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STRATEGIES FOR DISINFECTION OF FRESH PRODUCE WASH WATER

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

STRATEGIEËN VOOR DESINFECTIE VAN WASWATER VAN VERSE GROENTEN EN FRUIT

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VOORWOORD

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In het derde jaar van mijn opleiding (industrieel ingenieur) begon ik mij af te vragen wat te doen na mijn studies. Gaan werken in de industrie zag ik niet echt zitten en onderzoek stond me wel aan. Dus het plan was simpel: veel studeren om goede punten te behalen en zagen bij mijn toenmalige proffen (Katleen Raes en Imca Sampers) dat ik wou doctoreren. Blijkbaar werkte deze strategie en nadat een project van Katleen waarop ik zou werken er niet doorkwam, had Imca een onderzoeksplaats vrij op een project (Veg-i-Trade) en voilà. Dus in de eerste plaats wil ik Katleen en Imca bedanken voor hun goede wil en blijkbaar initieel vertrouwen in mij.

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AOP	advanced oxidation process
APC	aerobic psychrotrophic plate count
a_w	water activity
ClO_2	chlorine dioxide
COD	chemical oxygen demand
DBPs	disinfection by-products
DNA	deoxyribonucleic acid
DPD	N,N-diethyl-p-phenylenediamine
E. coli or EC	*Escherichia coli*
EU	European Union
FAO	Food and Agriculture Organization
FC	fecal coliforms
H_2O_2	hydrogen peroxide
HAAs	haloacetic acids
HACCP	Hazard Analysis and Critical Control Points
IARC	International Agency for Research on Cancer
IPW	industrial process water
L. monocytogenes	*Listeria monocytogenes*
LA	lactic acid
LAB	lactic acid bacteria
LP lamp	low-pressure lamp
MF	microfiltration
MP lamp	medium-pressure lamp
MWCO	molecular weight cutoff
NDMA	nitrosodimethylamine
NF	nanofiltration
O_3	ozone
ORP	oxidation reduction potential
PAA	peracetic acid
pKa	acid dissociation constant
RO	reverse osmosis
SPW	standardized process water
TC	total coliforms
THMs	trihalomethanes
TTHMs	total trihalomethanes
UF	ultrafiltration
US	ultrasound
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UV_{254}(F)	UV absorption at 254 nm (filtered)
UV-VIS	UV-Visible

WHO	World Health Organization
Y&M	yeasts and molds

SUMMARY/SAMENVATTING

SUMMARY

In a first part of **chapter 1** the microbial hazards of fresh produce and the link to water quality is described, followed by description of the possible water sources (and the associated microbial water quality) for use in pre- and postharvest practices. Because of the different inactivation mechanisms of different disinfection techniques and the differences in morphology and physiology of different microorganism types, microbial inactivation efficiency of disinfection techniques varies among different microorganism types. The physicochemical water quality (particles, dissolved (in)organic matter, pH, T) impacts the efficiency or normal operation of virtually all disinfection techniques. For chemical oxidants (focus of this PhD) the disinfection efficiency is mostly hindered by the decay of disinfectant due to reaction with water matrix components. As these reactions are disinfectant specific, both the disinfectant stability and qualitative/quantitative formation of DBPs is highly variable among chemical oxidants. In the literature study, both established disinfection technologies (chemical oxidants, ultraviolet irradiation (UV), ultrasound (US), membrane filtration) and alternative disinfection technologies (metals, sequential or combined use of chemical oxidants, advanced oxidation processes (AOPs)) are described.

Chapter 2 evaluated a sugar snaps washing process (washing for rehydration purposes) in a packaging company. A rapid build-up of aerobic psychrotrophic plate count (APC) (ca. 6.5 log CFU/100 mL), yeasts and molds (Y&M), and lactic acid bacteria (LAB) (both ca. 4.5 log CFU/100 mL) occurred in the wash water in the absence of water sanitizer, and a low build-up of COD (30 ± 5 mg O_2/L) and turbidity (5.2 ± 1.1 NTU). Lab-scale washing of sugar snaps in chlorine or organic acid formulations LA, NATRApHASE-ABAV®, and NATRApHASE-FVS®) was executed for extending the shelf-life. A significant higher reduction of APC on the sugar snaps compared to a water wash was achieved with LA in the range 0.8 to 1.6 %, ABAV 0.5 %, and free chlorine 200 mg/L. However, washing with disinfectant did not remove Y&M to a higher extent than a water wash. The use of water sanitizers could not extend the sensorial shelf-life. Microbial loads were not indicative/predictive for visual microbial spoilage (shelf-life limiting factor), whereas maturity and amount of damage at the calyx end of the pods were. The APC wash water contamination (5.2 log CFU/100 mL) was more efficiently reduced with free chlorine (to 1.4 log CFU/100 mL) than with organic acid formulations (to 3.4 – 2.7 log CFU/100 mL dependent on the concentration and formulation). Only the use of chlorine enabled a significant reduction of the Y&M wash water contamination (from 3.4 to 1.4 log CFU/100 mL). The low physicochemical build-up in the

sugar snaps wash water during the industrial washing process makes free chlorine attractive as water disinfectant to prevent bacterial and fungal cross-contamination, whereas the sanitizers based on organic acids are not, due to their weak water disinfection efficiency.

Chapter 3 assessed the use of PAA + LA and free chlorine for process water recycling and wash water disinfection of fresh-cut leafy vegetables wash water. The influence of COD, turbidity, filtered UV absorption at 254 nm (UV_{254}(F)), pH and contact time were assessed in oxidant demand free buffer or in standardized process water (SPW), i.e. artificial wash water consisting of a watery suspension of butterhead or iceberg lettuce. The inactivation of *E. coli* O157 in SPW by free chlorine was function of COD and chlorine concentration (pH and T kept constant), whereas the inactivation with PAA depended on UV_{254}(F), PAA concentration, contact time and pH (T kept constant). Models for process water recycling and wash water disinfection were constructed based on the inactivation trials in oxidant demand free and SPW conditions and validated with disinfection trials in fresh-cut leafy vegetables wash water from processing companies (industrial process water or IPW) and simulated lab-scale dynamic washing trials in SPW respectively. Maintaining a free chlorine residual of 1 mg/L during a 1h butterhead lettuce washing trial in SPW with COD 1000 mg O_2/L resulted in a significant production of total trihalomethanes (TTHMs) in the wash water (124.5 ± 13.4 µg/L) but not on the washed fresh-cut butterhead lettuce after rinsing with tap water.

In **chapter 4** a full-scale leafy vegetables (radicchio, sugar loaf, curled endive, lollo, lollo rosso) washing process was executed with continuous dosing of H_2O_2 in the washing bath to attempt to avoid the buildup of the APC and enterococci wash water contamination. The washing process consisted of 2 washing baths, with addition of H_2O_2 in the second washing bath. Despite addition of 300 L/h of 1.8 % H_2O_2 to a 450 L washing bath (333 ± 50 kg/h fresh-cut produce introduction speed), the H_2O_2 quickly decreased and a lower wash water contamination of APC and enterococci than without addition of H_2O_2 could not be maintained. The H_2O_2 treatment did not increase the APC removal from the fresh-cut leafy vegetables compared to those washed with water. In a second part, lab-scale experiments were performed to assess the impact of a commercial metal ion formulation (Bacsan®, containing a. o. Cu^{2+}, Zn^{2+}, Ag^{+}) on the stability of H_2O_2 in SPW (made from iceberg lettuce) and IPW. The H_2O_2 stability in SPW and IPW increased when adding Bacsan, according to a mechanism in which the inhibition of catalase by the metal ions is probably involved. H_2O_2/Bacsan synergistically increased the disinfection efficiency of APC and *E. coli* compared to H_2O_2 or Bacsan in a process water recycling setup. H_2O_2 is not suited as a wash

water disinfectant to avoid cross-contamination in fresh-cut leafy vegetables washing processes due to the slow water disinfection kinetics and the rapid H_2O_2 consumption.

In **chapter 5**, the removal of turbidity and COD from SPW by coagulation with biopolymers (gallotannins or chitosan) was assessed, with the goal of lowering the disinfectant demand and the DBPs formation, as such allowing longer reuse of the wash water. Chitosan was better at removing turbidity and COD than gallotannins. Coagulation of IPW with chitosan was more effective at lowered pH (pH 5). Although the turbidity removal was very high (> 90%), and as such also the removal of particulate COD, virtually no dissolved COD was removed. Coagulation lowered the disinfectant demand (of free chlorine and PAA) to some degree but did not decrease the TTHMs formation.

Chapter 6 consists of a selection tool for water disinfection methods for fresh produce pre- and postharvest practices. Selected disinfection technologies were characterized through literature study. From the collected information, general criteria were identified that influence the suitability of disinfection technologies for a target pre- or postharvest application. Criteria were divided into three principal components: i) criteria related to the technology and which relate to the disinfection efficiency, ii) attention points for the management and proper operation, and iii) necessities in order to sustain the operation with respect to the environment. A qualitative selection procedure was made based on knockout criteria (essential criteria) and additional criteria, in order to assess which technologies should be put to feasibility testing for water disinfection in a selected pre- and postharvest practice of the fresh produce chain.

In **conclusion**, process water recycling and water refreshing are strategies that do not prevent microbial cross-contamination during fresh produce washing. On the other hand, process wash water disinfection can greatly reduce the risk of microbial cross-contamination by guaranteeing a certain disinfectant residual (quantity dependent on disinfectant, target microorganism, and ultimately, the desired risk reduction) at all times, making each microorganism transferred from the fresh produce to the wash water an immediate and continuous target. When assessing cross-contamination prevention by wash water disinfectants, the impact of the used fresh produce contamination values on the cross-contamination event should be considered, and studies such as these should be linked to risk analysis studies. In a wash water disinfection process, the interconnected factors of importance are the physiochemical wash water quality, the disinfectant demand and residual, and the water refreshing rate. As such these factors should all be controlled/monitored when

studying the cost-effectiveness of fresh produce wash water disinfection. The validation in IPW is necessary for disinfection experiments to identify the influential parameters of which the influence was quantified in artificial water systems (SPW or oxidant demand free buffer). As the relation between the selection criteria ‘disinfection efficiency’ and ‘costs’ is not sufficiently characterized, no definite judgment can be made on technology selection and pilot or full scale tests are needed to determine which technology is most suited. Although the search for and assessment of new disinfection techniques is important, the author thinks that a clear understanding of the cost-effectiveness of the currently implementable technologies that show potential in these washing processes is of paramount importance for industrial application. Comparison of these studies should occur from a holistic approach, i.e. with regard to the interconnected factors physiochemical wash water quality, disinfectant demand and residual, and the water refreshing rate, and with comparison of all relevant technologies under similar conditions (i.e. the same full-scale postharvest washing process).

SAMENVATTING

In een eerste deel van **hoofdstuk 1** werden de microbiële gevaren van vers (versneden) groenten en fruit en de link met waterkwaliteit beschreven, gevolgd door de mogelijke waterbronnen (en de geassocieerde microbiële waterkwaliteit) voor gebruik in vers (versneden) groenten en fruit producerende toepassingen. Door de diversiteit in afdodingsmechanismen van verschillende desinfectietechnieken en de diverse morfologie en fysiologie van verschillende types micro-organismen is er aanzienlijke variatie tussen desinfectietechnieken inzake de afdodingsefficiëntie van types micro-organismen. De fysicochemische waterkwaliteit (deeltjes, opgeloste (an)organische materie, pH, T) heeft impact op de efficiëntie van nagenoeg alle desinfectietechnieken. Betreffende chemische oxidanten (de focus van dit PhD) wordt de efficiëntie hoofzakelijk negatief beïnvloed door de afbraak van desinfectans door reactie met componenten in de watermatrix. Aangezien deze reacties afhankelijk zijn van het desinfectans in kwestie, zijn zowel de stabiliteit van het desinfectans als de vorming van desinfectienevenproducten (DBPs) sterk afhankelijk van het betreffende chemische oxidans. In de literatuurstudie werden zowel gevestigde desinfectietechnieken (chemische oxidantia, ultraviolet straling, ultrasone drukgolven en membraanfiltratie) en alternatieve desinfectietechnieken (metalen, opeenvolgend of gecombineerd gebruik van chemische oxidantia, geavanceerde oxidatieprocessen) beschreven.

