAP (p=0.028). Also microbial concentrations (73 % of the total bacteria p=0.007, and 86 % of the HPC, p=0.026) decreased (III, Table 2). The decrease in contents of nutrients was associated with a decrease in the growth potential of heterotrophic bacteria (p=0.033) (III, Table 2). Waters produced in waterworks where ferric or alum salts were used in the precipitation stage were often toxic to the test bacterium *P. fluorescens*. PAC removed $AOC_{potential}$, while the alum salt coagulant even increased it.

5.2.2 Ozonation (II, III)

Ozonation increased strongly the content of $AOC_{potential}$ (p=0.001) (II, Fig. 2; III, Table 2). The increase varied between 0 and 847 % and was on average 310 %. The TOC concentration decreased on average by 6 % (p=0.008). The concentration of total phosphorus did not change with ozonation, but the content of MAP increased on average by 79 % (p=0.016) (II, Fig. 1; III, Table 2). The increase in the nutrient content was followed by an increased growth potential of heterotrophic bacteria, microbial growth in water sample was on average 4.6 times higher after ozonation than before it (II, III, Table 2) (p=0.004). Ozone decreased the numbers of total bacteria on average by 57 % (p=0.043) and HPC on average by 94 %, in two waterworks ozone did not decrease HPC (III, Table 2). In ozonated waters tere was a linear relationship between MAP and HGR, there 1 µg/l of MAP corresponded to 10^9 CFU.

5.2.3 Activated carbon filtration (III)

Ozonation is usually followed by activated carbon filtration (GAC), which removed 23 % of TOC (p=0.028) and 85 % of $AOC_{potential}$ concentrations in water (III, Table 2). Also the concentration of total phosphorus decreased below the detection limit of 2 µg P/l, and MAP by 47 %. The microbial growth potential (p=0.080) decreased with decreasing contents of MAP and $AOC_{potential}$. There were no changes in the total bacterial counts, but the concentration of HPC increased (III, Table 2).

5.2.4 pH-adjustment and chlorination (III)

In surfacewater works disinfection was generally combined with pH adjustment by lime $(Ca(OH)_2)$. In most of the studied waterworks, disinfection was carried out with chloramine. This finishing treatment increased slightly TOC and the growth potential of heterotrophic bacteria (III, Table 2), $AOC_{potential}$ increased by 26 % and total phosphorus concentration was not changed. However, the concentration of MAP increased by 260 % (p=0.068) during the combined disinfection and pH adjustment (III, Table 2). The concentration of HPC clearly decreased (p=0.080) (III, Table 2).

The effect of liming without disinfection could be analysed in two groundwater works, in one

artificially recharging groundwater works and in one surfacewater works. Liming did not change the concentrations of TOC or $AOC_{potential}$. In contrast, the average content of total phosphorus increased from below the detection limit up to 4 µg/l, and the MAP doubled (p=0.068) (III, Table 3). The increase in MAP was associated with an increase in the microbial growth potential (III, Table 3). In one waterworks (A1) using limestone filtration, the content of MAP decreased during the filtration.

5.2.5   Artificial recharge of groundwater (III)

Infiltration through soil effectively removed organic matter from the water. TOC decreased here on average by 56 %, $AOC_{potential}$ by 40 %, total phosphorus to below the detection limit and MAP by 67 % (III, Table 3). Also, the concentrations of total bacteria and HPC decreased (III, Table 3). Microbial growth potential with or without nutrient addition increased in slow sand filtration. Bank filtration applied in one waterworks (A3) removed effectively organic matter and bacteria but increased greatly the content of phosphorus in the water.

5.2.6.   UV-disinfection (IV)

UV_{254}-irradiation did not change the content of TOC or total phosphorus (IV, Table 1). In waterworks, and in the laboratory experiments, the disinfection efficiency was on average 89 % (1 log inactivation) with these low doses ($\leq$46 mWs/cm^2). UV_{254}-irradiation doses below 46 mWs/cm^2 decreased $AOC_{potential}$ on average by 29 % (range 7-50 %, p=0.028) (IV, Table 1). With higher UV_{254} doses, there was no further decrease in the $AOC_{potential}$ (IV, Table 1). In five cases, the sum of the molecular size fractions (SMSF) decreased in samples by 5-58 % during the UV_{254}-irradiation (IV, Table 1), in one sample the SMSF increased. There also were changes in the molecular size distribution this being seen especially in samples A and B, where the proportion of small fractions increased whereas the large fractions decreased (IV, Fig. 1).

In waterworks and in the laboratory experiments where the lowest UV_{254} doses (46 mWs/cm2) were applied, there was only a minor effect on MAP (IV, Table 1). The UV_{254} dose of 204 mWs/cm^2 in laboratory increased MAP on average by 55 % (range 8-82 %) (IV, Table 1). The highest increase in the content of MAP took place in waters containing the highest amounts of organic matter (E and F) (IV, Table 1). The effect of UV-disinfection on microbial growth

(HGR) was not clear, with the lowest doses of UV_{254}, microbial growth usually increased, in contrast to the highest doses, which inhibited microbial growth (IV, Table 1).

Table 2. Summary of the chemical and microbiological effects of the different water treatment processes on water quality.

	Coagulation	Ozonation	GAC	Disinfection + liming	Infiltration on soil	UV-disinfection*	Liming
TOC	⇩	⇩	⇩	⇒	⇩	⇒	⇒
AOC$_{potential}$	⇩	⇧	⇩	⇧	⇩	⇩	⇒
Total phosphorus	⇩	⇒	⇩	⇒	⇩	⇒	⇧
MAP	⇩	⇧	⇩	⇧	⇩	⇒	⇧
Total bacteria	⇩	⇩	⇒	⇩	⇩	n.a.	⇩
HPC	⇩	⇩	⇧	⇩	⇩	⇩	⇒
HGR max	⇩	⇧	⇩	⇒	⇧	⇧	⇧

Symbols: ⇩ decreased, ⇒ no changes, ⇧ increased, AOC$_{potential}$, assimilable organic carbon analysed with addition of nutrients; GAC, granular aactivated carbon filtration; MAP, microbially available phosphorus; n.a., not analysed; HPC, heterotrophic plate counts; HGR$_{max}$, maximum microbial growth potential in water; TOC, total dissolved organic carbon. * doses were < 46 mWs/cm^2.

5.3 Content of microbial nutrients in Finnish drinking waters, and the effect of MAP on microbial growth in water (III)

All of the surfacewater works applied chemical coagulation, which effectively removed organic matter and phosphorus. The average decrease in the TOC content during the complete purification process was 59 %. In purified drinking waters, TOC was 2.5 mg/l on average (III, Table 4). However, the AOC$_{potential}$ increased in most of the surfacewater works by 134 % on average. In drinking waters the AOC$_{potential}$ was 127 μg/l on average (III, Table 4). Total phosphorus and MAP were effectively removed by the surfacewater works (III, Table 4). Even though some parts of the process e.g. ozonation and liming increased MAP, during the complete purification process the concentration of MAP decreased on average by more than 90 % (III, Table 4). Artificial recharging of groundwater through soil effectively removed microbial

nutrients and microbes. On average half of TOC and of $AOC_{potential}$ in the raw water were removed in artificially recharging groundwater works, furthermore, the total phosphorus decreased below the detection limit, and MAP decreased by 73 % (III, Table 4).

Drinking waters produced in groundwater works had the highest concentrations of total phosphorus and MAP but lower contents of organic compounds than artificially recharged groundwater and water produced from surface water (III, Table 4). Also the microbial growth potential was higher in groundwaters than in waters produced from ARG or surface waters (III, Fig. 1). The content of MAP varied from 1 % to almost 100 % of the total phosphorus concentration. The relative availability of the MAP was highest in ground waters and lowest in drinking waters produced from surface waters.

In most of the drinking waters, microbial growth was limited by phosphorus availability (III, Fig. 1). In drinking water samples with low MAP concentration (<10 µg/l), MAP correlated strongly (r=0.90, n=14, p=0.000) with the heterotrophic growth potential (III, Fig. 2), there were no correlation between total phosphorus or $AOC_{potential}$ and heterotrophic growth potential.

5.4   The effect of phosphorus on the formation of biofilms (V)

Addition of 1 µg/l PO_4-P increased the viable counts of heterotrophic bacteria in biofilms (p=0.000) (V, Fig. 3). Further increase in the number of heterotrophic microbes with increasing phosphorus concentrations was minor. The number of bacteria was on average 3-4 times higher in the phosphorus treated biofilms than in the untreated biofilms (V, Fig. 3). Addition of 1 µg/l phosphorus doubled the concentration of total bacteria in the biofilm (p=0.000) (V, Fig. 4). The concentration of bacteria was highest with the highest phosphorus addition (5 µg/l PO_4-P). The total number of bacteria increased exponentially for up to 6 days, after which the growth was linear. In the linear growth phase, the biofilms treated with phosphorus had growth rates of 22 000 bacteria/cm^2/d (range 21 000-23 000) and the untreated biofilm produced 8 600 bacteria/cm^2/d. The enhanced microbial growth by added PO_4-P (1-5 µg/l) was also evident in the increase in the ATP content of the biofilms (V, Fig. 5). The content of ATP increased with increasing PO_4^{3-} concentrations (p=0.000 for all treatments).

Mean ATP concentrations per bacteria increased with increasing phosphorus additions. With 1, 2 and 5 µg/l PO_4^{3-}-P additions, ATP contents per cell (total numbers) were 2×10^{-17}, 2×10^{-17}, 5×10^{-17} and 6×10^{-17} g ATP/cell, respectively. If calculated with respect to the heterotrophic plate counts, then the mean ATP contents in the untreated, 1, 2 and 5 µg/l PO_4^{3-}-P treated biofilms were 4×10^{-17}, 3×10^{-17}, 7×10^{-17} and 1×10^{-16} g ATP/CFU, respectively.

6   DISCUSSION

6.1   Bioassay for MAP

There is a clear and obvious need to have a sensitive method for analysing MAP from drinking water. In previous studies from Finland, it was found that microbial growth in most Finnish drinking waters was limited by phosphorus and the content of total phosphorus is usually below the detection limit of the standard methods (2 μg/l) (Miettinen *et al.* 1997a).

A method to analyse assimilable organic carbon in drinking water was developed two decades ago (Van der Kooij *et al.* 1982b). This method was based on the growth of known bacterial strain (*P. fluorescens*) in a water sample and the cell numbers were converted to the assimilable organic carbon concentration with yield factors obtained from the standardization (Van der Kooij *et al.* 1982b). The bioassay developed here for analysing MAP in drinking water operates on a similar principle. Natural microbes in the water sample are first destroyed by pasteurization and the growth of inoculated test bacteria is converted to the concentration of microbially available phosphorus. The MAP bioassay is very sensitive, this being reflected in the high correspondence of *P. fluorescens* growth on the low phosphorus concentrations. In the AOC tests, growth yields for the *P. fluorescens* varied from 2.04×10^6 (Miettinen *et al.* 1999) to 4.1×10^6 CFU/ μg acetate-C (Van der Kooij *et al.* 1982b; Standard Methods 1995), while in the MAP bioassay, the growth yield for the same bacteria was 3.73×10^8 CFU/ μg PO_4-P. The ratio between these growth yields 183-91 is close to the ratio 100:1 for carbon and phosphorus required for optimum bacterial growth as suggested by Van der Kooij (1982b). The growth response was linear in the range from 0.05 to 10 μg/l PO_4-P, which is a reasonable range for use in drinking water microbiology, most drinking waters contained MAP less than 10 μg/l (I, III, IV). If drinking water contains MAP more than 10 μg/l it is obvious that microbial growth is not limited by phosphorus, because drinking waters in Finland contain always AOC/AOC$_{potential}$ less than 500 μg/l (III, Miettinen *et al.* 1997b and 1999).

Chemical coagulation with alum showed toxicity against the test bacterium *P. fluorescens*. Huck (1990) found that polyaluminum chloride (PAC) can inhibit the growth of *P. fluorescens* P17. According to our results, ferric and alum salts were more toxic than PAC against *P. fluorescens* P17. The toxicity appeared especially in the waters taken just after chemical coagulation (II).

In future, it would be worth to develope MAP bioassay by adding more bacterial species, as proposed by Juhna (2002), who found that the growth response of indigenous bacteria was higher for phosphorus than the bacterial strain (*P. fluorescens*) used in the bioassay. We also found that growth response of bacterial community was higher than that of *P. fluorescens.* The yield factor for the mixed populations of microbes was 5.9 x 10^8 (data in sub-study III) – 1 x 10^9 CFU/μg PO_4-P (II), which is higher than the yield for *P. fluorescens* in the MAP bioassay (3.7 x 10^8 CFU/μg PO_4-P). The higher yield factor of mixed populations based on microbial numbers does not strictly prove higher uptake of phosphorus by the mixed populations. If the cells of microbes in the mixture are smaller than the cells of *P. fluoresecens*, the biomass production and associated phosphorus uptake in the mixture are lower than the cell numbers predict.

Presently the chemical characteristics of phosphorus pool available for microbes in drinking water systems is not known. Various bacterial species probably differ in their capability to uptake the various forms of phosphorus, and therefore a microbial consortium consisting of several microbial species could more efficiently utilize phosphorus than a single species. Multi-species system also would diminish the problem of toxicity of some chemical compounds in the MAP analysis. A disadvantage of the multi-species system is the requirement to determinate the yield factor (standardization) and growth separately for every microbial species, thus making the test more laborious.

6.2 Effects of water treatment techniques on phosphorus, organic carbon and microbial growth

The various water purification techniques differed in their effects on microbial nutrients and microbial growth response. Chemical coagulation combined with rapid sand filtration was the most efficient phase in the removal of organic carbon and phosphorus from surface water. The removal percentage of organic carbon was higher for TOC, than for $AOC_{potential}$. These results are in agreement with the previous studies showing good removal of phosphorus (Cooke *et al.*, 1993; Nishijima *et al.* 1997) and AOC (Van der Kooij 1990; Charnock and Kjønnø 2000) with aluminum and iron salts. Nissinen *et al.* (2001) found that chemical coagulation was especially good at removing high molecular size molecules, though lower size fractions were removed less efficiently, which may explain the better removal of TOC than $AOC_{potential}$. There were difficulties encountered in the analysis of MAP and $AOC_{potential}$ in some samples as a result of

the toxic effects of coagulants on the test strain. Some raw waters before coagulation had a MAP concentration above the upper limit of the bioassay (dilution was needed).

These present results agree with others showing that ozonation increases strongly the content of AOC (Van der Kooij *et al.* 1982b; Miettinen *et al.* 1998; Escobar and Randall 2001) and decreases TOC (Kainulainen *et al.* 1995; Miettinen *et al.* 1998) as a result of degradation of organic matter. One novel finding here was that ozone increases the content of MAP in water (II, III). The origin of this MAP was considered to be the degrading organic matter. Martin (in Stevenson, 1982) suggested that inorganic phosphate can form stable complexes with soil organic matter and this phosphate is released after destruction of these complexes. Humus contains small amounts (0.1 – 0.46 %) of phosphorus, especially in association with cations such Fe^{2+}, Al^{3+} and Ca^{2+} (Thurman 1986; Holtan *et al.* 1988; Hens and Merckx 2002). The increase in content of MAP in ozonation is important because also the content of $AOC_{potential}$ increased strongly in ozonated water. The increase in $AOC_{potential}$ and MAP was associated with an increase in microbial growth in water (II). After ozonation, there was a good correlation between MAP and microbial growth rate (II), but no correlation between $AOC_{potential}$ and microbial growth was not found.

Ozonation is followed usually by activated carbon filtration (GAC/BAC), to enhance the removal of organic matter before distribution of drinking water. Here GAC effectively removed the nutrients ($AOC_{potential}$ and MAP) released in ozonation. According to previous studies, GAC has removed AOC by between 50 to 80 % (Vahala *et al*. 1998b; Hu *et al*. 1999; Liu *et al.* 2002). We found over 90 % efficiency in some waterworks. However, the concentrations of HPC and total bacteria increased during GAC as found previously (Servais *et al.* 1991; Pietari 1995; Van der Kooij *et al.* 1989). The reason for this increase is the export of bacteria from biologically activated carbon.

In combined chlorination and pH adjustment with lime, both the $AOC_{potential}$ and the MAP content increased and also the content of total phosphorus increased. The increase in MAP content was caused by the addition of lime, as found in the waterworks where disinfection was not used. The increase in nutrients was slightly affected the heterotrophic growth response, which also increased after disinfection and liming (III, Table 2).

In one water using limestone filtration, the MAP concentration decreased during the filtration, as found once earlier (Sallanko and Lakso 2000)

The $AOC_{potential}$ content in water increased by hypochlorite disinfection, as it did in chloramine disinfection, as a result of organic matter degradation by chlorine. Similar results have been reported in many previous studies (Van der Kooij 1990; Miettinen *et al.* 1998; Charnock and Kjønnø 2000; Okabe *et al.* 2001). It was previously found that the $AOC_{potential}$ concentration increased linearly with the added hypochlorite dose up to 2 mg/l, but there was no increase in the content of MAP with chlorination (Lehtola *et al.* 2001).

UV_{254}-radiation is known to degrade organic matter (Armstrong *et al.*, 1966; Corin *et al.* 1996; Kulovaara *et al*. 1996) and liberate phosphate (Armstrong *et al.* 1966; RonVaz *et al.* 1992; Vähätalo and Salonen 1996). With the doses of UV_{254} used in drinking water disinfection, no changes in TOC were found. However, there were changes in the $AOC_{potential}$ and molecular size distribution (IV, Table 1, Fig. 1). In molecular size fractions, the proportion of the small fractions increased and the proportion of large fractions decreased (IV, Fig. 1), as found previously with higher doses of UV_{254} (Backlund 1992). The reason for these changes could be the alterations in the UV absorbing chromophores of organic matter or in the amounts of organic matter. Degradation of large fractions to lower molecular size fraction can result in less UV absorptivity in the analysis. Kulovaara *et al*. (1996) found that UV_{254}-irradiation changed the high molecular weight organic matter to a more aliphatic character and with the appearance of more carboxyl and carbonyl atoms. Some of the microbially available organic compounds would be degraded by UV_{254} or precipitated as iron complexes as shown by Kulovaara (1996) and thus decrease the content of $AOC_{potential}$.

The release of MAP from organic matter required a higher dose (204 mWs/cm^2) of UV_{254}-radiation than that normally used in drinking water disinfection. The increase was highest in waters containing the highest amounts of organic matter. It has been reported that UV-radiation effectively released phosphorus (microbially available orthophosphate) bound in the ester form or via a C-P bond (Ron Vaz *et al*. 1992).

Surprisingly, the effect UV-disinfection on the heterotrophic plate counts was lower than expected (Harris *et al.* 1987; Wolfe 1990; Parrotta & Bekdash 1998). There were culturable

bacteria in all waters when the water was treated with doses less than 204 mWs/cm^2 (IV, Table 1), the disinfection efficiency in waterworks and in laboratory experiments with the doses of 46 mWs/cm^2 was on average 89 %. We found that the highest UV_{254} doses inhibited microbial growth in the HGR test. There are also some previous studies, showing prolonged inhibition (lasting for days) in microbial growth in water after UV_{254}-irradiation. The reason for that was supposed to be in hydroxyl radicals and hydrogen peroxide (H_2O_2) produced by UV_{254}-irradiation from humic substances (Gjessing and Källqvist 1991; Lund and Hongve 1994). Hydrogen peroxide has longer half life than hydroxyl radicals and may react with ferrous iron and humic substances to produce hydroxyl radicals (Lund and Hongve 1994).

Infiltration into soil was effective in removing both nutrients and microbes from water, which is in agreement with previous studies showing the good removal of bacteria and viruses (Farooq *et al.* 1994; Peters *et al.* 1998; Schijven *et al.* 2000;), AOC (Van der Kooij 1990; Miettinen *et al.* 2001b) and MAP (Miettinen *et al.* 2001b) in ground filtration.