Hoofdstuk 2 evalueerde een sugar snaps wasproces (met rehydratatie als hoofddoel) in een verpakkingsbedrijf. Een snelle opbouw van het aeroob psychrotroof kiemgetal (APC) (ca. 6.5 log KVE/100 mL), gisten en schimmels (Y&M), en melkzuurbacteriën (LAB) (beide ca. 4.5 log KVE/100 mL) gebeurde in het waswater in afwezigheid van waswaterdesinfectans en een lage opbouw van chemische zuurstofvraag (COD) (30 ± 5 mg O_2/L) en turbiditeit (5.2 ± 1.1 NTU) werd waargenomen. Het wassen van sugar snaps op laboschaal in chloor of samenstellingen van organische zuren (melkzuur (LA), NATRApHASE-ABAV®, and NATRApHASE-FVS®) werd uitgevoerd ter verlenging van de houdbaarheid. Een significant hogere reductie (dan door wassen in water) van APC werd bekomen met LA in de range 0.8 tot 1.6 %, ABAV 0.5 %, en 200 mg/L vrij chloor. Y&M werden niet beter verwijderd van de sugar snaps met behulp van desinfectantia in vergelijking met wassen in water. Het gebruik van waswaterdesinfectans had geen invloed op de sensorische houdbaarheid. De microbiële belasting was geen indicatie voor visueel microbieel bederf terwijl dit wel gold voor de maturiteit van de sugar snaps en de schade aan het calyx uiteinde van de peulen. De APC waswatercontaminatie (5.2 log KVE/100 mL) werd beter gereduceerd met vrij chloor (tot 1.4

log KVE/100 mL) dan met (samenstellingen van) organische zuren (tot 3.4 – 2.7 log KVE/100 mL afhankelijk van concentratie en samenstelling). Y&M in het water werden enkel significant verwijderd met chloor (van 3.4 tot 1.4 log KVE/100 mL). De lage fysicochemische opbouw in het waswater tijdens het industrieel wassen van sugar snaps maakt het gebruik van vrij chloor als waterdesinfectans aantrekkelijk voor het voorkomen van bacteriële en Y&M kruiscontaminatie, terwijl het gebruik van (samenstellingen van) organische zuren wordt afgeraden door de zwakke efficiëntie als waterdesinfectans.

In **hoofdstuk 3** werd het gebruik van perazijnzuur (PAA) + LA en vrij chloor in *process water recycling* en *wash water disinfection* van waswater van vers versneden bladgroenten getest. De invloed van COD, turbiditeit, gefilterde UV absorptie bij 254 nm (UV_{254}(F)), pH en contacttijd werd nagegaan in buffer (vrij van oxidans-vraag) of in gestandaardiseerd proceswater (SPW), d.w.z. artificieel was water dat bestaat uit een waterige suspensie van krop- of ijsbergsla. De afdoding van *E. coli* O157 in SPW door vrij chloor was functie van COD en chloorconcentratie (pH en T werden constant gehouden), terwijl de afdoding met PAA afhing van de UV_{254}(F), PAA concentratie, contacttijd en pH (T werd constant gehouden). Modellen voor *process water recycling* en *wash water disinfection* werden gemaakt, gebaseerd op de afdodings-experimenten in buffer en SPW en gevalideerd met experimenten in, respectievelijk, industrieel proceswater (IPW) van een producent van vers versneden bladgroenten en een gesimuleerd wasproces op laboschaal in SPW. Het behouden van 1 mg/L residu aan vrij chloor gedurende een uur durend wasproces (versneden kropsla) in SPW met COD 1000 mg O_2/L resulteerde in significante productie van totale trihalomethanen (TTHMs) in het waswater (124.5 ± 13.4 µg/L) maar niet op de gewassen, versneden kropsla na spoelen met leidingwater.

In **hoofdstuk 4** werd een wasproces voor versneden bladgroenten (radicchio, suikerbrood, krulandijvie, lollo, lollo rosso) op industriële schaal uitgevoerd waarbij H_2O_2 continu gedoseerd werd in het waswater om te trachten de microbiële waswatercontaminatie (APK en enterococci) onder controle te houden. Het wasproces bestond uit 2 wasbaden, met toevoeging van H_2O_2 aan het 2de wasbad. Ondanks de toevoeging van 300 L/h 1.8 % H_2O_2 in een 450 L wasbad (333 ± 50 kg/uur bladgroenten wassnelheid) verminderde de concentratie aan H_2O_2 snel en een lagere microbiële waswatercontaminatie kon niet aangehouden worden. De H_2O_2 behandeling verhoogde de APK verwijdering van de bladgroenten niet ten opzichte van wassen met water. In een tweede deel werden labo-schaal experimenten uitgevoerd om de invloed van een commercieel metaalionenmengsel (Bacsan®, bevat onder meer Cu^{2+}, Zn^{2+},

Ag^+) op de stabiliteit van SPW (van ijsbergsla) en IPW na te gaan. De stabiliteit van H_2O_2 verhoogde in SPW/IPW door toevoeging van Bacsan en dit volgens een mechanisme waarin de inhibitie van katalase door metaalionen waarschijnlijk betrokken is. De afdoding van APC en *E. coli* door gecombineerd H_2O_2/Bacsan was synergetisch in een *process water recycling* opstelling. H_2O_2 is niet geschikt als waswaterdesinfectans om kruiscontaminatie te vermijden in wasprocessen van vers versneden bladgroenten door de trage waterdesinfectiekinetiek en de snelle H_2O_2 afbraak.

In **hoofdstuk 5** werd de verwijdering van de turbiditeit en COD door coagulatie met biopolymeren (gallotannines of chitosan) bestudeerd, met als doel de afbraak van desinfectans en de vorming van DBPs te verminderen, en aldus een langer hergebruik van het waswater te bekomen. Chitosan verwijderde turbiditeit en COD beter dan gallotannines. Coagulatie van IPW met chitosan was beter bij lagere pH (pH 5). Ondanks dat de verwijdering van turbiditeit zeer hoog was (> 90%) en dusdanig het deel van de COD dat vaste stof was, werd nagenoeg geen opgeloste COD verwijderd. Coagulatie verlaagde de afbraak van desinfectans (vrij chloor en PAA) enigszins, maar verminderde de vorming van TTHMs niet.

Hoofdstuk 6 bestaat uit een selectie tool voor waterdesinfectiemethoden om te gebruiken in vers (versneden) groenten en fruit producerende toepassingen. Geselecteerde desinfectietechnieken werden gekarakteriseerd door middel van literatuurstudie. Vanuit de verzamelde informatie werden algemene criteria geïdentificeerd die van invloed zijn op de toepasbaarheid van desinfectietechnieken voor een bepaalde vers (versneden) groenten en fruit producerende toepassing. De criteria werden onderverdeeld in drie hoofdcomponente: i) criteria gerelateerd aan de technologie en van invloed op de afdodings-efficiëntie, ii) aandachtspunten voor het management en een goed functioneren van de desinfectietechniek en iii) benodigdheden voor het blijven functioneren van de desinfectietechniek en dit op een duurzame wijze. Een kwalitatieve selectieprocedure werd opgesteld, gebaseerd op knockout criteria (lees essentiële criteria) en bijkomende criteria, met als doel om na te gaan welke technieken getest moeten worden inzake hun toepasbaarheid voor waterdesinfectie in een bepaalde processtap in de productie van vers (versneden) groenten en fruit.

Om te **besluiten**, *process water recycling* en waterverversing zijn strategieën die microbiële kruiscontaminatie niet beletten tijdens het wassen van vers (versneden) groenten en fruit. Daarentegen, *process wash water disinfection* kan het risico van kruiscontaminatie sterk verminderen door te allen tijde de aanwezigheid van een residu aan desinfectans (kwantiteit

afhankelijk van desinfectans, micro-organisme, beoogde risico-reductie) te garanderen, zodat elk micro-organisme dat in het waswater terecht komt een ogenblikkelijk en continu doelwit is. Wanneer de preventie van kruiscontaminatie door middel van desinfectantia wordt nagegaan, dan zou de impact van de microbiële belasting van het beginproduct in rekening gebracht moeten worden, en dergelijke studies moeten gelinkt worden aan risicoanalyses. Tijdens *process wash water disinfection* zijn de onderling verbonden factoren de fysicochemische waterkwaliteit, de consumptie van desinfectans en residu, en de snelheid van waterverversing. Daarom dienen deze factoren gecontrolleerd/gemeten te worden wanneer de kosteffectiviteit van waswaterdesinfectie bestudeerd wordt. De validatie in IPW is noodzakelijk voor desinfectie-experimenten om de beïnvloedende parameters te identificeren van de welke de invloed gekwantificeerd werd in artificieel water (SPW of buffer). Aangezien het verband tussen de selectiecriteria ‘desinfectie-efficiëntie’ en ‘kosten’ niet voldoende gekarakteriseerd is, kan geen definitief besluit genomen worden inzake selectie van desinfectietechniek en testen op pilootschaal of industriële schaal zijn nodig om te bepalen welke de beste beschikbare techniek is. Alhoewel het onderzoek naar nieuwe desinfectietechnieken van belang is, denkt de auteur dat een goed begrip van de kosteffectiviteit van de huidig implementeerbare technieken in deze wasprocessen van het grootste belang is voor de industrie. Dergelijke vergelijkbare studies moeten uitgevoerd volgens een holistische aanpak, d.w.z. met betrekking van de fysicochemische waterkwaliteit, het verbruik aan desinfectans en het residu, en de snelheid van waterverversing. Vergelijking van de relevante desinfectietechnieken moet dan ook uitgevoerd worden in vergelijkbare condities (een industrieel wasproces met dezelfde setup).

INTRODUCTION

INTRODUCTION

Fresh fruit and vegetables are recognized to be an important part of a healthy diet, as they provide necessary nutrients such as vitamins, minerals, fibers and antioxidants (De Giusti et al., 2010). Because the population becomes increasingly aware of this fact, the consumption of these food products has augmented. As convenience is a desired quality parameter these days, increased consumption is especially the case for pre-packed, ready-to-eat fresh-cut produce (Heaton & Jones, 2008). Minimal processing of produce consists of a series of possible steps including harvesting, cold storage, trimming, shredding, washing/rinsing, draining, packaging, cold storage and distribution (Tirpanalan et al., 2011). The washing is done to remove dirt, foreign materials, tissue fluids from cut surfaces, and microorganisms. As fresh-cut produce is not subject to any inactivation technology during processing, washing is the only processing step that reduces the microbial load on the lettuce (Artes et al., 2009). Fresh produce is relatively vulnerable to microbial pathogen contamination (Luo et al., 2011) and leafy greens are amongst the most frequently implicated fresh produce in produce associated outbreaks (Nou & Luo, 2010; Luo et al., 2011; Olaimat & Holley, 2012). Also changing consumer habits are of influence on the manifestation of fresh produce associated outbreaks. The demand for a wide choice of exotic fruit and vegetables, supplied year-round and made possible by increased global trade, has increased the number of potential commodities implicated in foodborne disease. In addition, consumers tend to buy fresh produce more often at large supermarkets instead of local shops, which makes it possible for a batch of pathogen infected produce to reach a larger number of consumers (Johannessen et al., 2002; Heaton & Jones, 2008).

Washing with potable water removes microorganisms to some degree, a process which can be enhanced by using sanitizers for disinfection of produce, i.e. decontamination (Beuchat, 1998). The success of these washing processes to remove naturally present microorganisms from fresh-cut produce is limited (1-2 log reduction), i.e. microbial reductions occur but total removal cannot be achieved. This is because part of the microorganisms are quite firmly attached to the surface, sometimes in hard to reach crevices or irregular surface structures. In addition they might form biofilms, or become internalized within the plant tissues through stomata, cut surfaces or other tissue wounds, or in the preharvest stage via the root system, although the significance of the latter is far from confirmed (Sapers et al., 2001; Keskinen et al., 2009; Lopez-Galvez et al., 2010a; Holvoet et al., 2012; 2013).

Washing produce is also a potential pathway for spreading contamination among crops (Holvoet et al., 2012) and the risk of cross-contamination is not removed by using large quantities of water (Lopez-Galvez et al., 2009). Chemical oxidants (a. o. chlorine, chlorine dioxide (ClO_2), ozone (O_3), hydrogen peroxide (H_2O_2), peracetic acid (PAA)) are much more effective for inactivation of bacterial pathogens in wash water than for removal of these pathogens from fresh produce (Sapers, 2001; Gil et al., 2009). In addition, once cross-contamination has occurred, rewashing the newly infected lettuce in chemical oxidant solutions proves unable to completely remove the newly attached *E. coli* O157, even shortly after the contamination event (Lopez-Galvez et al., 2009, 2010a; Luo et al., 2011). The efficiency of wash water disinfection is not limited by the issues that plague decontamination, but the effectiveness of chemical oxidants is rather hindered by the presence of organic matter in the wash water.

As avoiding cross-contamination during the washing process is a viable option to contribute to increased safety and quality of fresh produce, the main goal of this PhD study was to understand the factors that influence the efficiency of water disinfection in fresh-cut produce washing processes, by studying the impact of physicochemical parameters (i.e. chemical oxygen demand (COD), turbidity, pH etc.), the location of water disinfection (in or outside of the washing bath) and the influence of water refreshing. In order to achieve this main goal, objectives were defined. The research outline is presented in Figure 0.1.

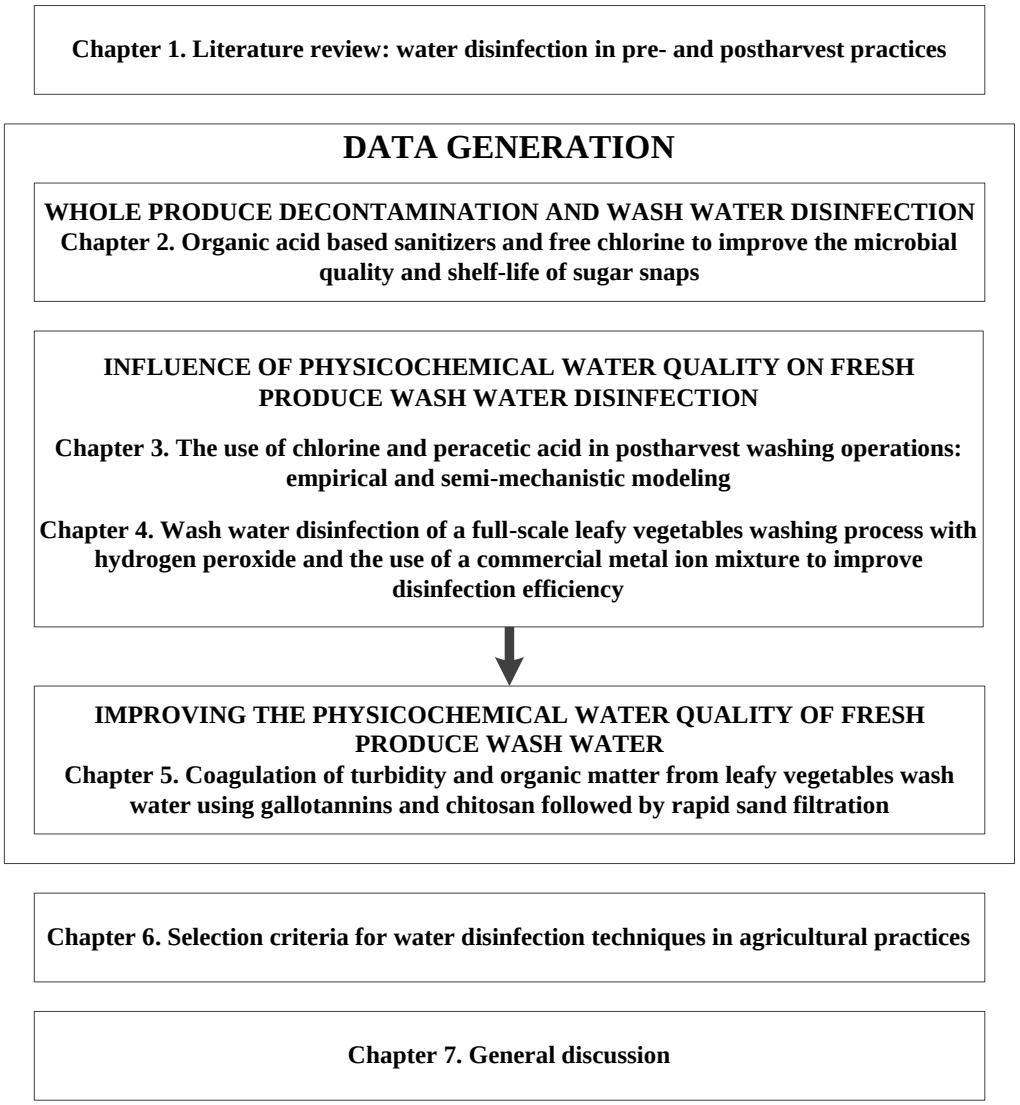

Figure 0.1. Overview of the research topics in this PhD study

Chapter 1 reviews the scientific literature concerning the microbial hazards of fresh produce that are linked to water and the properties of water disinfectants related to the reaction with water matrix constituents, antimicrobial mechanisms and disinfection efficiency, and the production of disinfection by-products (DBPs).

In **chapters 2 – 4**, the impact of the physicochemical quality of fresh produce wash water on water disinfection is assessed. As washing fresh-cut produce leads to transfer of exudates to the wash water, both washing of whole produce (lower expected physicochemical load) and fresh-cut produce (higher expected physicochemical load) were studied. In **chapter 2**, a whole (i.e. uncut) produce process, namely a sugar snaps washing process, and the use of organic acids and free chlorine to disinfect the wash water and prolong the shelf life of the sugar snaps are studied. In **chapter 3**, free chlorine and PAA+ lactic acid (LA) are compared

as wash water disinfectants to inactivate *Escherichia coli* (*E. coli*) O157 in fresh-cut leafy vegetables wash water. The impact of the physicochemical wash water quality, concentration and contact time on disinfection efficiency are quantified and implemented in prediction models. **Chapter 4** concerns the use of H_2O_2 to control the wash water contamination in a full-scale leafy vegetables washing process and explores the potential synergetic effect of combining H_2O_2 with selected metal ions to improve the microbial inactivation of *E. coli*.

Because the physicochemical load of the water can have an impact on the disinfectant stability and therefore the disinfection efficiency, **Chapter 5** explores the use of the biopolymers chitosan and gallotannins as coagulants to remove turbidity and organic matter from fresh-cut leafy vegetables wash water and assesses the impact on disinfectant decay and DBPs production.

Chapter 6 describes a selection tool for water disinfection methods for fresh produce pre- and postharvest practices. A variety of water disinfection technologies is available on the market and no single technology is the best choice for all applications. It can be difficult for end users to choose the technology that is best fit for a specific application. Therefore, the different technologies are characterized in order to identify criteria that influence the suitability of a technology for pre- or postharvest applications.

In the **general discussion**, some key observations regarding water disinfection strategies (disinfection in the washing bath, disinfection before reuse), cross-contamination via water, and the influence of organic matter and water refreshing on disinfection efficiency are discussed. Furthermore, the usability of the selection tool for preliminary decision making, regarding the choice of disinfection treatments is illustrated, and finally some future perspectives are explained.

CHAPTER 1

LITERATURE REVIEW: WATER DISINFECTION IN PRE- AND POSTHARVEST PRACTICES

1. LITERATURE REVIEW: WATER DISINFECTION IN PRE- AND POSTHARVEST PRACTICES

1.1. HAZARDS OF FRESH PRODUCE AND LINKS TO WATER

Fresh fruit and vegetables (fresh produce) are recognized to be an important part of a healthy diet, as they provide necessary nutrients such as vitamins, minerals, fibers and antioxidants (De Giusti et al., 2010). Because the population becomes increasingly aware of this fact, the consumption of these food products has augmented. As convenience is a desired quality parameter these days, increased consumption is especially the case for pre-packed, ready-to-eat fresh-cut produce (Heaton & Jones, 2008). Due to the increased fresh produce consumption, the risk of produce associated pathogens has become more relevant. Also changing consumer habits are of influence on the manifestation of fresh produce associated outbreaks. The demand for a wide choice of exotic fruit and vegetables, supplied year-round and made possible by increased global trade, has increased the number of potential vectors of food disease (Johannessen et al., 2002; Heaton & Jones, 2008). Fresh-cut produce is minimally processed, as it mostly involves only trimming, cutting and washing, and thus contamination is not easily removed during processing. Therefore, the probability that this produce, once contaminated, reaches the consumer in contaminated state is relatively large.

There are a multitude of pathogens that have been associated with fresh produce including bacteria (*Salmonella* spp., *Listeria monocytogenes* (*L. monocytogenes*), *E. coli* O157 and non O157 VTEC, *Shigella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Clostridium* spp., *Bacillus cereus*., *Staphylococcus aureus*.), protozoa (*Cyclospora cayetanensis, Cryptosporidum* spp., *Giardia* spp.), viruses (Hepatitis A, norovirus) and helminths (Suslow et al., 2003; Steele & Odumeru, 2004; Chaidez et al., 2005; Doyle & Erickson, 2008; Pielaat et al., 2008; Mota et al., 2009; Barton Behravesh et al., 2011; Danyluk & Schaffner, 2011; Keskinen & Annous, 2011**;** MacDonald et al., 2011; Verhoeff-Bakkenes et al., 2011**)**. In the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Meeting on Risk Assessment of Microbiological Hazards in Foods in 2008 concerning microbial hazards in fresh fruits and vegetables, the majority of the participating countries identified leafy greens as primary food group of concern, and either *Salmonella* spp., *E. coli* O157:H7 or norovirus as the pathogens of greatest concern, due to their major occurrence (Doyle & Erickson, 2008; FAO/WHO, 2008; Franz & van Bruggen, 2008; Heaton & Jones, 2008). The recent outbreak caused by verotoxin producing *E. coli* O104 associated with fenugreek

sprouts in Europe illustrated the health, political and economic impact food outbreaks can cause, especially due to the geographically widespread nature of these incidents in a consistently increasing economic globalization (Buchholz et al., 2011; Mothershaw et al., 2013).

Water is an important raw material in the fresh produce chain, as it is used in considerable amounts in many operations, including irrigation and application of pesticides and fertilizers in primary production, but also for rinsing, cooling, washing and as transport medium in postharvest practices (FDA, 1998) (Figure 1.1).

stage	water use	practices involving water disinfection
preharvest	-irrigation -applying fertilizers -applying pesticides -hydroponics -cleaning & disinfection	-control of microbial load in irrigation water (pond and well treatment, river or canal water treatment, irrigation channel networks) -disinfection of solutions containing fertilizers, pesticides -prevention of biofilm in piping and biofilm cloggin of drippers -disinfection of wastewater reuse in agriculture -disinfection of (recycling) hydroponic solutions
@harvest	-at harvest rinse or prewash before loading -cleaning & disinfection	-control of microbial load in prewash water
postharvest	-transporting -cooling -washing -rehydration -cleaning & disinfection	-control of microbial load in transport, cooling, rehydration and wash water -decontamination of produce -disinfecting process wastewater for reuse

Figure 1.1. The use of water in fresh produce production and processing

Pathogen contamination on fresh produce can be due to a. o. contaminated seeds and seedlings, manure and soil, via animals, and water (Holvoet, 2014). The used water needs to be of sufficient **initial** microbial quality. Water can serve as source of or as a vector for transport of pathogens to plants and crops (Steele & Odumeru, 2004; Chaidez et al., 2005; Mota et al., 2009; Barton Behravesh et al., 2011; Holvoet et al., 2012).

Several outbreaks have been traced back to the use of contaminated irrigation water. Irrigation water was believed to be involved in a European outbreak in 2006 of *Salmonella* Thompson caused by consumption of Italian rucola lettuce (Nygard et al., 2008). In a multistate outbreak of *Salmonella* Newport in the United States of America (USA) in 2005, associated with eating tomatoes, the strain responsible for the outbreak was isolated from pond water used to irrigate tomato fields (Greene et al., 2008). A nationwide outbreak of *Salmonella* enteric serotype Saint Paul in the USA in 2008 was traced back to agricultural water and serrano peppers on a Mexican farm, as well as jalapeño peppers (Barton Behravesh et al., 2011). A large outbreak of *E. coli* O157 occurred in Sweden in 2005. The most likely source of the outbreak was lettuce that was irrigated by water from a small stream, which contained the strain involved with the outbreak (Söderström et al., 2008). In 2006, a multistate *E. coli* O157 outbreak occurred in the USA that was associated with the consumption of bagged spinach. The outbreak strain was isolated from one of the fields that produced the implicated spinach and in addition from river water, cattle feces, and wild pig feces on a ranch under one mile from the spinach field. A potential cause for the *E. coli* O157:H7 contamination of the spinach was that the river water functioned as vector between the contaminated feces and the irrigation wells used for irrigating the spinach field (CFERT, 2007). Outbreaks where norovirus was involved are numerous, and vehicles of norovirus include berries, fresh-cut fruits, lettuce, tomatoes, green onions and other vegetables. One of the major routes of potential contamination of norovirus is the use of contaminated water for irrigation (Butot et al., 2009; De Giusti et al., 2010; Predmore & Li, 2011).

In recirculating hydroponic water systems, at harvest rinsing/washing, and postharvest water operations, the microbial water quality needs to be **maintained** in order to avoid cross-contamination via the water. At harvest, prewashing the produce before transport can minimize field soil on the produce, bins and pallets and remove plant exudates from harvest cuts or wounds. As such, cross-contamination during transport can be reduced, and the disinfectant demand during postharvest washing lowered (Suslow, 2000a). When cross-contamination via wash water occurs, subsequent decontamination of the produce is less efficient than the inactivation of suspended microorganisms in wash water (Suslow, 2004a; Lopez-Galvez et al., 2010a; Luo et al., 2011). Therefore, the foremost strategy of containing contamination in postharvest practices should be maintaining a good microbial quality of the process water. Effective use of water disinfection also allows higher recycling ratios of the

processing water, which will become increasingly important as water prizes continue to rise (Casani et al., 2005; Olmez & Kretzschmar, 2009).

1.2. WATER SOURCES AND MICROBIAL WATER QUALITY USED IN FRESH PRODUCE PRODUCTION AND PROCESSING

Approximately 70 % of the global fresh water use is for irrigation practices (Pedrero et al., 2010). In developing countries, this increases to 95% of the available fresh water (Malato et al., 2009; Pedrero et al., 2010). Water used for irrigation may originate from multiple sources: rain water, ground water, surface water, wastewater and in (semi) arid areas desalinated seawater or brackish groundwater are potential water sources for irrigation practices. The concept of water being a never-ending resource with a limitless renewable capacity belongs to the past (Beekman; 1998). To adapt to the increasing water shortages due to ground water depletion, applying alternative water sources for irrigation will gain importance in the future. Application of alternative water sources causes higher probability towards presence of pathogens and increases the pressure on governing water quality. Reconditioned wastewater and surface water are two abundant sources with potential to replace ground water as supplementary irrigation source for rainfall in periods of drought.