Microbial growth potential increased in infiltration into soil, in contrary to the decrease in the contents of microbial nutrients. The reason can be the changes in the microbial community (Preuß and Nehrkorn 1996). Also the grazing of protozoa in untreated lake or river water can retard the growth of bacteria populations (Hahn and Höfle 2001).

6.3   Biofilms

Formation of biofilms was studied with the PVC slides in a laboratory experiment. Our results showed that the availability of phosphorus regulated not only the development rate of biofilms but also microbial numbers during steady state. Recycling of phosphorus in biofilms with water of low MAP content cannot maintain a maximal microbial growth as suggested by LeChevalllier (1990).

The number of culturable bacteria in biofilms rapidly reached the steady state and their concentration increased with phosphorus addition of 1 μg/l, but there was only a minor subsequent increase with further phosphorus addition. Total bacterial concentrations grew continuously to the end of the experiment. Phosphorus was affecting also the growth rate, which was higher in phosphorus treated biofilms if compared to untreated biofilms. During the

maturation of the biofilm, the viability of bacteria deeper in the biofilm may decrease. In some earlier studies it was found that the numbers of culturable bacteria in biofilms were 1-2 order of magnitude lower than the microbial numbers assessed with direct microscopical counting (Block *et al.* 1993; Schwartz *et al.* 1998). In our results, almost half of the total bacteria were culturable, which may indicate that steady state in total bacteria had not been reached, but also may reflect the optimal conditions for biofilm formation. It was also found that addition of phosphorus enhanced the culturability of microbes in biofilm.

The content of ATP in biofilms increased with the increase in phosphorus concentration up to 5 μg/l. The mean content of ATP/cell or CFU increased with increasing phosphorus concentration, which is an indication of the limiting effect of phosphorus availability. In the previous literature, it has been found that phosphorus limitation significantly decreased the cellular ATP pool of microbes (Karl 1980). The ATP content in biofilm microbes receiving 5 μg/l phosphorus in water was close to that found earlier (10^{-16} – 10^{-15} g ATP/cell) for active bacterial cells (Webster *et al.* 1985; Stanley 1989).

These results showed that in phosphorus limiting water also the formation of biofilms was affected by the availability of phosphorus. Very low additions of phosphate in water increased the microbial concentrations in biofilms. There are also some other studies agreeing with these findings. Phosphorus enhanced bacterial growth in drinking water biofilms in a Japanese study, where addition of phosphorus significantly increased the growth of heterotrophic bacteria in a rotating biofilm membrane reactor (Okabe *et al.* 2001). Critchley *et al*. (2001) found in a copper corrosion study that phosphate concentrations in drinking water correlated with total biomass in biofilms. In a recent study from Latvia, Juhna (2002a) found that the formation of biofilms in distribution networks was lowest with the lowest concentrations of MAP in humus rich drinking water.

According to the recent results, phosphorus can influence also the microbial community structure in biofilms, characterized by phospholipid fatty acids and lipopolysaccharide hydroxy fatty acids (Keinänen *et al*. 2002). The addition of phosphorus increased the proportion of gram-negative bacteria in biofilms and also changed the community structure of gram-negative bacteria (Keinänen *et al*. 2002). However, the greatest difference in phospholipid fatty acids was found between drinking waters and biofilms (Keinänen *et al*. 2002).

There are many areas where microbial growth in drinking water is known to be limited by organic carbon (Van der Kooij *et al.* 1982b; LeChevallier 1990; Appenzeller *et al.* 2001; Chandy and Angles 2001). There also the formation of biofilms was not increased by the addition of phosphate to the water (Rompré *et al.* 2000; Appenzeller *et al.* 2001; Chandy and Angles 2001). In highly corroded iron pipes, addition of notably higher concentration (1-5 mg/l) of phosphate than we used in our experiments (eg. zinc orthophosphate and ortho-polyphosphate) decreased microbial activity on the pipe surface and corrosion products in water (Abernathy and Camper 1998; Rompré *et al*. 2000; Appenzeller *et al.* 2001 and 2002). Phosphorus reacts with iron and iron corrosion products like $FeOOH$ forming $FeOOH\text{-}PO_4$ complexes and $FePO_4$. Since $FePO_4$ and bacteria both have negative charges, this will depress the biofilm densities and also reduce the humic adsorption capacity (Abernathy and Camper 1998). Since phosphorus reacts with iron compounds (Cooke *et al.* 1993; de Jonge *et al.* 1993; Abernathy and Camper 1998; Appenzeller *et al*. 2002), iron may also affect the content and availability of phosphorus in drinking water distribution networks.

Our recent results suggest that phosphorus accumulates together with iron, organic matter (AOC) and microbes in loose deposits of iron pipes, and a change in water flow then drives all these components into the water phase (Lehtola *et al*. Submitted). A question is, would the anaerobic conditions in biofilm induce microbial iron reduction (Fe III > Fe II) and associated phosphate release from iron-phosphorus complexes, a mechanism known to occur in anaerobic lake sediments (Cooke *et al*. 1993; Gächter and Meyer 1993). In theory phosphorus would both enhance and diminish corrosion of iron pipes. Small extra phosphorus (μg) in phosphorus limited water can increase biofilm formation on iron pipes and microbial induced corrosion. However, it remains to be shown if the high phosphorus concentrations used in anticorrosion treatment (mg) would reduce iron pipe corrosion even in phosphorus limited water distribution systems by modifying the surface properties of iron corrosion products. This question is very important at waterworks planning to apply phosphate based anticorrosion treatment.

6.4   Phosphorus limitation in drinking water

Finnish lake waters contain a high concentration of organic matter (Kortelainen 1993). According to our results, one important reason for the phosphorus limitation in Finnish drinking waters is usually the more effective phosphorus removal than the removal of $AOC_{potential}$. In

many cases the $AOC_{potential}$ content even increases during the drinking water purification process, especially if the waterworks utilizes ozonation. Microbial growth in raw waters of the studied surface waterworks was not always clearly limited by phosphorus (results not shown). Carbon limitation of heterotrophic bacteria in aquatic systems has been an important paradigm in microbial ecology, but phosphorus limitation has rarely even been considered (Vadstein 2000). In most studies in aquatic systems where phosphorus limitation has been tested, however, phosphorus has been found to be a limiting factor for the growth of heterotrophic bacteria (Haas *et al*. 1988; Vadstein 2000). In river water, also the formation of biofilms increased with increasing phosphorus concentrations (Mohamed *et al.* 1998). Therefore it is obvious that especially in regions where phosphorus limits microbial growth in raw water, also after water purification, phosphorus still may be the limiting factor for microbial growth. Efficient water purification also can change the water so that it becomes phosphorus limiting, as found in the present study (II, III).

After the first findings on phosphorus limitation in drinking waters (Miettinen *et al.* 1996b; Sathasivan *et al.* 1997) some other direct and indirect findings have been published concerning phosphorus limitation in drinking water (Charnok and Kjønnø 2000; Juhna and Nikolajeva 2000; Szewzyk *et al.* 2000). Recently opposite results have also been published (Rompré *et al.* 2000; Appenzeller *et al.* 2001; Chandy and Angles 2001; Frias *et al*. 2001; Niquette *et al*. 2001). These results show that phosphorus vs. organic carbon limitation varies geographically and should be tested in each individual waterworks. In waterworks, where microbial growth is limited by organic carbon, small changes in phosphorus concentration had no effects on the microbial quality of drinking water. The ratio of $AOC_{potential}$ to MAP in phosphorus limited waters varied in different types of drinking waters. In ground waterworks, the $AOC_{potential}$:MAP ratio was on average 27:1 and in surface waterworks it was on average 633:1 (III, Table 4). Because $AOC_{potential}$ and MAP analyses are based on the growth of one or two bacterial strains, there can be organic carbon or phosphorus available for microbes but not shown by the tests (see above). This hampers the exact determination of the critical $AOC_{potential}$ to MAP ratio where either phosphorus or carbon limits the growth of bacterial community. Important is, that the C:P ratio is completely different in drinking waters in Finland than e.g. in USA, where the average C:P ($AOC:PO_4\text{-}P$) ratio was 0.4:1 and 2.3:1 and microbial growth in drinking waters is usually limited by the carbon content (Zhang and DiGiano 2002). The required C:P ratio for optimum microbial growth is 100:1 (Van der Kooij 1982; Zhang and DiGiano 2002). The C:P ratio in

bacteria may vary, normally it is 20, but if the phosphorus supply is sufficient and redox conditions are appropriate, the C:P ratio can decrease down to 5 (Gächter and Meyer 1993). Also, the phosphorus content in bacteria depends on the specific growth rate of the bacteria (Vadstein 2000).

In our study, the phosphorus limitation was tested with native bacteria communities, which vary in different waters. Phosphorus requirements for the growth of heterotrophic bacteria can vary (Vadstein 2000). It has been reported that phosphorus availability affect the bacterial community structure in biofilms (Keinänen *et al*. 2002). Also, adding of phosphorus may enhance the culturability of bacteria in drinking water. Block *et al*. (2002) presented that the HPC values depended on environmental stress factors like e.g. nutrient starvation. In a recent study with phosphorus limited water, addition of phosphorus enhanced the survival of coliform bacteria (Pitkänen *et al*. 2002). All these reasons may affect also the HPC numbers in HGR test and thus the correlation of MAP and HGR. However, the MAP bioassay proved to be useful analytical tool in studying phosphorus limited waters where MAP correlated with microbial growth potential of the native bacteria in the drinking water. There were no correlation between MAP and total phosphorus, indicating that in these waters total phosphorus was not a good parameter in describing the usability of phosphorus for microbial growth.