Rain and ground water are generally of good (yet less than potable) quality. The quality of rainwater depends in part on the microbial quality of the recipient e.g. in the case of roof-harvested rainwater it can be contaminated by pathogenic bacteria and protozoan parasites through the presence of bird and animal droppings on the roofs, especially right after relatively long periods of drought. Ground water (or bore hole water) is generally of good microbial quality although presence of pathogens is possible due to septic discharges, leaking sewer lines or infiltration of surface water (Burch & Thomas, 1998; Ahmed et al., 2011). For example, analysis of groundwater from 448 samples in 35 states of the USA resulted in 4,8 % positive samples for human enteric viruses (Abbaszadegan et al., 2003). Another study screened 50 household groundwater wells in Wisconsin, of which four were positive for human enteric viruses, three of them containing Hepatitis A virus (Borchardt et al., 2003). Although ground water usually contains less organics than surface water, it may contain higher amounts of inorganic loads causing unpleasant colors and odors (e.g. hydrogen sulfide) and which also creates a certain oxidant demand towards disinfection (Burch & Thomas, 1998; Suslow, 2004b).

Surface water is of lesser and more variable microbial and physicochemical quality as it is an open system, subjected to discharges of (treated) wastewater, storm water runoff, fecal contamination from animals etc. e.g. the Missouri river may have fecal coliforms/100 ml that vary 4 log over a 100 mile stretch. Also for turbidity, great variations exist (Anderson & Davidson, 1997; Burch & Thomas, 1998; Steele & Odumeru, 2004; Gil et al., 2013). A WHO world survey indicated that most European rivers contain mean fecal coliform counts of 3-4 log/100 ml. Yet none of these countries has restricted the use of such water for irrigation practices (Mara & Horan, 2003). Lakes tend to have better water quality than rivers, although the lakes are also subjected to surrounding sources of contamination like river inflow. Guidelines for microbial water quality of surface water for irrigation are usually less stringent than those of wastewater effluent used for unrestricted irrigation, due to the assumption that enteric viruses and other human pathogens are present in lesser amounts (Steele & Odumeru, 2004) or in the context of microbial criteria, fecal coliforms in surface water may originate from other sources than sewage or waste effluents, certainly in hot climate regions (Carr et al., 2004).

As other surface waters, **seawater** is subjected to industrial and municipal waste discharges and river or stream runoff, possibly containing a wide range of human enteric pathogens (Dionisio et al., 2002). There are some crops such as wheat and barley with high salt tolerance, a property that can be enhanced by selecting and breeding and which have potential for irrigation with diluted seawater (Ghadiri et al., 2006). However, in virtually all cases, seawater needs to be properly desalinated by thermal processes or reverse osmosis (RO) before use in agriculture (Guler et al., 2010), which also results in a very high removal of microorganisms. Desalinated seawater is increasingly considered as a water source for irrigation practices. Although the costs of RO membranes are high, the use of desalinated seawater might be economically feasible for high-value crops like greenhouse vegetables and flowers (Yermiyahu et al., 2007). Brackish groundwater (i.e. groundwater containing salt, yet in lesser amounts than seawater) can also be applied for irrigation when desalinated. Applying brackish groundwater for desalination is less energy consuming than applying seawater. However, the fact that groundwater is a limited resource, as opposed to seawater, should be taken into consideration (Munoz et al., 2008).

Wastewater is of very poor physicochemical and microbial quality and requires intensive treatment (WHO, 1989; Steele & Odumeru, 2004; Pedrero et al., 2010). Primary and secondary water treatment processes can eliminate 1 to 3 log of enteric microorganisms with

an additional 2 log by tertiary treatments, such as filtration techniques. Disinfection techniques should be applied if further elimination is required for reuse in irrigation practices (Liberti et al., 1999, 2000a, 2000b; Dell'Erba et al., 2004; Koivunen & Heinonen-Tanski, 2005a, 2005b; Ksibi, 2006; Falsanisi et al., 2006).

Indications on appropriate microbial quality for irrigation water can be obtained by consulting legal/regulatory requirements or in guides of good agricultural practices (Table 1.1). Overall, the requirements for microbial water quality increase as the product progresses from field to final processing (Suslow, 2004b). Requirements enabling the use of water for unrestricted irrigation (i.e. for crops that are to be eaten uncooked) are usually more stringent than those for restricted irrigation (i.e. crops that are not to be eaten uncooked) (Steele & Odumeru, 2004). Most guidelines and regulations are heavily based on defined microbial standards, but ad hoc risk assessment strategies for managing health risks may also be effective (WHO, 1989; Blumenthal & Peasey, 2002; Carr et al., 2004) e.g. the latest guidelines by the WHO (anno 2006) for use of wastewater in agriculture have been revised substantially. The fecal coliform guideline has been replaced by guidelines based on attributable risks and disability-adjusted life years, and governments in developing countries have been given greater flexibility in applying guidelines (WHO, 2006).

Another issue concerning microbial water quality is the change in microbial water quality during its delivery to crops. i.e. when flowing through pipes and in residual standing water in pipes. Stagnant water conditions form a better environment for biofilm formation (Soini et al., 2002). Biofilms **(**section 1.4.3**)** are known to form and survive in irrigation water distribution systems. The material the pipes consist of could influence the persistence of biofilms. Aluminum, steel pipe and cast iron showed to be a better substrate for biofilm manifestation compared to plastic pipes (Kerr et al., 1998; Yu et al., 2010; Shelton et al., 2013). On that note, the increase in surface roughness due to corrosion of aging metal pipes could form a more hospitable environment for biofilm formation (Lehtola et al., 2004). The physicochemical quality of the water to be transported is also of influence. Regrowth of *Legionella* and *Aeromonas* in treated effluent was attributed to the relatively high concentrations of assimilable organic carbon in these waters (Jjemba et al., 2010).

Besides the microbial concerns, there are other water quality parameters which are of great importance for crop yield and proper operation of irrigation practices. High salt contents may decrease the water uptake by the plant and reduce the rate at which water moves into the soil.

Some specific ions such as boron are phytotoxic above certain concentrations, the tolerance level varying widely among crops. Also, possible formation of calcium scale, presence of particles or algae, originating from the water, can cause clogging of sprinkler orifices. The nutrient content of irrigation water may be advantageous or disadvantageous (over-fertilization) on crop yield (Ayers & Westcot, 1989; Grattan, 2002; ILSI, 2008; Pedrero et al., 2010). Another concern is the presence of organic micropollutants in irrigation water, including pharmaceuticals, personal care products, hormones, surfactants etc., that are continuously released into the environment. These contaminants may be present at very low concentations (pg/L to ng/L) and conventional wastewater treatment plants are not designed to remove these contaminants (Schwarzenbach et al., 2006; Calderon-Reciado et al., 2011). Uptake in agricultural crops of organic contaminants present in irrigation water, including pharmaceuticals, has been reported (Calderon-Preciado et al., 2011).

Washing of fresh produce occurs mainly by submerging the produce or fresh-cut produce in a sequence of washing baths. The water is reused and refreshed during the washing operations, causing microbial build-up in the water. Water turbulence, to induce a scrubbing effect, is created by mechanical means or influx of pressurized air (Pao et al., 2012). At the end of the washing process, the produce is often rinsed with water of drinking water quality, to allow a final rinse with water that has not come into contact with produce on beforehand. **Water used in produce processing** traditionally needed to be of potable quality (Council Directive 98/83/EC; USEPA, 2009), although more recently some flexibility has been provided by allowing the use of water of lesser quality if this use does not compromise food safety of the end product (FDA, 1998; Regulation (EC) 852/2004; 21CFR110, 2010).This can be understood as “suitability for intended use”, meaning to adjust the water quality to the particular application. However, when determining suitability the judgment needs to be based on risk assessment for chemical and microbiological hazards, and the water use integrated in the Hazard Analysis and Critical Control Points (HACCP) principles (ILSI, 2008). Sources of potable water quality used in produce processing are mostly tap water obtained from municipalities and (treated) ground water (Nicolaisen, 2003; Xu et al., 2010). It is a normal practice to reuse water by recuperating, possibly treating (reconditioning) and recycling the washing and cooling water in processing operations and flume water in packing houses (Codex Alimentarius, 1999; Casani & Knochel, 2002; Lopez-Galvez et al., 2010b; Jacxsens, 2011).

Table 1.1. Overview of some water quality guidelines and regulations for irrigation water

Country/region	Water type	Regulation/ Guideline	Criteria [a, b]	Reference
Australia & New Zealand	Wastewater	Guideline	< 1 EC[c], < 100 EC[d]	Australian Natural Resource Management Ministerial council, 2000
Canada	All	Guideline	< 1000 TC/< 100 FC	Steele & Odumeru, 2004
Canada (Alberta)	Surface water	Guideline	< 1000 TC/< 100 FC	Steele & Odumeru, 2004
Canada (British Colombia)	All	Guideline	< 200 FC/< 77 EC/< 20 fecal streptococci	Steele & Odumeru, 2004
Canada (Saskatchewan)	Surface water	Guideline	< 1000 TC/< 100 FC	Steele & Odumeru, 2004
Italy	Wastewater	Regulation	< 10 EC/Salmonellae absent	Angelakis et al., 2007
Spain [c]	Wastewater	Regulation	< 100 EC/< 1 nematode egg/ 10 l	Iglesias et al., 2010
USA (USEPA, 1973)	Surface water	Guideline	< 1000 TC	Gerba & Choi, 2006
USA (USEPA, 1992)	Wastewater	Guideline	FC absent	Shuval et al., 1997
USA (FDA)[c]	All	Regulation (proposal)	<235 EC (single sample); <126 EC (5 samples rolling geometric mean)	FDA, 2013
WHO	Wastewater	Guideline	< 1000 FC/< 1 nematode egg/l	WHO, 1989
California (USA)		Regulation	< 2,2 TC/FC absent	Steele & Odumeru, 2004
WHO (proposed alteration)		Guideline	< 1000 FC/< 0,1 nematode egg/l	Blumenthal & Peasey, 2002

[a] All values per 100 ml, unless otherwise stated, TC = total coliforms, FC = fecal coliforms, EC = *E. coli*. [b] Specifics of sample value calculation, such as geometric mean, minimal number of samples, period of sampling, percentage of samples that may deviated from the target value etc. are not mentioned here. [c] Commercial food crops consumed raw or unprocessed. [d] Commercial food crops.

In the context of water treatment in food operations, specific concepts have been defined or need defining. Relevant definitions have been defined by the Codex Alimentarius Commission (Codex Alimentarius, 1999) (Table 1.2). Water disinfection in fresh-cut industry is carried out in washing tanks (immersion washers), where fresh-cut vegetables are washed, and agitation applied by water, air, sound or mechanical devices (Pao et al., 2012). Alternatively, non-immersion washers that wash produce by spraying or rinsing can be applied but the latter is not the focus of this PhD thesis. Water disinfection can also be used to recycle process water. The process water recycling or reconditioning usually takes place outside the processing line. Basically, disinfection processes in this context can be divided as: i) process wash water disinfection in the washing tank and ii) process water recycling outside the washing tank (Figure 1.2).

Figure 1.2. Two types of water disinfection: process water recycling or reconditioning and process wash water disinfection in the washing tank

Reconditioning is, by definition, any water treatment process which improves the microbial, chemical or physical quality of used water in order to allow reuse. In the context of disinfection, **process water recycling or reconditioning** is defined as inactivation of microorganisms in process water outside the processing line before reuse in the washing process. **Process wash water disinfection** concerns the inactivation of incoming microorganisms by keeping a disinfectant residual in the washing tank.

Table 1.2. Definition of water treatment/usage terms in food manufacturing operations by Codex Alimentarius Commission (1999)

Definition	
Reuse	The recovery of water from a processing step, including from the food component itself; its reconditioning treatment, if applicable; and its subsequent use in a food manufacturing operation.
Reconditioning	The treatment of water intended for reuse by means designed to reduce or eliminate microbiological, chemical, and physical contaminants, according to its intended use.
Recycled water	Water, other than first use or reclaimed water, that has been obtained from a food manufacturing operation and has been reconditioned when necessary such that it may be reused in a subsequent manufacturing operation
Reclaimed water	Water that was originally a constituent of a food, has been removed from the food by a process step, and has been subsequently reconditioned when necessary such that it may be reused in a subsequent manufacturing operation
Reused water	Recycled and reclaimed water
Food manufacturing operation	Any operation intended to clean, sort, process, or package a food product or its ingredients including the cleaning of equipment and facilities

1.3. PHYSICAL AND CHEMICAL PROPERTIES OF WATER DISINFECTION TECHNOLOGIES

1.3.1. Chlorine

Chlorine is a green-yellow gas at room temperature, with a melting point of -102 °C, a boiling point of -35 °C and an oxidation reduction potential (ORP) of 1.36 V. Chlorine is available as solid ($Ca(OCl)_2$), aqueous solution (NaOCl) or chlorine gas (Cl_2) and is mainly produced in electrolytic cells. The degradation of a NaOCl solution follows second order kinetics, meaning more active component is lost in function of time when the initial solution is more concentrated (White, 2010). To illustrate the stability: at 20°C a NaOCl solution will degrade from 12.5 % to 10% NaOCl in 100 days. $Ca(OCl)_2$ loses about 3 to 5 % of its free chlorine content per year. Cl_2 gas is much more stable than hypochlorite (OCl^-) formulations during

storage. When added to water, Cl_2 forms hypochlorous acid (HOCl), which deprotonates to OCl^-, depending on the pH (reactions 1 & 2). Chlorine gas becomes noticeable by humans at 0.014 to 0.054 ppmv in air and is rapidly lethal for most animals at 1000 ppmv in air. As chlorine gas is hazardous and corrosive, it is important to assure complete dissolution in the water. The dissolution of chlorine gas in water increases with decreasing T and ionic strength (White, 2010).

$$Cl_2 + H_2O \rightarrow HCl + HOCl \quad (1.1)$$

$$HOCl \rightarrow OCl^- + H^+ \quad \text{(acid dissociation constant (pKa)} \approx 7.5\text{; White, 2010)} \quad (1.2)$$

NaOCl and $Ca(OCl)_2$ also form HOCl upon contact with water, but in this process alkaline compounds are formed. To take advantage of the stronger HOCl acid form and yet to minimize corrosion of the equipment, a pH value between 6 and 7 is desired (Parish et al., 2003; Wang et al., 2006a; Tirpanalan et al., 2011). Free chlorine refers to all molecular species where the chlorine atom is in the 0 or +1 oxidation state that are not combined with ammonia or organic nitrogen. In theory, this implies Cl_2, HOCl, OCl^-, and Cl_3^-. However, in water, the residual free chlorine consists almost entirely of HOCl and OCl^-, and as such free chlorine is often defined as the sum of these two compounds (White, 2010).

Chlorine reacts through oxidation, addition and electrophilic substitution reactions with organic substances in the water. Usually electrophilic substitution (i.e. chlorination) is the predominant mechanism. Reactions with double bonds, alcohols and ketones are rather slow, whereas reaction with ammonia, aliphatic amines, amino acids and peptides (N atom of terminal amino-function is the target) occurs fast. Reaction rates with compounds containing reduced sulfur moieties are especially fast and this includes the amino acids cysteine and methionine, proteins containing these amino acids, and the reducing compound gluthatione (Donnermair & Blatchley, 2003; Deborde & von Gunten, 2008). Chlorine can also react with several inorganic species present in water, such as reduced iron, arsenic and manganese, halides, and sulfide, etc. (Deborde & von Gunten, 2008). Inorganic chloramines and organic chloramines can be formed, due to reaction with ammonia and organic nitrogen compounds (particularly organic amino compounds) respectively (Donnermair & Blatchley, 2003).

1.3.2. Chlorine dioxide

ClO_2 is highly volatile, unstable in highly concentrated solution and is therefore produced on-site. Three methods of generating ClO_2 are most commonly used: i) reacting sodium chlorate

($NaClO_3$) with HCl, ii) sodium chlorite ($NaClO_2$) with Cl_2 or iii) $NaClO_2$ with NaOCl and HCl. ClO_2 gas is greenish-yellow with a chlorine like odor and detectable at 0.1 ppmv in air. It can cause tears or throat irritation at 0.3 ppmv and at 5 ppmv it can be fatal for humans. It is 10 times more soluble in water than chlorine (above 11°C) and exists mostly in a gaseous state in water (boiling point 11 °C) (Anderson et al., 1982; USEPA, 1999a; Hofmann et al., 2002; Ayyildiz et al., 2009; Hosni et al., 2009). ClO_2 is usually reduced to chlorite (ORP = 0.95 V) which itself can potentially be reduced to chloride (ORP = 0.37 V). In total this yields 5 electrons and based on molecular weight it has 2.5 times the oxidative capacity of chlorine. However, ClO_2 is rarely reduced to chloride in water treatment, and the one electron shift to form chlorite generally occurs.

ClO_2 is a selective oxidant that reacts by electrophilic abstraction and not by substitution. It attacks electron-rich centers in organic molecules, does not readily react with ammonia or primary amines, but does oxidize nitrite to nitrate, and reacts with ferrous iron, manganese and sulfides. Complete mineralization of organics generally does not occur (Gordon & Rosenblatt, 2005; White, 2010).

1.3.3. Hydrogen peroxide

H_2O_2 is a strong oxidant (ORP = 1.78 V) that decomposes into safe by-products (H_2O, O_2). It is mainly produced by the anthraquinone process, i.e. production of H_2O_2 when reacting anthraquinones with O_2, but can also be produced by electrolysis of sulfates or sulfuric acid. Although it exists in almost pure form (98%), it is commercially available as an aqueous solution, shipped as 31%, 35%, 50%, and 70%, but stored at 50% or less (Drogui, et.al., 2001; Wagner et al., 2002; Ukuku et al., 2012). Commercial solutions typically lose less than 1% active component per year. However, the decomposition rate increases about 2.3 times per 10°C rise, so cold storage is advised. Also, the use of solutions of ≤ 35 % is encouraged as under those circumstances it is unlikely that detonable compositions will form (Jones, 1999; Kitis, 2004).

There are different types of reactions in which H_2O_2 is involved: i) oxidation-reduction reactions with decomposition of H_2O_2 to water and O_2, ii) peroxide group transfer, iii) addition-compound formation (Wagner et al., 2002). Also, H_2O_2 stability can be severely compromised by the presence of catalase (Ukuku et al., 2012).Despite the high oxidation potential and active oxygen content, it is a rather slow oxidant in the absence of a catalyst (transition metals, O_3, UV to induce HO• radical formation) or an activator (formation of

peroxycarboxylic acids) (Juven & Pierson, 1996; Neyens et al., 2002; Richardson et al., 2000).

1.3.4. Peracetic acid

PAA is the peroxide of acetic acid, is a clear colorless liquid, and has a strong pungent acetic acid odor. It is produced by reacting acetic acid or acetic anhydride with H_2O_2, catalyzed by 1% sulfuric acid (Falsanisi et al., 2008; Gonzalez-Aguilar et al., 2012). PAA is commercially available in a quaternary equilibrium mixture (reaction 3). It consists of acetic acid, H_2O_2, PAA and water. The stability of PAA is lower than that of H_2O_2. Dilute solutions (e.g. 1%) lose half their concentration through hydrolysis in 6 days, while a 40 % solution loses 1 to 2 % of active ingredient per month. However, commercially available PAA solutions of 10 – 15 % are much more stable than higher and lower concentrated solutions (Kitis, 2004). PAA has strong oxidizing properties; its ORP (1.81 V) is larger than that of chlorine, ClO_2 and H_2O_2 (Kitis, 2004; Santoro et al., 2007; De Luca et al., 2008; Pedersen et al., 2009).

$$CH_3COOH + H_2O_2 \leftrightarrows CH_3COOOH + H_2O \quad (1.3)$$

PAA is consumed in aqueous medium in multiple ways (reaction 4-6) (Liberti et al., 1999; Koivunen & Heinonen-Tanski, 2005b; Koivunen, 2007, Santoro et al., 2007):

$$2\ CH_3COOOH \rightarrow 2\ CH_3COOH + O_2 \quad (1.4)$$

$$CH_3COOOH + H_2O \rightarrow CH_3COOH + H_2O_2 \quad (1.5)$$

$$CH_3COOOH + \text{metal} \rightarrow O_2 + \text{other decomposition products} \quad (1.6)$$

The interaction of PAA with organic compounds functions through HO• radical formation. Interaction of the radicals with organics results in formation of organic radicals (eq. 1.7) which in turn further react with other compounds, while the PAA is degraded to H_2O and acetic acid (Appels et al., 2011).

$$RH + HO\bullet \rightarrow H2O + R\bullet \quad (1.7)$$

A second proposed mechanism is the release of active O_2 (eq. 1.8):

$$2\ CH_3COOOH \rightarrow 2\ CH3COOH + O_2 \quad (1.8)$$

1.3.5. Ozone

O_3 is an allotropic form of O_2. At room temperature O_3 is a colorless, highly odorous gas. O_3 is a powerful oxidant (ORP = 2.07 V) in water treatment, second only to the HO• radical (ORP = 2.80 V) (Audenaert, 2012). It is produced commercially from pure oxygen or dry air by corona discharge of electricity (most cost-effective) or through photochemical reactions if low amounts are required, (e.g. in laboratories). O_3 can be smelled at 0.01 to 0.04 ppmv in air, which is well below harmful levels. At concentrations higher than 0.5 ppmv long irritation, fatigue and chest pain arise. Concentrations ≥ 30 % in gas are unstable and can be explosive. Once produced, gaseous O_3 is transferred into the water. Because of the short half-life and the reactive and toxic nature, O_3 has to be produced on site (Doull, et.al., 1980; USEPA, 1999a; Rakness, 2005; White, 2010). O_3 is not readily soluble in water (chlorine is 12 times more soluble). Consequently, O_3 concentrations in water treatment are typically rather low (Doull, et.al., 1980; USEPA, 1999a; Cheremisinof, 2002; Rakness, 2005).

Aqueous O_3 reacts with (in)organic compounds through a direct oxidation with molecular O_3 or an indirect oxidation with HO• radicals, formed from O_3 decomposition (von Gunten, 2003; LeChevallier & Au, 2004;). Formation of HO• radicals from O_3 is promoted by increasing the pH (reaction 9 and 10) or with addition of H_2O_2 or by photolysis. (Choi et.al., 2007; Kim, 2007).

$$O_3 + OH^- \rightarrow O_2 + HO_2^- \quad (1.9)$$

$$O_3 + HO_2^- \rightarrow HO\bullet + O_2\bullet^- + O_2 \quad (1.10)$$

Because the use of O_3 for oxidation purposes in water treatment is widespread, considerable knowledge has been gathered regarding rate constants of O_3 and HO• radical reactions with dissolved compounds in the water matrix. O_3 reacts primarily with activated aromatic structures, carbon–carbon double bonds and non-protonated amines. Carbohydrates and fatty acids react only slowly with O_3 while amines, amino acids, nucleic acids, proteins and protein functional groups react more rapidly. Reaction of O_3 with inorganic compounds occurs mainly through the transfer of an oxygen atom to the inorganic compound in a two electron oxidation of said compound. The high reactivity of HO• radicals leads to near diffusion controlled reaction rates with water matrix constituents (von Gunten, 2003).

1.3.6. Electrolyzed water

Electrochemical disinfection (= a. o. electrolytic disinfection, anodic oxidation, functional water, electrochemically activated water) is the inactivation of microorganisms through use of

an electric current passing through water by means of suitable electrodes. At the phase boundary between the electrodes chemical oxidants are produced. Electrochemical disinfection comprises the generation of free chlorine (electrochlorination) and/or other free oxidants, depending on the solute content of the water and the type of electrodes used (Kraft, 2008). There are two types of electrochlorination, from a brine solution in an electrolytic generator (indirect), or directly from chloride ions present in the treated water itself (Martinez-Huitle & Brillas, 2008; Tsolaki et al., 2010). Active chlorine species formed at the anode are Cl_2, HOCl, OCl^- and ClO_2 (Martinez-Huitle & Brillas, 2008):

The kinetics of the produced free chlorine species and reactive oxygen species depend on the applied electrode materials. Efficiency of free chlorine production is higher with titanium (Ti) anodes coated with mixed oxides based on iridium/ruthenium oxides (RuO_2, IrO_2 and IrO_2/RuO_2), with high production even at low chloride levels, compared to platinum (Pt) and boron-doped diamond (BDD) materials and these last are therefore less suited for electrochlorination (Jeong et al., 2009; Khelifa et al., 2004; Kraft, 2008; Martinez-Huitle & Brillas, 2008). However, BDD anodes produce much higher amounts of reactive oxygen species and also other oxidizing species like peroxodisulfate, peroxodicarbonate, and peroxodiphosphate, provided the needed anions are present (Martinez-Huitle & Brillas, 2008; Jeong et al., 2009). For oxygen production Pt electrodes are most efficient, and for O_3 and H_2O_2 the use of BDD and graphite electrodes respectively (Jeong et al., 2009; Kraft, 2008).

1.3.7. Ultraviolet irradiation

UV radiation is the part of the electromagnetic spectrum consisting of photons with a wavelength from 100 to 400 nm: UV-A (315 to 400 nm), UV-B (280 to 315 nm), UV-C (200 to 280 nm) and vacuum UV (100 to 200 nm). Light emission from UV lamps depends on electrical power input, radiant power efficiency, lamp dimensions, and the characteristics of the quartz sleeve (if present). The emittance of the lamp is the radiant power emitted per unit area (W/m^2) of the lamp. The quantity of radiation that strikes a target is denoted by the irradiance or fluence rate, signifying the UV power per unit area (W/m^2) of a flat surface target or per unit cross- sectional area of a spherical target respectively. When integrating the fluence rate over time, the fluence or UV dose (J/m^2) is obtained (Bolton & Cotton, 2011).