If chlorine is used for the disinfection, the disinfectant residuals have a stronger influence on bacterial regrowth, than temperature, AOC and water residence time (Zhang and DiGiano 2002). There are several large waterworks in Europe where the microbial growth in the distribution system is controlled without chlorine disinfection (Van der Kooij *et al*. 1998; Szewzyk *et al*. 2000; Uhl *et al*. 2001). Also the use of UV-disinfection is becoming an increasingly common way to disinfect drinking water. Even without disinfectants bacterial regrowth can be controlled by removing microbial nutrients so effectively that the water becomes biologically stable, if there is a short residence time in distribution system and a low initial bacterial concentration in the water as it leaves the waterworks (Van der Kooij 1990; LeChevallier *et al*. 1991; Van der Kooij *et al*. 1998; Szewzyk *et al*. 2000; Uhl *et al*. 2001). Our results show that also phosphorus has to be considered when the regrowth of bacteria and hygienic quality of drinking water in distribution system is controlled by nutrients. There are many areas in Finland, and probably in the world, where the content of phosphorus is so low and the content of organic carbon so high, that microbial growth in drinking water is limited by

phosphorus availability (Miettinen *et al.* 1996b, 1997a; Sathasivan *et al.* 1997; Charnock and Kjønnø 2000; Juhna and Nikolajeva 2000; Szewzyk *et al.* 2000).

The present drinking water purification techniques such as chemical coagulation and infiltration in soil are effective in removing of MAP from drinking water. It may be difficult to a achieve a further increase in their efficiency since there are economical and technical problems. Therefore, it is probably more beneficial to develop or avoid the present water purification techniques which increased the nutrient concentrations. One way is to develop pH adjustment techniques to which would not necessitate the use of compounds causing phosphorus contamination. One possible way could be limestone filtration, which seemed even decrease the MAP concentration in one studied waterworks. This treatment is worth of studying in the future. The effects of oxidative disinfection on the microbial nutrients, both MAP and AOC, have to be considered critically.

In ground waterworks, the MAP concentrations and microbial growth potential were higher than in surface waterworks, as a result of less treatments. In some ground waterworks it could be necessary to utilize some treatment step for removing phosphorus from water to avoid high microbial growth in drinking water, especially if there is not used chlorination.

The experiments in this thesis were performed in waterworks with real water samples. One biofilm study was done in the laboratory scale. In the future, more research will be needed to study the role of phosphorus in old biofilms present in a full scale distribution system or in a pilot scale distribution system with different pipe materials. Also, the role of phosphorus on survival and growth of pathogenic microbes in drinking water and biofilms should be studied.

7   CONCLUSIONS

- In phosphorus limited drinking waters very low amounts of phosphorus affect microbial growth. A new sensitive bioassay for microbially available phosphorus (MAP) permits studies on the role of phosphorus in drinking water and the effects of water treatment techniques on phosphorus availability. The bioassay detects that part of phosphorus, which is readily available for microbes. The detection limit for the method is 0.08 μg P/l.

- Chemical coagulation, activated carbon filtration and infiltration in soil effectively remove microbial nutrients (MAP and $AOC_{potential}$) and reduce the microbial growth potential in the water, while ozonation increases them. Liming increases MAP and disinfection with chlorine increases $AOC_{potential}$. UV-disinfection does not increase the content of MAP, but slightly decreases $AOC_{potential}$.

- The content of MAP and microbial growth potential is higher in drinking water produced from natural ground water than in drinking waters produced from surface water due to the more effective water purification of surface waters.

- In most Finnish drinking waters, microbial growth is limited by phosphorus. In these waters MAP correlates with the growth potential of microbes in the water.

- In phosphorus limited drinking water, low additions (1-5 μg/l P) of phosphorus increase the concentration of microbes present in biofilms.

8   REFERENCES

Abernathy C. G. and Camper A. K. 1998. The effect of phosphorus based corrosion inhibitors and low disinfection residuals on distribution biofilms. Proceeding of Water quality technology conference, AWWA. CD-Rom. San Diego, USA.

Albrechtsen H-J., Boe-Hansen R., Henze M. and Mikkelsen P. S. 1998. Microbial growth and clogging in sand column experiments simulating artificial recharge of groundwater. Proc. Artificial recharge of groundwater. Jos H. Peters *et al.* (eds.) A. A. Balkema/ Rotterdam/ Brookfield. Netherlands. pp. 73-80.

Ammerman J. W. 2002. Phosphorus cycling in aquatic environments: role of bacteria. In Encyclopedia of Environmental Microbiology, Vol 5. G. Bitton (ed.). John Wiley & Sons, Inc. New York, USA. pp. 2448-2453.

Anderson J. P. E. and Domsch K. H. 1980. Quantities of plant nutrients in the microbial biomass of selected soils. Soil Science. 130(4):211-216.

Anghern M. 1984. Ultraviolet disinfection of water. Aqua. 2:109-115.

Angles M. L., Marshall K. C. and Goodman A. E. 1993. Plasmid transfer between marine bacteria in the aqueous phase and biofilms in reactor microcosms. Applied and Environmental Microbiology. 59(3):843-850.

Anselme C., Suffet I. H. and Mallevialle J. 1988. Effects of ozonation on tastes and odor. Journal AWWA. 10:45-51.

Appenzeller B. M. R., Batte M., Mathieu L., Block J-C., Lahoussine V., Cavard J. and Gatel D. 2001. Effect of adding phosphate to drinking water on bacterial growth in slightly and highly corroded pipes. Water Research. 35(4):1100-1105.

Appenzeller B. M., Duval Y. B., Thomas F. and Block J-C. 2002. Influence of phosphate on bacterial adhesion onto iron oxyhydroxide in drinking water. Environmental Science & Technology. 36(4):646-652.

Armstrong A. J., Williams P. M. and Strickland J. D. H. 1966. Photo-oxidation of organic matter in sea water by ultra-violet radiation, analytical and other applications. Nature. 211:481-483.

Augoustinos M. T., Venter S. N. and Kfir R. 1995. Assesment of water quality problems due to microbial growth in drinking water distribution systems. Environmental Toxicology and Water Quality. 10:295-299.

Backlund P. 1992. Degradation of aquatic humic material by ultraviolet light. Chemosphere. 25(12):1869-1878.

Baldwin D. S. 1998. Reactive "organic" phosphorus revisited. Water Research. 32(8):2265-2270.

Bellar T. A., Lichtenberg J. J. and Kroner R. C. 1974. The occurrence of organohalides in chlorinated drinking water. Journal AWWA. 66:703-706.

Block J. C. 1992. Biofilms in drinking water distribution systems. *In* Biofilms Science and Technology. L.F. Melo *et al*. (eds.). Kluwer Academic Publishers. Netherlands.

Block J. C., Haudidier, K., Paquin, J. L., Miazga, J., and Levi Y. 1993. Biofilm accumulation in drinking water distribution systems. Biofouling 6: 333-343.

Block J. C. 2002. Counting what? Stressing environments change the meaning of HPC in drinking waters. Proceeding of the NSF International / WHO Symposium on HPC Bacteria in drinking water – Public health implications? April 22-24. Geneva, Switzerland. pp. 525-533.

Boere J. A. 1992. Combined use of ozone and granular activated carbon (GAC) in potable water treatment; effects on GAC quality after reactivation. Ozone Science & Engineering. 14:123-137.

Boorman G. A., Dellarco V., Dunnick J. K., Chapin R. E., Hunter S., Hauchman F., Gardner H., Cox M. and Sills R. C. 1999. Drinking water disinfection byproducts: Review and approach of toxicity evaluation. Environmental Health Perspectives. 107 (supplement I):207-217.

Boualam M., Mathieu L., Fass S., Cavard J. and Gatel D. 2002. Relationship between coliform culturability and organic matter in low nutritive waters. Water Research. 36:2618-2626.

Bull R. J., Birnbaum L. S., Cantor K. P., Rose J. B., Butterworth B. E., Pegram R. and Tuomisto J. 1995. Water chlorination: Essential process or cancer hazard. Fundamental and Applied Toxicology. 28:155-166.

Buswell C. M., Herlihy Y. M., Lawrence L. M., McGuiggan J. T. M., Marsh P. D., Keevil C. W. and Leach S. A. 1998. Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and rRNA staining. Applied and Environmental Microbiology. 64(2):733-741.

Camper A. K., LeChevallier M. W., Broadway S. C. and McFeters G. A. 1986. Bacteria associated with granular activated carbon particles in drinking water. Applied and Environmental Microbiology. 52(3):434-438.

Camper A. K., Jones W. L. and Hayes J. T. 1996. Effect of growth conditions and substratum composition on the persistence of coliforms in mixed-population biofilms. Applied and Environmental Microbiology. 62(11):4014-4018.

Chandy J. P. and Angles M. L. 2001. Determination of nutrients limiting biofilm formation and the subsequent impact on disinfectant decay. Water Research. 35(11):2677-2682.

Chapelle F. H. 1992. Groundwater microbiology and geochemistry. John Wiley & Sons Inc. New York, USA.

Characklis W. G. 1990. Microbial biofouling control. In: Biofilms. Characklis W. G. and Marshall K. C. (eds.) John Wiley & Sons, Inc. USA.

Characklis W. G. and Marshall K. 1990. Biofilms: A basis for an interdisciplinary approach. In: Biofilms. Characklis W. G. and Marshall K. C. (eds.) John Wiley & Sons, Inc. USA.

Charnock C. and Kjønnø O. 2000. Assimilable organic carbon and biodegradable dissolved organic carbon in Norwegian raw and drinking waters. Water Research. 34(10):2629-2642.

Clancy J. L., Bukhari Z., Hargy T. M., Bolton J. R., Dussert B. W. and Marshall M. M. 2000. Using UV to inactivate Cryptosporidium. Journal AWWA. 92(9):97-104.

Cooke G. D., Welch E. B., Martin A. B., Fulmer D. G., Hyde J. B. and Schrieve G. D. 1993. Effectiveness of Al, Ca and Fe salts for control of internal phosphorus loading in shallow and deep lakes. Hydrobiologia. 253:323-335.

Coveney M. F. and Wetzel R. G. 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. Applied and Environmental Microbiology. 58(1):150-156.

Corin N., Backlund P. and Kulovaara M. 1996. Degradation products formed during UV-irradiation of humic waters. Chemosphere. 33(2):245-255.

Council directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official Journal of European Communities. 330:32-54.

Critchley M. M., Cromar N. J., McClure N. and Fallowfield H. J. 2001. Biofilms and microbially influenced cuprosolvency in domestic copper plumbing systems. Journal of Applied Microbiology. 91:646-651.