UV photons have sufficient energy to excitate outer shell electrons to higher excitation states. Interaction of molecules and ions with UV photons leads to absorption when the photon energy equals a change in molecular energy level due to excitation of outer shell electrons.

Although these energy states that electrons can attain are discrete, UV irradiation is not absorbed at discrete wavelengths but over wider wavelength ranges because electron excitation is accompanied by changes in vibrational states within each electronic state as well as rotational sublevels (Burgess, 2007).

Because of the absorption peak of nucleotides near 260 nm , low-pressure (LP) UV lamps are most commonly applied for disinfection as 85% of the emission occurs at 253.7 nm. Passage of an electrical arc through a mercury vapor generates the UV energy. Conversion of electricity to light in LP lamps is between 35 and 40 %. The use of medium-pressure (MP) lamps has gained popularity because of the higher intensity output, allowing one MP lamp to replace 6 to 16 LP lamps. Contrary to the monochromatic nature of LP lamps, MP lamps emit photons in a spectrum ranging from 185 to over 1400 nm. Although the required power to deliver a germicidal UV dose is higher for a MP lamp than for a LP lamp, the fact that fewer lamps are needed, results in savings on equipment costs, lamp replacement and cleaning practices (Lingireddy, 2002; Mofidi et al., 2001). In contrast to the described continuous-wave emission technologies, pulsed-UV lamps produce high-intensity pulses from a xenon-gas lamp. The emission is controlled by microsecond electrical discharges of high energy, which heat the xenon gas, leading to photon emission in a broad wavelength range (185 to over 800 nm). The big advantage of pulsed-UV is the very fast microbial inactivation (Gomez-Lopez, 2012; Mofidi et al., 2001).

1.3.8. Membrane filtration

Membranes are thin films of synthetic inorganic (ceramic, metallic) or organic (polymeric) materials that separate two phases, selectively restricting transport of various substances. Membranes can be neutral, or charged. Membrane filtration is the separation of substances from a fluid by passing the mixture through a membrane in a pressure driven process. Certain substances are entrapped in the matrix or retained at the surface (Madaeni, 1999; USEPA, 2005; Wang et al., 2006a).

Membrane filtration functions through multiple mechanisms:

- sieve retention based on pore size,
- depth filtration (when the pores or interstices between filter media are larger than the particles),

- solution-diffusion (in membranes with very small pores, i.e. nanofiltration (NF) and RO), meaning permeants dissolve in the membrane material and subsequently diffuse through the membrane down a concentration gradient,
- electrostatic repulsion and adsorption sequestration i.e. capturing particles into the membrane matrix. This implies the importance of the membrane material (pore size distribution, charge, hydrophilic character etc.) for adsorption efficiency. Particles, carrying opposite charge compared to the membrane stick to the membrane matrix, possibly leading to removal of particles smaller than the membrane pores (Wijmans & Baker, 1995; Madaeni, 1995, 1999; Sadr Ghayeni et al., 1999; USEPA, 2005; Langlet et al., 2009).

Types of membrane filtration are defined based on pore size: in decreasing order of pore size: microfiltration (MF), ultrafiltration (UF), NF, RO (Madaeni, 1999; Cheremisinoff, 2002; Wang et al., 2006a). Some basic properties of membrane types are summarized in Table 1.3. Due to relatively great pore size, the removal mechanism of MF and UF is mainly based on size exclusion. NF and RO both remove salts via solution-diffusion. Contrary to RO, NF membranes contain fixed charged functional groups, resulting in different selectivity for monovalent and bivalent ions between the two processes (Boerlage, 2001). Almost all membranes used for drinking water production are based on polymeric material, due to the lower production costs compared to other materials (USEPA, 2005).

1.3.9. Ultrasound

US refers to pressure waves with a frequency of 20 kHz or more (Piyasena et al., 2003). Ultrasonic waves (in practice in the range 16 kHz to 100 MHz) induce a sinusoidal pressure variation on the medium, thus alternately increasing and decreasing the local density of the molecules. Decrease of the local fluid density during a decreasing pressure cycle results in formation of cavities (voids). The resulting microscopic bubbles are slowly filled with vaporized liquids till a critical size is reached, whereupon violent collapse occurs. A single bubble has a lifetime on the order of microseconds and a radius on the order of micrometers (Bruce & Nareddy, 2005; Gogate & Pandit, 2004; Hua & Thompson 2000). Cavitational bubble implosions result in extreme temperatures (in the range of 1900 K and 5200 K in the interfacial region and cavity respectively) and pressures (330 atmosphere) at the collapsed bubble center (Hua & Thompson, 2000; Mahamuni & Adewuyi, 2010). These extreme conditions result in radical formation (Figure 1.3), and as such cavitation can lead to oxidation

of water matrix constituents, as well as pyrolysis reactions in the cavity interior and the gas-liquid interface (Adewuyi, 2001; Naddeo et al., 2007). Also, formed hydrodynamic shear forces can mechanically rupture larger polymeric structures (Henglein & Gutierrez, 1990).

Table 1.3. Basic properties of membrane filtration (Boerlage, 2001; Cheremisinoff, 2002; Rautenbach et al., 1997; USEPA, 2005; Van der Bruggen & Vandecasteele, 2003; Wang et al., 2006a)

Membrane type	Pore size	Removes	Pressure (atm)	Mechanism
MF	0.1-10 µm	protozoa, bacteria, viruses to some degree (adsorption, electrostatic repulsion)	1 to 3	sieve retention
UF	5-100 nm	all microorganisms	2 to 10	sieve retention
	MWCO[a] = 1000-500 000 daltons (Da)	macromolecules (e.g. proteins)		
NF	0.1-5 nm	all microorganisms	5 to 20	sieve retention
	MWCO = 200-1000 Da	organics (MWCO limited), 95 % divalent salts, 40 % monovalent salts		solution-diffusion[b]
RO	no detectable pore size	all microorganisms	10 to 70	solution-diffusion
	MWCO < 200	organics (MWCO limited), 95-99% inorganic salts		

[a] Molecular weight cutoff (MWCO): the molecular weight of a compound that is rejected by 90% (Van der Bruggen & Vandecasteele, 2003). [b] The transition between sieve retention and solution-diffusion is believed to be in the NF range, NF combining both mechanisms (Wijmans & Baker, 1995; Boerlage, 2001).

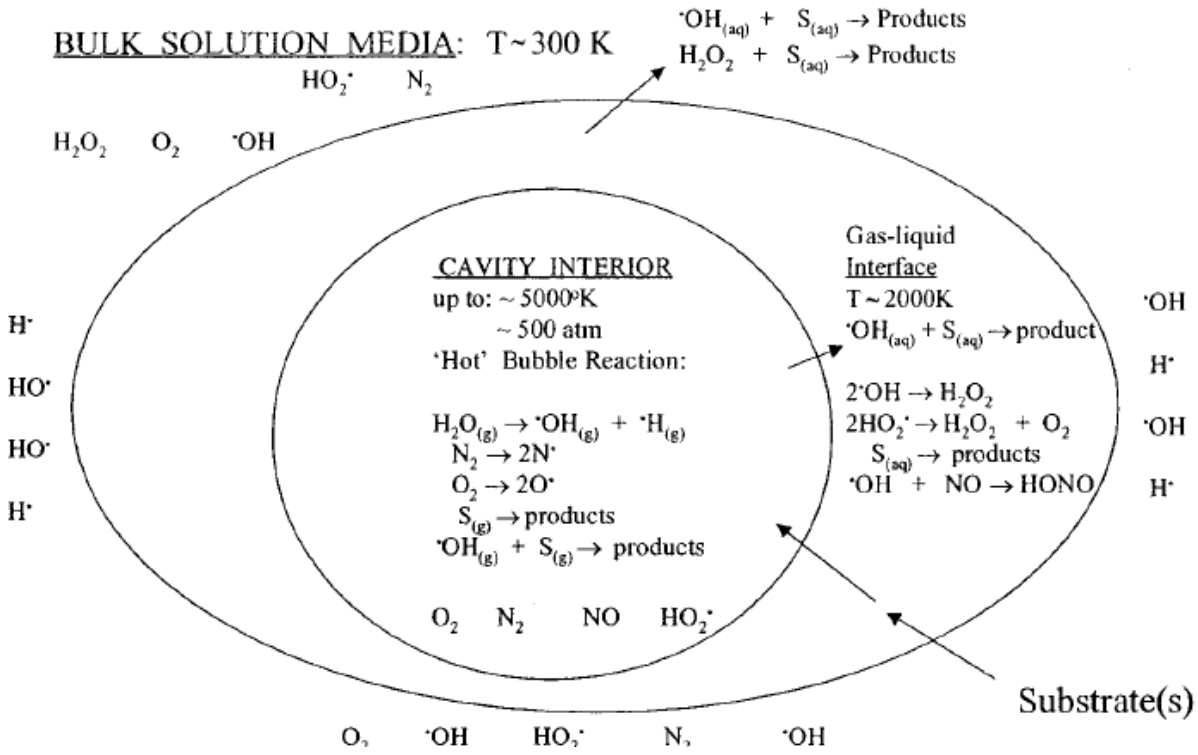

Figure 1.3. The chemical reaction zones in the cavitation process (adapted from Adewuyi, 2001)

Different designs of US reactors have been developed, comprising sonochemical and hydrodynamic cavitation reactors. Gogate (2007) made a comprehensive description of different reactor designs.

1.4. DISINFECTION BY-PRODUCTS

Disinfection technologies interact with water matrix constituents. As such, chemical changes in the water can occur with generation of new molecular species, which are generally called DBPs. A downside of disinfection is that some of these chemical species can be harmful to aquatic life and other life forms that consume the treated water.

1.4.1. Chlorine

Chlorine reacts with many water matrix components, creating halogenated compounds and non-halogenated organic oxidation products such as organic acids, ketones and aldehydes (Legay et al., 2010; Wang et al., 2006a). Some bromate (section 1.4.5) can be formed if bromide is present (which is enhanced in sunlight), but under normal circumstances no significant amounts are generated (Huang et al., 2008;. Richardson et al., 2007).

A variety of halogenated organics are produced by reaction of chlorine with organic matter: a. o. trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles and chlorophenols, of which THMs (especially chloroform) and HAAs are the two most prevalent classes in chlorinated drinking water, the others mostly occurring in trace amounts (Goslan et al., 2009; Hua & Reckhow, 2007; Legay et al., 2010; Nieuwenhuijsen et al., 1999; Wang et al., 2006a). Some THMs (i.e. chloroform, bromodichloromethane, chlorodibromomethane and bromoform), HAAs (trichloroacetic acid, dibromoacetic acid and dichloroacetic acid) and trichloroacetaldehyde are likely carcinogens. Other hazardous health effects attributed to one or more produced halogenated organics include hepatotoxicity, renal toxicity, neurotoxicity, spontaneous abortions, stillbirth, developmental issues and metabolic disturbances (Richardson et al., 2007; Tang et al., 2014). On the other hand, incoming compounds that already possess endocrine-disrupting properties, such as some estradiol species can become less estrogenic due to chlorination (Sedlak & von Gunten, 2011).

Furthermore, a few non-halogenated organic DBPs of chlorine disinfection are potential carcinogens: some nitrosamines and aldehydes (i.e. formaldehyde and acetaldehyde) (Nieuwenhuijsen et al., 1999; Richardson et al., 2007; Wang et al., 2006a). N-nitrosodimethylamine (NDMA) is a very potent carcinogen, with an associated risk three orders of magnitude higher than THMs and HAAs. It is predominately formed through reaction of chloramines with amine-based precursors. Chloramines can be used as disinfectant or unwillingly formed due to presence of ammonia in the water when dosing free chlorine (Mitch et al., 2003; Shen, 2013). Nonetheless, in the absence of ammonia, free chlorine can produce NDMA by reacting with secondary amines, although the rate of formation is an order of magnitude lower than is the case with monochloramine (Mitch et al., 2003). NDMA is regulated in drinking water in several states of the USA and provinces in Canada. Also, drinking water guidelines by the WHO, Health Canada and Australian guidelines have been suggested (Shen, 2013). Chlorination of antibiotics can result in elevated genotoxicity, as was observed with the chlorination of e.g. cefazolin (Li et al., 2013; Sedlak & von Gunten, 2011).

OCl^- solutions are known to contain impurities such as bromate (section 1.4.5), chlorite (section 1.4.2), chlorate (section 1.4.2), and perchlorate; further formation of the latter two can also occur during storage of OCl^- solutions (Greiner et al., 2008).

In the European Union (EU) and the USA, some DBPs are regulated in drinking water due to their detrimental effects on human health (Table 1.4).

Table 1.4. European Communities (drinking water) regulations and United States Environmental Protection Agency (USEPA) DBPs rule (adapted from Eureopean Communities, 2007; Richardson et al., 2007; USEPA, 2009)

DBP	EC regulations (MCL[c] µg/L)	USEPA regulations (MCL µg/L)
TTHMs[a]	100	80
5 HAAs[b]		60
bromate	10	10
chlorite		1000

[a] total THMs (TTHMs): the sum of concentrations of four THMs: chloroform, bromoform, bromodichloromethane and chlorodibromomethane. [b] five HAAs: monochloro-, dichloro-, trichloro-, monobromo-, and dibromoacetic acid. [c] maximum contaminant level.