Culp G. L. and Culp R. L. 1974. New concepts in water purification. Van Nostrand Reinhold Environmental Engineering Series, New York.

De Jonge V. N., Engelkes M. M. and Bakker J. F. 1993. Bio-availability of phosphorus in sediments of western Dutch Wadden Sea. Hydrobiologia. 253:151-163.

DeLaat J., Bouanga F. and Dore M. 1985. Influence of microbiological activity in granular activated carbon filters on the removal of organic compounds. The Science of the Total Environment. 47:115-120.

Dennet K. E., Amirtharajah A., Moran T. F. and Gould J. P. 1996. Coagulation: its effect on organic matter. Journal AWWA. 88(4):129-142.

Edberg S. C., Gallo P. and Kontnick C. 1996. Analysis of the virulence characteristics of bacteria isolated from bottled, water cooler, and tap water. Microbial Ecology in Health and Disease. 9:67-77.

Edberg S. C. and Allen M. J. 2002. Virulence and risk of HPC bacteria in human population groups. Proceeding of the NSF International / WHO Symposium on HPC Bacteria in drinking water – Public health implications? April 22-24. Geneva, Switzerland. pp. 287-299.

Eighmy T. T., Collins M. R., Spanos S. K. and Fenstermacher J. 1992. Microbial populations, activities and carbon metabolism in slow sand filters. Water Research 26(10):1319-1328.

Ekholm P. 1998. Algal-available phosphorus originating from agriculture and municipalities. Monographs of the boreal environment research 11. Finnish Environment Institute. Helsinki, Finland.

Escobar I. C. and Randall A. A. 2001. Case study: Ozonation and distribution system biostability. Journal AWWA. 10:77-89.

Escobar I. C., Randall A. A. and Taylor J. S. 2001. Bacterial growth in distribution systems: Effect of assimilable organic carbon and biodegradable dissolved organic carbon. Environmental Science and Technology. 35:3442-3447.

Exall K. N. and Vanloon G. W. 2000. Using coagulants to remove organic matter. Journal AWWA. 92(11):93-102.

Farooq S., Al-Yousef A.K., Al-Layla R. I. and Ishaq A. M. 1994. Tertiary treatment of sewage effluent via pilot scale slow sand filtration. Environmental Technology. 15:15-28.

Fielding M. and Hutchison J. 1995. Formation of bromate and other ozonation by-products in water treatment. Water Supply. 13(1):71-84.

Finnish Standards Association. 1986. Determination of total phosphorus in water, digestion with peroxodisulfate. SFS 3026. Finnish Standards Association SFS, Helsinki, Finland.

Ford T. E. 1999. Microbiological safety of drinking water: United States and global perspectives. Environmental Health Perspectives. 107 (supplement I):191-206.

Fransolet G., Depelchin A., Villers G., Goossens R. and Masschelein W. J. 1988. The role of bicarbonate in bacterial growth in oligotrophic waters. Journal AWWA. 57-61.

Frias J., Ribas F. and Lucena F. 1992. A Method for the measurement of biodegradable organic carbon in waters. Water Research. 26(2):255-258.

Frias J., Ribas F. and Lucena F. 1995. Comparison of methods for the measurement of biodegradable organic carbon and assimilable organic carbon in water. Water Research. 29(12):2785-2788.

Frias J., Ribas F. and Lucena F. 2001. Effects of different nutrients on bacterial growth in a pilot distribution system. Antonie Van Leeuwenhoek. 80(2):129-138.

Gavriel A. A., Landre J. P. B. and Lamb A. J. 1998. Incidence of mesophilic Aeromonas within a public drinking water supply in north-east Scotland. Journal of Applied Microbiology. 84:383-392.

Gerba C. P., Wallis C. and Melnick J. L. Viruses in water: the problem, some solutions. Environmental Science and Technology. 9(13):1122-1126.

Ghico J-M. 2001. Natural conjugative plasmids induce bacterial biofilm development. Nature. 412(26):442-445.

Gibbs R. A., Scutt J. E. and Croll B. T. 1993. Assimilable organic carbon concentrations and bacterial numbers in a water distribution system. Water Science and Technology. 27(3-4):159-166.

Gilbert P., Allison D. G. and McBain A. J. 2002. Biofilms *in vitro* and *in vivo*: do singular mechanisms imply cross-resistance? Journal of Applied Microbiology Symposium Supplement. 92:98S-110S.

Gjessing E. T. and Källqvist T. 1991. Algicidal and chemical effect of u.v.-radiation of water containing humic substances. Water Research. 25(4):491-494.

Glaze W. H. 1987. Drinking water treatment with ozone. Environment Science and Technology. 21(3):224-230.

Gächter R. and Meyer J. S. 1993. The role of microorganisms in mobilization and fixation of phosphorus in sediments. Hydrobiologia. 253:103-121.

Haas C. N., Bitter P. and Scheff P. A. 1988. Preliminary determination of limiting nutrients for indigenous bacteria in Chicago intake water. Water Air and Soil Pollution. 37:65-72.

Hahn M. W. and Höfle M.G. (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiology Ecology. 35:113-121.

Hansen B. Foundation course in chemical precipitation 1 and 2. (22.2.2001) http://kw.kemira.com/img/pdf/Grundkurs%201%2Epdf, http://kw.kemira.com/img/pdf/Grundkurs%202%2Epdf

Harris G. D., Adams D., Sorensen D. L. and Curtis M. S. 1987. Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria. Water Research. 21(6):687-692.

Hatva T. 1989. Iron and manganese in groundwater in Finland: Occurrence in glacifluvial aquifers and removal by biofiltration. Publications of the water and environment research institute 4. National board of waters and the environment, Finland. Helsinki. pp. 3-12.

Hatva T. 1996. Artificial groundwater recharge in Finland. Proc. of an international symposium of Artificial recharge of groundwater. Kivimäki A-L and Suokko T. (eds.) NHP Report No. 38. Helsinki, Finland.

Hausner M. and Wuertz S. 1999. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Applied and Environmental Microbiology. 65(8):3710-3713.

Hens M. and Merckx R. 2002. The role of colloidal particles in the speciation and analysis of "dissolved" phosphorus. Water Research. 36:1483-1492.

Hobbie J. E., Daley R. J. and Jasper S. 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology. 33:1225-1228.

Hochstädter S. 2000. Seasonal changes of C:P ratios of seston, bacteria, phytoplankton and zooplankton in a deep mesotrophic lake. Freshwater Biology. 44(3):453-463.

Holtan H., Kamp-Nielsen L. and Stuanes A. O. 1988. Phosphorus in soil, water and sediment: an overview. Hydrobiologia 170:19-34.

Hu J. Y., Wang Z. S., Ng W. J. and Ong S. L. 1999. The effect of water treatment processes on the biological stability of potable water. Water Research. 33(11):2587-2592.

Hubaux, A., and G. Vos. 1970. Decision and detection limits for linear calibration curves. Analytical Chemistry. 42:849-855.

Huck P. M. 1990. Measurement of biodegradable organic matter and bacterial growth potential in drinking water. Journal AWWA. 82(7):78-86.

Hudson J. J., Taylor W. D., Schindler D. W. 2000. Phosphate concentration in lakes. Nature. 406:54-56.

Huisman L. and Olsthoorn T. N. 1983. Artificial groundwater recharge. Pitman Books Limited Boston. p. 312.

Hunter P. 2002. Epidemiological evidence of disease linked to HPC bacteria. Proceeding of the NSF International / WHO Symposium on HPC Bacteria in drinking water – Public health implications? April 22-24. Geneva, Switzerland. pp. 231-245.

Jacks G. and Frycklund C. 1996. Liming of infiltration ponds as a measure to increase hardness and alkalinity in groundwater. Proc. of an international symposium of Artificial recharge of groundwater. Eds. Kivimäki A-L and Suokko T. NHP Report No. 38. Helsinki, Finland. pp. 179-186.

Jansson M., Olsson H. and Pettersson K. 1988. Phosphatases; origin, characteristics and function in lakes. Hydrobiologia. 170:157-175.

Jiang J-Q. 2000. Development of coagulation theory and new coagulants for water treatment: its past, current and future trend. Conference preprint Book 2, drinking water treatment. 1st world water congress of the international water association (IWA). Paris, France. 132-139.

Jones R. I., Salonen K. and De Haan H. 1988. Phosphorus transformations in the epilimnion of humic lakes: abiotic interactions between dissolved humic materials and phosphate. Freshwater Biology. 19:357-369.

Jones R. D. 1997. Phosphorus cycling. In *Manual of Environmental Microbiology*. Hurst, J. C., Knudsen, G. R., McInerney, M. J., Stetzenbach, L. D., Walter, M. V., (eds.); ASM Press: Washington D. C. pp 343-348.

Joret J. C., Levi Y. and Volk C. 1991. Biodegradable dissolved organic carbon (BDOC) content of drinking water and potential regrowth of bacteria. Water Science and Technology. 24(2):95-101.

Juhna T. 1999. Sorption of humic substances and microbial activity in the course of artificial recharge of groundwater. Licentiate thesis. Luleå University of Technology. Department of Environmental Engineering. 99/25. 20p.

Juhna T. and Nikolajeva V. 2000. Bacterial growth in humus-rich drinking water with a high mineral content. The abstracts of the posters of The Fourth Valamo conference on environmental and risk assesment 13-16.9.2000. Finnish research programme on environmental health – SYTTY.

Juhna T. 2002a. Effect of phosphorus removal from humus-rich drinking water on biofilm formation. Proceeding of International Specialiced Conference on Biofilm Monitoring. March 17-20, 2002. Porto, Portugal. pp. 245-248.

Juhna T. 2002b. Aspects of drinking water supply in areas of humic water. Doctoral thesis. Luleå University of Technology. 2002:27. 44 p.

Kainulainen T., Tuhkanen T., Vartiainen T., Heinonen-Tanski H. and Kalliokoski P. 1994. The effect of different oxidation and filtration processes on the molecular size distribution of humic material. Water Science and Technology. 9:169-174.