1.4.2. Chlorine dioxide

ClO_2 decomposes primarily in chlorite (about 70% of applied ClO_2) in treated water, but also chlorate (about 10% of applied ClO_2) and chloride ions (Chang et al., 2001; Hofmann et al., 2002; Hua & Reckhow, 2007). Redox reactions with organic matter play the dominant role in formation of chlorite from ClO_2 in drinking water. Chlorate can appear for a variety of reasons when ClO_2 is applied (Chang et al., 2001; Hofmann et al., 2002; USEPA, 1999a). When relatively high doses are present in drinking water, chlorite and chlorate can cause anaemia in some animals and it has been demonstrated that moderately high levels are harmful on thyroid function (Hebert et al., 2010; Stampi et al., 2002). ClO_2 and chlorite are determined as unclassifiable as to human carcinogenicity by the International Agency for Research on Cancer (IARC) and the USEPA. IARC noted that no data is available to evaluate carcinogenicity of chlorate to humans (ATSDR, 2004; Health Canada, 2008). Bromate formation can also occur to some extent (although less severe than with ozonation, section 1.4.5), particularly in the presence of sunlight (Richardson et al., 2007).

ClO_2 produces fewer potentially carcinogenic halogenated DBPs than chlorine and much lower acute toxicity has been observed after ClO_2 disinfection. ClO_2 reacts through oxidation contrary to chlorine which reacts both through oxidation and chlorination, the latter reaction mechanism forming halogenated DBPs. No significant amounts of THMs and THAAs are produced by ClO_2. If however, THMs are produced, this is often attributed to the presence of excess chlorine (used for generating ClO_2) due to poor ClO_2 generator performance (Chang et al., 2000, 2001; Doull et al., 1980; Hofmann et al., 2002; Hua & Reckhow, 2007; Richardson

et al., 2000; Stampi et al., 2002). Considerable amounts of halogenated DBPs, especially dihaloacetic acids can be formed by ClO_2 (Hofmann et al., 2002; Hua & Reckhow, 2007; Ölmez & Kretzschmar, 2009). When iodide is present in the water, more iodinated DBPs, especially iodoform, are formed with ClO_2 than with chlorine. Toxicological studies suggest that iodinated DBPs may be more toxic than chlorinated DBPs (Hua & Reckhow, 2007).

Non halogenated organic DBPs, like carboxylic acids, aldehydes, ketones, aromatic compounds and esters are formed to a certain degree and formaldehyde and acetaldehyde are the most prevalent (Chang et al., 2000, 2001; Richardson et al., 2000).

1.4.3. Hydrogen peroxide

H_2O_2 does not significantly produce toxic DBPs. Reaction of H_2O_2 with organic matter in water results in formation of low levels of aldehydes and ketones (USEPA, 1997). Further research concerning the nature of formed DBPs has, as far as the author knows, not been conducted, and mostly it is simply assumed that the formation of toxic DBPs by H_2O_2 is limited (Ukuku et al., 2012). It is known however that hydrogen peroxide can assist in avoiding the formation of brominated organics as it reduces hypobromous acid to bromide (von Gunten & oliveiras, 1997) and chlorinated organics as it reduces hypochlorous acid to chloride, and it has been proposed as free chlorine quencher when chlorine residuals are unwanted (Shams El Din & Mohammed, 1998).

1.4.4. Peracetic acid

PAA produces predominantly carboxylic acids. However, the formation of toxic/mutagenic DPBs has been suggested due to the strong oxidizing properties of PAA. PAA cannot oxidize chloride to HOCl acid, but it is able to oxidize bromide to hypobromous acid, which can theoretically lead to brominated organics, but none were observed and it can be concluded that the potential of PAA to produce toxic DBPs is much lower compared to chlorine, ClO_2 and O_3 (Dell'Erba et al., 2007; Kitis, 2004; Santoro et al., 2007). Aldehydes, supposedly hepatotoxins at the mg/L level, were observed at negligible levels after disinfection with PAA in municipal wastewaters (Dell'Erba et al., 2007).

1.4.5. Ozone

For ozonation, the main DBP of concern is bromate which results from oxidation of bromide to hypobromous acid. Hypobromous acid is further oxidized to bromite and bromate

(Rakness, 2005; von Gunten, 2003a). Bromide concentrations in natural waters are highly variable. Low concentrations of bromide (< 20 µg/L) are not considered problematic. Higher concentrations of bromide (50-100 µg/L) result in excessive bromate formation and bromate becomes a serious problem for levels of bromide > 100 µg/L (Camel & Bermond, 1998; von Gunten, 2003b). Bromate induces deoxyribonucleic acid (DNA) damage and is found to be a human carcinogen (Camel & Bermond, 1998; Kim, 2007; von Gunten, 2003b; Wert, et.al., 2008). Bromate is particularly problematic because it is not biodegradable (Wert et.al., 2008).

Bromo-organic DBPs have been identified (Huang, et.al., 2005). They are formed by the reaction of hypobromous acid with organic matter. However, the concentrations of these bromo-organic compounds are usually far below current drinking water standards (von Gunten, 2003b). Non-brominated organic compounds result from the oxidative breakdown of organic matter. Alkenes, activated aromatic systems, amines and sulphur-containing organic compounds can lead to the fast formation of low molecular weight organic compounds (e.g. organic acids, aldehydes, ketones, alcohols and esters) (Doull, et.al., 1980; Hammes, et.al., 2006; Huang et.al., 2005). After the initial (fast) formation, the concentrations of these compounds increase continuously but very slowly (Hammes, et.al., 2006). The biggest fraction of low molecular weight organic compounds constitutes of organic acids whereas aldehydes and ketones are only formed in small amounts (Huang et.al., 2005). Many of these low molecular weight compounds are easily biodegradable and can support microbial growth (Camel & Bermond, 1998; Lehtola, et.al., 2001; Xu, et.al., 2002). Iodate is the main by-product formed by direct oxidations with molecular O_3 in iodide containing waters. Iodate is considered non-problematic because it is transformed back to iodide in the human body (Doull, et.al., 1980; von Gunten, 2003b). Amines are highly reactive towards O_3 which leads to the formation of odorous agents (isovaleraldehydes, phenylacetaldehydes, isobutyraldehydes and 2-methylbutyraldehyde). These compounds are recognized as problematic in the food industry (Hammes, et.al., 2006; Huang et.al., 2005).

1.4.6. Electrolyzed water

Reaction of electrochemically generated chlorine species with dissolved organic carbon produces halogenated organics. However, lower amounts are formed compared to classic chlorination because mixed oxidants are produced (e.g. ClO_2 produces less halogenated organics) (Deborde & von Gunton, 2008; Fang et al., 2006; Perez et al., 2010; Schmalz et al., 2009; Tsolaki et al., 2010). Chlorite, chlorate and perchlorate can be produced by electrochlorination (Fang et al., 2006; Kraft, 2008; Palmas et al., 2007). Temperatures below

40°C will prevent chlorate production and also the type of anode is of importance (e.g. Ti/RuO_2 produces lower amounts of chlorate compared to Ti/Pt or graphite anodes) (Khelifa et al., 2004).

1.4.7. Physical disinfection techniques

UV doses up to 200 mJ/cm² do not change the pH, turbidity, dissolved organic carbon level, color, nitrate, nitrite, bromide, iron, or manganese of the water being treated (Malley et al., 1996). At typical UV doses in drinking water (< 140mJ/cm²), no significant DBPs formation was observed but low levels of non-regulated DBPs (e.g. aldehydes) are formed if UV light is applied at doses > 400 mJ/cm² to wastewater and raw drinking water sources (Kashinkunti et al., 2003; Liu et al., 2002). Nitrate is a very strong absorber of UV below 250 nm. MP lamps with spectral output below 250 nm, can induce production of nitrite from nitrate. However, this is unlikely to be high enough to violate USA (1 mg/L (as N)) and Canadian water quality standards (3.2 mg/L nitrite) except in situations where nitrate is present in excess of its USA legal threshold of 10 mg/l (as N) or when UV doses far above those required for disinfection are applied. However, the EU standard may potentially be violated (0.5 mg/L (0.11 mg/L as N)) when using these lamps, even when the water contains levels of nitrate within regulatory limits (Sharpless & Linden, 2001; Sharpless et al., 2003).

Although US can alter or decompose water matrix constituents, it does not produce harmful DBPs (Hulsmans et al., 2010; Kim et al., 2007; Naddeo et al., 2007).

1.5. ANTIMICROBIAL MECHANISMS AND INHERENT WATER DISINFECTION EFFICIENCY

When assessing inactivation mechanisms with disinfection techniques, the primary targets for inactivation are often looked for. Possible targets can be those compounds which exhibit structural, enzymatic or hereditary function (as is shown for vegetative bacteria in Figure 1.4). The classical approach to identify antimicrobial targets is to look for correlations between concentration of biocides that initiate failure of certain cellular structures or functions (Denyer & Stewart, 1998). However, when assessing disinfectants, i.e. assessing inactivation of microorganisms, correlations between failure of functions due to the disinfectant and actual inactivation of the microorganism are looked for. As failure of these targets results in death or inability to reproduce, these are the primary targets for microbial inactivation.

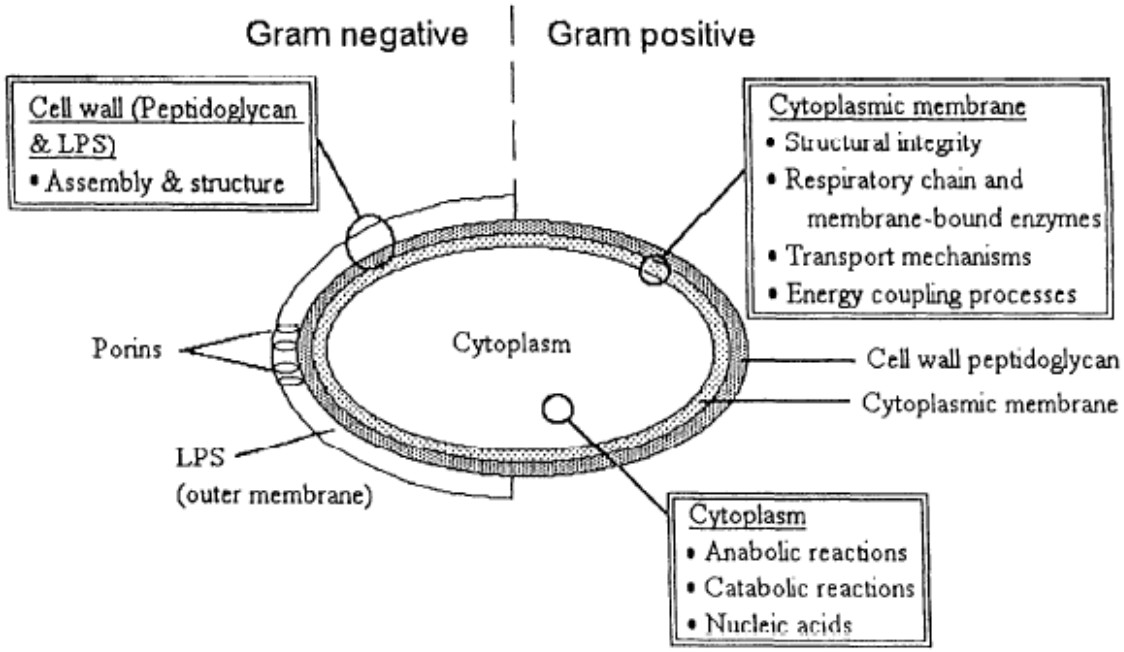

Figure 1.4. Bacterial targets for disinfectant action (Adapted from Denyer & Stewart, 1998)

1.5.1. Chemical oxidants

For chemical oxidants, the resistance generally increases in the following order: vegetative bacteria < viruses < *Giardia* cysts < bacterial spores and *Cryptosporidium* oocysts (USEPA, 1999a; Vandekinderen et al., 2009; Kitis, 2004).

1.5.1.1. Vegetative bacteria

As the interaction of the described chemical oxidants with organic molecules differs (Section 1.3), a difference in microbial targets or in affinity towards microbial targets can be expected. Recognizing that extreme exposures do not contribute to understanding the primary inactivation mechanisms, Cho et al. (2010) conducted a study to elucidate the damage that *E. coli* sustains when achieving a 1 log inactivation during 60 s at 20°C; with free chlorine, ClO_2, or O_3. Microscopic analysis revealed that when achieving 1 log reduction, the surface damage to the *E. coli* cell was in increasing order: chlorine < ClO_2 < O_3 (Figure 1.5). In addition, lipid peroxidation, protein release and cell permeability change were quantified in order to assess the mechanisms in molecular terms. Analogous to the spectroscopic observations, the destruction of cell components was largest with O_3, followed by ClO_2 and chlorine. As described in section 1.3, O_3 is an aggressive oxidant, ClO_2 is more selective in oxidation targets, and free chlorine functions predominately through chlorination rather than

oxidation. As such, O_3 causes more damage to the cell structure during the process of penetration into the cell, whereas chlorine enters the cell with much less oxidation events, while ClO_2 has an intermediary character.