Kainulainen T. K., Tuhkanen T. A., Vartiainen T. K. and Klliokoski P. J. 1995. Removal of residual organics from drinking water by ozonation and activated carbon filtration: A pilot plant study. Ozone Science & Engineering 17:449-462.

Kajosaari E. 1981. Vesihuolto. Suomen rakennusinsinöörien liitto. RIL 124. Helsinki, Finland. pp. 48-55.

Karl, D. M. 1980. Cellular nucleotide measurements and applications in microbial ecology. Microbiological Reviews. 44(4):739-796.

Karl D. M. and Tien G. 1992. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnology and Oceanography. 37(1):105-116.

Karpel N., Leitner V., Miquel M., Dore M. and Legube, B. 1996. Oxidation of natural organic matter by ozone and hydroxyl radicals. *In* Proceedings of IWSA Natural organic matter workshop. Poitiers, France, pp. 25:1-5.

Keevil C. W. Chemostat models of human and aquatic corrosive biofilms. 1989. Proceedings of Recent advances in microbial ecology, ISME 5. Hattori T. *et al*. (eds.). Japan scientific societes press. Japan.

Keinänen M. M., Korhonen L. K., Lehtola M. J., Miettinen I. T., Martikainen P. J., Vartiainen T. and Suutari M. H. 2002. The microbial community structure of drinking water biofilms can be affected by phosphorus availability. Applied and Environmental Microbiology. 68(1):434-439.

Kerneis A., Nakache F., Deguin A. and Feinberg M. 1995. The effects of water residence time on the biological quality in a distribution network. Water Research. 29(7):1719-1727.

Kivimäki A-L. 1992. Tekopohjavesilaitokset Suomessa (The artificial groundwater plants in Finland, abstract in English). The national board of waters and environment, Finland. Series A 98. Helsinki.

Kivimäki A-L., Lahti K., Hatva T., Tuominen S. M. and Miettinen I. T. 1998. Removal of organic matter during bank filtration. Proc. Artificial recharge of groundwater. Ed. Jos H. Peters *et al.* A.A.Balkema/Rotterdam/Brookfield. Netherlands. pp. 107-112.

Klavins M., Juhna T. and Eglite L. 2000. Removal of humic substances during treatment of drinking water using sorbents. Vatten. 56:79-83.

Kortelainen P. 1993. Content of total organic carbon in Finnish lakes and its relationship to catchment characteristics. Canadian Journal of Fisheries and Aquatic Sciences. 50:1477-1483.

Kramer M. H., Quade G., Hartemann P. and Exner M. 2001. Waterborne diseases in Europe-1986-96. Journal AWWA. 1:48-53.

Kruithof J. C., Van der Leer R. C., Hijnen W. A. M. 1992. Practical experiences with UV disinfection in the Netherlands. Water SRT-Aqua. 41(2):88-94.

Kuehn W. and Mueller U. 2000. Riverbank filtration, an overview. Journal AWWA. 12:60-69.

Kukkula M., Maunula L., Silvennoinen E. and von Bonsdorf C-H. 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by norwalk-like viruses. The Journal of Infectious Diseases. 180:1771-1776.

Kulovaara M., Corin N., Backlund P. and Tervo J. 1996. Impact of UV_{254}-radiation on aquatic humic substances. Chemosphere. 33(5):783-790.

Kulovaara M. 1996. Light-induced degradation of aquatic humic substances by simulated sunlight. International Journal of Environmental Analytical Chemistry 62(2):85-95.

Lahti K. and Hiisvirta L. 1995. Causes of waterborne outbreaks in community water systems in Finland: 1980-1992. Water Science and Technology. 31(5-6):33-36.

Langlais B., Reckhow D. A. and Brink D. R. (Eds.). 1991. Ozone in Water Treatment, Application and Engineering. CRC Press/Lewis Publishers, USA.

Laurent P., Servais P. and Randon G. 1993. Bacterial development in distribution networks . study and modelling. Water Supply 11(3/4):387-398.

LeChevallier M. W. and McFeters G. A. 1985. Interactions between heterotrophic plate count bacteria and coliform organisms. Applied and Environmental Microbiology. 49(5):1338-1341.

LeChevallier M. W., Babcock T. M. and Lee R. G. 1987. Examination and characterization of distribution systems biofilms. Applied and Environmental Microbiology. 53(12):2714-2724.

LeChevallier M. W. Cawthon C. D. and Lee R. G. 1988. Inactivation of biofilm bacteria. Applied and Environmental Microbiology. 54:2492-2499.

LeChevallier M. W. 1990. Coliform regrowth in drinking water: A Review. Journal AWWA. 32:74-86.

LeChevallier and McFeters. 1990. Microbiology of activated carbon. *In* Drinking Water Microbiology. Ed. McFeters G. A. Springer-Verlag. New York. USA.

LeChevallier M. W., Lowry C. D. and Lee R. G. 1990. Disinfecting biofilms in a model distribution system. Journal AWWA. 82(7):87-99.

LeChevallier M. W., Schultz W. and Lee R. G. 1991. Bacterial nutrients in drinking water. Applied and Environmental Microbiology. 57(3):857-862.

LeChevallier M. W., Becker W. C., Schorr P. and Lee R. G. 1992. Evaluating the performance of biologically active rapid filters. Journal AWWA. 84(4):136-146.

LeChevallier M. W. 1999. The case for maintaining a disinfectant residual. Journal AWWA. 91(1):86-94.

Lehtola M. J., Miettinen I. T., Vartiainen T. and Martikainen P. J. 2001. The effect of chlorination on availability of microbial nutrients in drinking water. IWA 2nd World Water Congress, Berlin, 15-19.10.2001. CD-Rom.

Lehtola M. J., Nissinen T.K., Miettinen I. T., Vartiainen T. and Martikainen P. J. Removal of loose deposits from distribution system improves the drinking water quality. SUBMITTED.

Lemke M. J., Churchill P. F. and Wetzel R. G. 1995. Effect of substrate and cell surface hydrophobicity on phosphate utilization in bacteria. Applied and Environmental Microbiology. 61(3):913-919.

Literathy P. and Laszlo F. Processes affecting the quality of bank-filtered water. 1996. Proceedings of an international symposium of Artificial recharge of groundwater. Eds. Kivimäki A-L and Suokko T. NHP Report No. 38. June 3-5. 1996, Helsinki, Finland. pp. 53-64.

Lippy E. C. and Waltrip S. C. 1984. Waterborne disease outbreaks-1946-1980: a thirty-five-year perspective. Journal AWWA. 77(2):60-67.

Liu W., Wu H., Wang Z., Ong S. L., Hu J. Y. and Ng W. J. 2002. Investigation of assimilable organic carbon (AOC) and bacterial regrowth in drinking water distribution system. Water Research. 36:891-898.

Logsdon G. S. 1990. Microbiology and drinking water filtration. In *Drinking water microbiology, progress and recent developments*. McFeters G.A. (ed.). pp. 120-145. Springer-Verlag, Michigan USA.

Lucena F., Frias J. and Ribas F. 1990. A New dynamic approach to the determination of biodegradable dissolved organic carbon in water. Environmental Technology. 12:343-347.

Lund V. and Hongve D. 1994. Ultraviolet irradiated water containing humic substances inhibits bacterial metabolism. Water Research. 28(5):1111-1116.

Mathieu L., Paquin J. L., Block J. C., Randon G., Maillard J. and Reasoner D. 1992. Parameters governing bacterial growth in water distribution systems (summary in English). Revue des Sciences de L´eau. 6:91-112.

Mathieu L., Block J. C., Dutang M., Maillard J. and Reasoner D. 1994. Control of biofilm accumulation in drinking water distribution systems. Water Supply. 11:365-376.

Matin A. and Harakeh S. 1990. Effect of starvation on bacterial resistance to disinfectants. In McFeters G. A. (ed.) Drinking water microbiology, progress and recent developments. Springer-Verlag. New York. USA. pp. 88-103.

McFeters G. A. 1990. Drinking water microbiology, progress and recent developments. Springer-Verlag. New York. USA.

Miettinen, I. T., Vartiainen T., and Martikainen P. J. 1996a. Changes in water microbiology and chemistry during bank filtration of humus-rich lake water. Proc. of an international symposium of Artificial recharge of groundwater. Eds. Kivimäki A-L and Suokko T. NHP Report No. 38. June 3-5, 1996. Helsinki, Finland. pp. 53-64.

Miettinen, I. T., Vartiainen T., and Martikainen P. J. 1996b. Contamination of drinking water. Nature 381:654-655.

Miettinen I. T., Vartiainen T. and Martikainen P. J. 1997a. Phosphorus and bacterial growth in drinking water. Applied and Environmental Microbiology. 63:3242-3245.

Miettinen I. T., Vartiainen T. and Martikainen P. J. 1997b. Microbial growth and assimilable organic carbon in Finnish drinking water. Water Science and Technology. 35(11-12):301-306.

Miettinen I. T., Vartiainen T. and Martikainen P. J. 1998. Microbial growth in drinking waters treated with ozone, ozone/hydrogen peroxide or chlorine. Ozone Science & Engineering. 20:303-315.

Miettinen I. T., Vartiainen T. and Martikainen P. J. 1999. Determination of assimilable organic carbon in humus-rich drinking waters. Water Research. 33(10):2277-2282.

Miettinen I. T., Zacheus O., von Bonsdorff C-H. and Vartiainen T. 2001a. Waterborne epidemics in Finland in 1998-1999. Water Science and Technology. 43(12):67-71.

Miettinen I. T., Lehtola M., Vartiainen T., Hatva T. and Martikainen P. J. 2001b. Microbial nutrients and growth in artificially recharged ground water. IWA 2nd World Water Congress, Berlin, 15-19.10.2001. CD-Rom.

Mohamed M. N., Lawrence J. R. and Robarts R. D. 1998. Phosphorus limitation of heterotrophic biofilms from the Fraser river, British Columbia, and the effect of pulp mill effluent. Microbial Ecology. 36:121-130.

Myllykangas T., Nissinen T. and Vartiainen T. 2000. Bromate formation during ozonation of bromide containing drinking water – a pilot scale study. Ozone Science & Engineering. 22:487-499.

Nedwell D. B. 1999. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature, Minireview. FEMS Microbiology Ecology. 30:101-111.

Niquette P., Servais P. and Savoir R. 2000. Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. Water Research. 34(6):1952-1956.

Niquette P., Servais P. and Savoir R. 2001. Bacterial dynamics in the drinking water distribution system of Brussels. Water Research. 35(3):675-682.

Nishijima W., Shoto E. and Okada M. 1997. Improvement of biodegradation of organic substance by addition of phosphorus in biological activated carbon. Water Science and Technology. 36(12):251-257.

Nissinen T. K., Miettinen I. T., Martikainen P. J. and Vartiainen T. 2001. Molecular size distribution of natural organic matter in raw and drinking waters. Chemosphere. 45:865-873.

Nissinen T. K., Miettinen I. T., Martikainen P. J. and Vartiainen T. 2002. Disinfection by-products in Finnish drinking waters. Chemosphere. In Press.

Noble P. A., Clark D. L. and Olson B. H. 1996. Biological stability of ground water. Journal AWWA. 88(5):87-96.

Odell L. H., Kirmeyer G. J., Wilczak A., Jacangelo J. G., Marcinko J. P. and Wolfe R. L. 1996. Controlling nitrification in chloraminated systems. Journal AWWA. 88(7):86-98.

Okabe S., Kokazi T. and Watanabe Y. 2001. Biofilm formation potentials in drinking waters treated by different advanced treatment processes. IWA 2nd World Water Congress, Berlin, 15-19.10.2001. CD-Rom.

Oppenheimer J. A., Jacangelo J. G., Laine J-M. and Hoagland J. E. 1997. Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards. Water Environment Research 69(1):14-24.

O´Toole G., Kaplan H. B. and Kolter R. 2000. Biofilm formation as microbial development. Annual Reviews in Microbiology. 54:49-79.

Pantsar-Kallio M. and Manninen P. K. G. 1995. Application of capillary electrophoresis in the analysis of phosphate in lake water. Chemosphere. 31(7):3699-3707.

Paquin J. L., Block J. C., Haudidier K., Hartemann P., Colin F., Miazga J. and Levi Y. 1992. Effect of chlorine on the bacterial colonisation of a model distribution system (summary in English). Revue des Sciences de L´eau. 5:399-414.

Parrotta M. J. and Bekdash F. 1998. UV-disinfection of small groundwater supplies. Journal AWWA. 90(2):71-81.

Pavlov D., de Wet C. M. E., Grabow W. O. K. and Ehlers M. M. 2001. Determination of cytotoxicity and invasiveness of heterotrophic plate count bacteria isolated from drinking water. IWA 2nd World Water Congress, Berlin, 15-19.10.2001. CD-Rom.

Payment P., Franco E. and Siemiatycki J. 1993. Absence of relationship between health effects due to tap water consumption and drinking water quality parameters. Water Science and Technology. 27(3-4):137-143.

Payment P., Coffin E. and Paquette G. 1994. Blood agar to detect virulence factors in tap water heterotrophic bacteria. Applied and Environmental Microbiology. 60(4):1179-1183.

Percival S. L. and Walker J. T. 1999. Potable water and biofilms: a review of the public health implications. Biofouling. 42(2):99-115.

Peters J. H., Schijven J. F., Hoogenboezem W., vanBaar M. J. C., Bergsma J., Nobel P. J., Stakelbeek A. and Vogelaar A. J. 1998. Fate of pathogens and consequences for the design of artficial recharge systems. Proc. Artificial recharge of groundwater. Jos H. Peters *et al.* (eds.). A.A.Balkema/Rotterdam/Brookfield. Netherlands. pp. 141-148.

Pietari J. 1995. Biological activated carbon (BAC) treatment of surface water at low temperatures. Master of science thesis. Tampere University of Technology.

Pitkänen T. M., Korhonen L. K., Lehtola M. J., Miettinen I. T., Hänninen M-L. and Martikainen P. J. 2002. Survival of *Campylobacter jejuni, Escherichia coli* and *Klebsiella pneumoniae* in drinking water. In Proceedings of International Symposium on Waterborne Pathogens. IWA-AWWA. September 22-25, 2002, Cascais/Lisbon, Portugal. CD-Rom.

Preuß G. and Nehrkorn A. 1996. Succession of microbial communities during bank filtration and artificial groundwater recharge. In *Artificial recharge of groundwater*. Kivimäki A-L. and Suokko T. (eds.). Proceedings of an international symposium, Helsinki, Finland.

Prévost M., Rompré A., Coallier J., Servais P., Laurent P., Clément B. and Lafrance P. 1998. Suspended bacterial biomass and activity in full-scale drinking water distribution systems: impact of water treatment. Water Research. 32(5):1393-1406.

Raassina S. and Suokas T. 2001. Kalkkikivialkalointia käyttävät vesilaitokset Suomessa: nykytilan kartoitus/laitostiedot (Waterworks utilising limestone alkalinisation in Finland: current situation and plant specific data. Finnish Environment Institute, abstract in English). Mimeograph series of Finnish Environment Institute no. 207. Helsinki.

Reasoner D. J. and Geldreich E. E. 1985. A new medium for the enumeration and subculture bacteria from potable water. Applied and Environmental Microbiology. 49:1-7.

Reasoner D. J. 2002. Heterotrophic plate count (HPC) methodology in the United States. Proceeding of the NSF International / WHO Symposium on HPC Bacteria in drinking water – Public health implications? April 22-24. Geneva, Switzerland. pp. 147-159.

Ribas F., Frias J., Huguet J. M. and Lucena F. 1997. Efficiency of various water treatment processes in the removal of biodegradable and refractory organic matter. Water Research. 31(3):639-649.

Roberts P. V. and Valocchi. 1981. Principles of organic contaminant behavior during artifical recharge. The Science of the Total Environment. 21:161-172.

Robertson W. and Brooks T. 2002. What role should heterotrophic plate count bacteria play in the treatment of distribution of drinking water. Proceeding of the NSF International / WHO Symposium on HPC Bacteria in drinking water – Public health implications? April 22-24. Geneva, Switzerland. pp. 511-524.

Rompré A., Prevost M., Coallier J., Brisebois P. and Lavole J. 2000. Impacts of implementing a corrosion control strategy on biofilm growth. Water Science and Technology. 41(4-5):287-294.

Ron Vaz M. D., Edwards A. C., Shand C. A. and Cresser M. 1992. Determination of dissolved organic phosphorus in soil solutions by an improved automated photo-oxidation procedure. Talanta 39(11):1479-1487.

Rook J. J. 1974. Formation of haloforms during chlorination of natural water. Water Treatment and Examination. 23:234-243.

Rusin P. A., Rose J. B., Haas C. N. and Gerba C. P. 1997a. Risk assesment of opportunistic bacterial pathogens in drinking water. Reviews of Environmental Contamination and Toxicology. 152:57-38.

Rusin P. A., Rose J. B. and Gerba C. P. 1997b. Health significance of pigmented bacteria in drinking water. Water Science and Technology. 35(11-12):21-27.

Sallanko J. and Lakso E. 2000. Alkaloiva märkäsuodatus rautapitoisten pohjavesien käsittelyssä (Alkalizing wet filtration in the treatment of ferrous groundwater, abstract in English) University of Oulu, publications of water resources and environmental engineering laboratory A17. Oulu, Finland.

Sathasivan A., Ohgaki S., Yamamoto K. and Kamiko N. 1997. Role of inorganic phosphorus in controlling regrowth in water ditribution system. Water Science and Technology. 35(8):37-44.

Sathasivan A. and Ohgaki S. 1999. Application of new bacterial regrowth potential method for water distribution system – A clear evidence of phosphorus limitation. Water Research. 33(1):137-144.

Schijven J. F., Medema G. J., Vogelaar A. J. and Hassanizadeh S. M. 2000. Removal of microorganisms by deep well injection. Journal of Contaminant Hydrology. 44: 301-327.

Schlegel H. G. 1997. General Microbiology, 7th edition. Cambridge University Press. Cambridge, United Kingdom.

Schmidt K. 1996. Water quality aspects of artificial groundwater recharge – General overview as a keynote. Proc. of an international symposium of Artificial recharge of groundwater. Kivimäki A-L and Suokko T. (eds.). NHP Report No. 38. Helsinki, Finland. pp. 145-154.

Scholz M. and Martin R. J. 1997. Ecological equilibrium on biological activated carbon. Water Research 31(12):2959-2968.

Schwartz, T., Hoffman, S., and Obst, U. 1998. Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. Water Research 32(9): 2787-2797.

Schwarzenbach R.P., Giger W., Hoehn E. and Schneider J. K. 1983. Behavior of organic compounds during infiltration of river water to groundwater. Field studies. Environmental Science and Technology. 17:472-479.

Servais P., Billen G. and Hascoet M-C. 1987. Determination of the biodegradable fraction of dissolved organic matter in waters. Water Research. 21(4):445-450.

Servais P., Billen G., Ventresque C. and Bablon G. P. 1991. Microbial activity in GAC filters at the Choisy-le-Roi treatment plant. Journal AWWA. 83(2):62-68.

Servais P., Billen G., Bouillot P. and Benezet M. 1992. A pilot study of biological GAC filtration in drinking-water treatment. J Water SRT – Aqua. 41(3):163-168.

Servais P., Laurent P. and Randon G. 1995. Comparison of the bacterial dynamics in various French distribution systems. J Water SRT-Aqua. 44(1):10-17.

Shaw J. P., Malley Jr. J. P., Willoughby S. A. 2000a. Effects of UV irradiation on organic matter. Journal AWWA. 92(4):157-167.

Shaw P. J., Jones R. I. and De Haan H. 2000b. The Influence of humic substances on the molecular weight distributions of phosphate in epilimnic lake waters. Freshwater Biology. 45(4):383-393.

Sibille I., Sime-Ngando T., Mathieyu L. and Block J-C. 1998. Protozoan bacteriovory and Escherichia coli survival in drinking water distribution systems. Applied and Environmental Microbiology. 64(1):197-202.

Singer P. C. 1990. Assessing ozonation research needs in water treatment. Journal AWWA. 82(10):78-88.

Sinsabaugh R. L., Hoehn R. C., Knocke W. R. and Linkins A. E. 1986. Removal of dissolved organic carbon by coagulation with iron sulfate. Journal AWWA. 78(5):74-83.

Smeds A., Vartiainen T., Mäki-Paakkanen J. and Kronberg L. 1997. Concentrations of Ames mutagenic chlorohydroxyfuranones and related compunds in drinking waters. Environmental Science and Technology. 31:1033-1039.

Soveltamisopas talousvesiasetukseen 461/2000. 2000. Sosiaali- ja terveysministeriön asetus 461/2000 talousveden laatuvaatimuksista ja valvontatutkimuksista. Vesi- ja viemärilaitosyhdistys, Suomen kuntaliitto. Helsinki 2000, Finland.

Stainton M. P. 1980. Errors in molybdenum blue methods for determining orthophosphate in freshwater. Canadian Journal of Fisheries and Aquatic Science. 37:472-478.

Standard Methods for the examination of water and wasterwater, 19th Edition. 1995. Assimilable organic carbon (proposed) 9217. American Public Health Association. Franson M. A. H. (ed.). Washington, USA. pp. 9-39-43.

Standard Methods for the examination of water and wasterwater, 19th Edition. 1995. Phosphorus 4500-P. American Public Health Association. Franson M. A. H. (ed.).Washington, USA. pp. 4-106-115.

Stanley, P.E. 1989. A Concise beginner´s guide to rapid microbiology using adenosine triphosphate (ATP) and luminescence. *In* ATP luminescence rapid methods in microbiology. Stanley P.E., McCarthy B. J., and Smither R. (eds.). pp. 1-9.

Stevenson F. J. 1982. Humus chemistry – genesis, composition, reactions. John Wiley & Sons. USA.

Storey M. V. and Ashbolt N. J. 2001. Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. Water Science and Technology. 43(12):133-138.

Stuyfzand P. J. and Kooiman J. W. 1996. Elimination of pollutants during artificial recharge and bank infiltration: a comparison. Proc. of an international symposium of Artificial recharge of groundwater. Kivimäki A-L and Suokko T. (eds.). NHP Report No. 38. Helsinki, Finland. pp. 223-232.

Szewzyk U., Szewzyk R., Manz W. and Schleifer K.-H. 2000. Microbiological safety of drinking water. Annual Review of Microbiology. 54:81-127.

Thurman E. M. 1986. *Organic geochemistry of natural waters*, 2nd ed. Martinus Nijhoff/Dr. W. junk Publishers. The Netherlands.

Tranvik L. J. 1998. Degradation of dissolved organic matter in humic waters by bacteria. In Aquatic humic substances. Hessen D. O. and Tranvik L. J. (eds.). Springer, Germany. Ecological studies Vol. 133.

Trussel R. R. 1998. An overview of disinfectant residuals in drinking water distribution systems. Preprint of specialized conference on drinking water distribution with of without disinfectant residual. Gerlach M. and Gimbel R. (eds.). IWW Rheinisch-Westfälisches Institut fur Wassenforschung. Mulheim an der Ruhr, Germany.

Tuhkanen T. A., Kainulainen T. K., Vartiainen T. K. and Kalliokoski P. J. 1994. The effect of preozonation, ozone/hydrogen peroxide treatment, and nanofiltration on the removal of organic matter from drinking water. Ozone Science & Engineering. 16:367-383.

Uhl W., Schaule G. and Gimbel R. 2001. Preventing bacterial regrowth in old distribution systems without disinfection. IWA 2nd World Water Congress, Berlin, 15-19.10.2001. CD-Rom.

Vadstein O. 2000. Heterotrophic, planktonic bacteria and cycling of phosphorus. Phosphorus requirements, competitive ability, and food web interactions. Advances in Microbial Ecology. 16:115-167.

Vahala R., Mormarco V., Niemi R. M., Rintala J. and Laukkanen R. 1998a. The effects of nutrients on natural organic matter (NOM) removal in biological activated carbon (BAC) filtration. Acta Hydrochim. Hydrobiol. 26(3):196-199.

Vahaha R., Ala-Peijari T., Rintala J. and Laukkanen R. 1998b. Evaluating ozone dose for AOC removal in two-step GAC filters. Water Science and Technology. 37(9):113-120.

Vahala R., Niemi R. M., Kiuru H. and Laukkanen R. 1999. The effect of GAC filtration on bacterial regrowth and nitrification in a simulated water main. Journal of Applied Microbiology Symposium Supplement. 85:178S-185S.

Van der Kooij D., Visser A. and Oranje J. P. 1982a. Multiplication of *fluorescent pseudomonads* at low substrate concentrations in tap water. Antonie van Leeuwenhoek 48:229-243.

Van der Kooij D., Visser A. and Hijnen W. A. M 1982b. Determination the concentration of easily assimilable organic carbon in drinking water. Journal AWWA. 74:540-545

Van der Kooij D. and Hijnen W. A. M. 1984. Substrate utilization by an oxalate-consuming *Spirillum* species in relation to its growth in ozonated water. Applied and Environmental Microbiology. 47:551-559.

Van der Kooij D., Hijnen W. A. M. and Kruithf J. C. 1989. The effects of ozonation, biological filtration and distribution on the concentration on easily assimilable organic carbon (AOC) in drinking water. Ozone Science & Engineering 11:297-311.

Van der Kooij D. 1990. Assimilable organic carbon (AOC) in drinking water. In *Drinking water microbiology, progress and recent developments*. McFeters, G.A. (ed.). Springer-Verlag, Michigan USA. pp. 57-87.

Van der Kooij D. 1992. Assimilable organic carbon as an indicator of bacterial regrowth. Journal AWWA. 84(2):57-66.

Van der Kooij D., Veenendaal H. R., Baars-Lorist C., Van der Klift D. W. and Drost Y. C. 1995. Biofilm formation on surfaces of glass and teflon exposed to treated water. Water Research. 29(7):1655-1662.

Van der Kooij D., van Lieverloo H., Schellart J. and Hiemstra P. 1998. Distributing drinking water without disinfectant: highest achievement or height of folly? *In*: Proceedings of specialized conference on drinking water distribution with or without disinfectant residual. Muelheim an der Ruhr, Germany. 28-30.9.1998. Gerlach M and Gimbel R. (eds.). IWW Rheinisch-Westfälisches Institut fur Wasserforschung. Muelheim

Van der Wende E., Characklis W. G. and Smith D. B. 1989. Biofilms and bacterial drinking water quality. Water Research. 23(10):1313-1322.

Vartiainen T. and Liimatainen A. 1986. High levels of mutagenic activity in chlorinated drinking water in Finland. Mutation Research. 169:29-34.

Vartiainen T., Liimatainen A., Kauranen P. and Hiisvirta L. 1988. Relations between drinking water mutagenicity and water quality parameters. Chemosphere. 17(1):189-202.

Volk C., Bell K., Ibrahim E., Verges D., Amy G. and LeChevallier. 2000a. Impact of enhanced and optimized coagulation on removal of organic matter and its biodegradable fraction in drinking water. Water Research. 34(12):3247-3257.

Volk C., Dundore E., Schiermann J. and LeChevallier M. 2000b. Practical evaluation of iron corrosion in a drinking water distribution system. Water Research. 34(6):1967-1974.

Vähätalo A. and Salonen K. 1996. Enhanced bacterial metabolism after exposure of humic lake water to solar radiation. Nordic Humus Newsletter. 3(3):36-43.

Waara T., Jansson M. and Pettersson K. 1993. Phosphorus composition and release in sediment bacteria of the genus Pseudomonas during aerobic and anaerobic conditions. Hydrobiologia. 253:131-140.

Watnick P. and Kolter R. 2000. Minireview: Biofilm, city of microbes. Journal of Bacteriology. 182(10):2675-2679.

Webster, J. J., Hampton, G.J., Wilson, J. T., Ghiorse, W. C., and Leach, F. B. R. 1985. Determination of microbial cell numbers in subsurface samples. Ground Water 23(1):17-25.

White G. C. 1986. The handbook of chlorination. 2nd Edition. Van Nostrand Reinhold Company. New York, USA.

WHO. 1996. Guidelines of drinking water quality. Vol. 2. Health criteria and other supporting information. World Health Organization. Geneva.

Wilczak A., Jacangelo J. G., Marcinko J. P., Odell L. H., Kirmeyer G. J. and Wolfe R. L. 1996. Occurence of nitrification in chloraminated distribution systems. Journal AWWA. 88(7):74-85.

Wolfe R. L., Ward N. R. and Olson B. H. 1985. Inactivation of heterotrophic bacterial populations in finished drinking water by chlorine and chloramines. Water Research. 19(11):1393-1403.

Wolfe R. L. 1990. Ultraviolet disinfection of potable water. Environment Science and Technology. 24(6):768-773.

Zacheus O. M. and Martikainen P. J. 1995. Occurrence of heterotrophic bacteria and fungi in cold and hot water distribution systems using water of different quality. Canadian Journal of Microbiology. 41:1088-1094.

Zacheus O. M., Iivanainen E. K., Nissinen T. K., Lehtola M. J. and Martikainen P. J. 2000. Bacterial biofilm formation on polyvinyl chloride, polyethylene and stainless steel exposed to ozonated water. Water Research. 34(1):63-70.

Zacheus O. M., Lehtola M. J., Korhonen L. K. and Martikainen P. J. 2001. Soft deposits the key site for microbial growth in drinking water distribution networks. Water Research. 35(7):1757-1765.

Zhang W. and DiGiano F. A. 2002. Comparison of bacterial regrowth in distribution systems using free chlorine and chloramine: a statistical study of causative factors. Water Research. 36:1469-1482.

Zullei-Seibert N. 1996. Pesticides and artificial recharge of groundwater via slow sand filtration – elimination potential and limitations. Proc. of an international symposium of Artificial recharge of groundwater. Kivimäki A-L and Suokko T. (eds.). NHP Report No. 38. Helsinki, Finland. pp. 247-256.