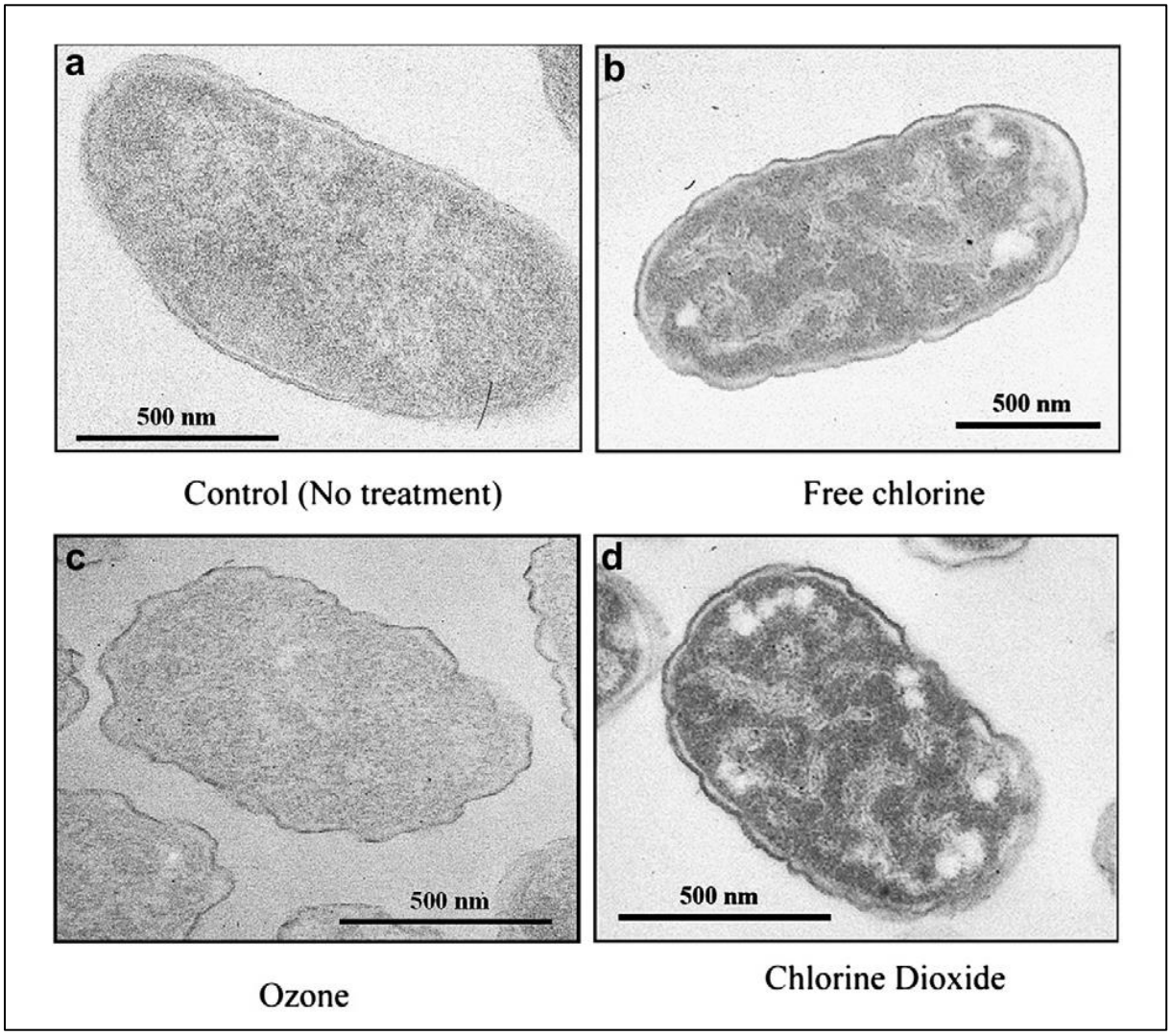

Figure 1.5. Transmission electron microscopy images before and after 1 log inactivation of *E. coli* by free chlorine, ClO_2 and O_3 (buffer, pH 7.1, 20°C) (Adapted from Cho et al. (2010))

Thanomsub et al. (2002) also observed cell wall destruction as mechanism for inactivation of Gram-negative and Gram-positive bacteria with O_3. More 'subtle' damage than structural destruction of vegetative bacteria by chemical oxidants (e.g. PAA, O_3, H_2O_2, chlorine, ClO_2) has often been attributed to reaction with, or oxidation of thiol groups, essential for activity of many enzymes (Imlay, 2003; Liberti et al., 2000a; Roller et al., 1980; Santoro et al., 2007).

ClO_2 disinfection of bacteria does not involve membrane rupture and loss of protein or DNA damage as the primary inactivation mechanism (although these types of damage also occur as described above). Dehydrogenase enzymes are inactivated but they are not primarily responsible for the inactivation of the microorganisms as complete inactivation of these enzymes leaves a part of the bacteria viable. There is evidence that blocking protein synthesis is involved, but it did not correlate with cell death and as such does not seem to be the primary inactivation mechanism (Benarde et al., 1967; Roller et al., 1980).

Sublethal chlorination affects the physiological status of *E. coli*, and previous studies suggested that active transport and respiration systems for glucose and amino acids are targets for chlorine (Lisle et al., 1999, McFeters & Camper, 1983). Other studies confirmed that actual destruction of membrane or cell wall integrity is not necessary for *E. coli* as well as *L. monocytogenes* inactivation and suggested that the primary action of chlorination is not on the cell surface of the prokaryotic cell but mostly on the interior (Cho et al., 2010; Virto et al., 2005).

Little is known about the exact inactivation mechanism of bacteria when exposed to PAA. When *Pseudomonas aeruginosa* was treated with sublethal concentrations of PAA, genes associated with cellular protective processes were induced, genes involved in metabolic pathways were repressed and transcription of genes encoding small molecule transporters and membrane proteins were altered (Chang et al., 2005).

H_2O_2 is a non-radical reactive oxygen species that is capable of penetrating all biological membranes yet directly inactivate only few enzymes (Atli et al., 2006). It is the production of HO• radicals through the Fenton reaction with free intra- and extracellular iron that enables damage to membrane structures, DNA, and proteins (a. o. oxidation of Fe-S cluster proteins and more generally of cysteine residues in proteins) (Brudzynski et al., 2011; Imlay, 2003; Raffellini et al., 2011). Imlay and Linn (1986) observed that at low H_2O_2 concentrations ($\leq$ 85 mg/L) and only in metabolically active *E. coli* cells, H_2O_2 induced DNA damage. At higher concentrations, the inactivation showed an uncharacterized multiple target mechanism (membrane lipids, proteins, nucleic acids) dependent on both H_2O_2 concentration and contact time and effective against both metabolically active and inactive cells (Imlay and Linn, 1986; Imlay et al., 1988; Raffellini et al., 2011). Raffellini et al. (2011) observed that exposure of *E. coli* to high concentration of H_2O_2 (0.5 to 6 %) resulted in loss of membrane integrity (rupture of outer and cytoplasmic membrane). Research has shown that H_2O_2 is less effective against microorganisms with high catalase activity level. As such, higher initial microbial numbers

can lead to more microbial catalase in solution, and the requirement for higher H_2O_2 exposure (Table 1.5) (Armon et al., 2000; Lambert et al., 1999; Sacchetti et al., 2009; Watts et al., 2003).

Electrolyzed water can, besides inactivation by generating oxidizing species, disinfect by direct electron transfer between electrode surface and cells, as such oxidizing cellular constituents; and electric fields themselves may cause irreversible permeabilization of the cell membrane (Okochi et al., 1997; Drogui et al., 2001; Feng et al., 2004; Liang et al., 2005; Polcaro et al., 2007). Electrochlorination outperforms classic chlorination (when sufficient oxidants can be produced for the respective water volume, water flow, or influx of microbial contamination), because of the production of reactive oxygen species next to free chlorine. This was observed for vegetative bacteria (but also for bacterial spores, yeasts, viruses and protozoa) (Venczel et al., 1997; Li et al., 2004a; Fang et al., 2006; Bergmann et al., 2008). Electrochemical disinfection of solutions containing other electrolytes than chloride (e.g. phosphate, sulfate) seems to require longer contact times/applied currents to achieve a similar efficiency as electrochlorination (Jeong et al., 2007, 2009; Polcaro et al., 2007).

For chlorine, ClO_2, O_3, and electrochlorination, inactivation studies showed higher susceptibility of Gram-negative compared to Gram-positive bacteria (Doull, et.al., 1980; Kim et al., 2000; Park et al., 2004; USEPA, 1999a; Vandekinderen.et al., 2009; von Gunten et al., 2003b).In some studies Gram-negative bacteria (i.e. *E. coli*, fecal and total coliforms) were more susceptible to PAA than Gram-positive bacteria (enterococci) (Stampi et al., 2002; Zanetti et al., 2007; De Luca et al., 2008; Falsanisi et al., 2008) while other studies showed no considerable differences between the two (Stampi et al., 2001; Koivunen & Heinonen-Tanski, 2005a, 2005b).

As it is fairly established that the targets of bacterial inactivation with chlorine and ClO_2 are located in the interior of the cell, it can be considered that the difference in resistance between Gram-negative and Gram-positive species might be dependent predominately on differences in resistance to the mass transfer of these disinfectants across Gram-positive and -negative cell surface layers. On that note, a comparison between resistance of Gram-positive and Gram-negative species based solely on the thickness of the peptidoglycan layer is an oversimplification due to the spatial difference of these layers in the cell surface of both bacterial groups as well as the difference in composition of the Gram-positive and Gram-negative bacterial cell walls (Dalrymple et al., 2010). Vegetative bacteria are very susceptible

to chlorine, ClO_2, PAA and O_3 (Table 1.6-1.8) and to lesser extent to H_2O_2 (Table 1.5) (Collivignarelli et al., 2000; Kitis, 2004).

1.5.1.2. Bacterial spores

The resistance of bacterial spores to chemical oxidants can be explained by i) the spore coat that restricts diffusion into the spore, ii) the low permeability of the spore's inner membrane to hydrophilic molecules, iii) the low water content in the spore's core, iv) the protected DNA due to specific DNA binding proteins, v) the DNA repair mechanisms during spore germination. As diffusion is inhibited, the necessity for oxidative damage towards structural components of the spore increases (Cortezzo & Setlow, 2005; Vandekinderen, 2009; Young & Setlow, 2003).

O_3 has high sporicidal activity. It damages and alters the structure of the bacterial spore, i.e. the outer surface layer and the outer spore coat (Figure 1.6). *Bacillus subtilis* was more susceptible to O_3 at alkaline pH (which has not been observed for vegetative bacteria or viruses), attributed to the production of HO• radicals at that pH, and was confirmed because radical scavengers undid this enhanced effect on inactivation at alkaline pH (Cho et al., 2003; Larson & Marinas, 2003). As the outer spore layers are targets for O_3 disinfection, it is assumable that the powerful, nonselective oxidation by HO• radicals has a significant impact on the outer structure of the spore, as such enhancing the oxidation of structural cellular compounds, which is clearly involved in the inactivation of bacterial spores as described above.

H_2O_2 causes similar damage as O_3 but exuberantly high exposure is necessary, e.g. H_2O_2 at a 10000 times higher exposure than O_3 was less effective for inactivation of *Bacillus* spores (Khadre & Yousef, 2001; Shin et al., 1994).

Chlorine and ClO_2 damage *Bacillus subtilis* by attacking the spore's inner membrane and no DNA damage occurs. Chlorine prevents germination of the spores, whereas ClO_2 cannot inhibit initiation of germination in the presence of nutrients, although the spores cannot complete the germination process, rendering them *de facto* unviable (Young & Setlow, 2003).

Disinfection efficiency against bacterial spores can be ranked according to increased efficiency as following: H_2O_2 < chlorine < PAA <?> ClO_2 < O_3 (Table 1.5-1.8) (Alasri et al., 1993; Hosni et al., 2009; Toledo et al., 1973; von Gunten, 2003b; Young & Setlow, 2003).

1.5.1.3. Protozoan parasites

Protozoan parasites (*Cryptosporidium, Giardia, Cyclospora*) are obligate parasites. As such they can only reproduce within the host and subsequently take on a dormant, protected form which is excreted in the feces into the 'hostile' environment outside the host, i.e. a cyst or oocyst form. Similar to bacterial spores, these dormant life stages of protozoa are relatively highly resistant against chemical oxidants; cysts and oocysts consist of multilayered cell walls that need to be penetrated or damaged by chemical oxidants in order to be inactivated (Fraise et al., 2012; Rose & Slifko, 1999).

Similar as for bacterial spores resistance of protozoan parasites against chemical oxidants is according to the following order: H_2O_2 < chlorine < ClO_2 < O_3 (Table 1.5-1.8). Notable is the very high resistance of *Cryptosporidium parvum* against chlorine. PAA is weak against *Giardia* and *Cryptosporidium parvum* (oo)cysts (Kitis, 2004; Koivunen, 2007; Santoro et al., 2007; Wagner et al., 2002). There is little kinetic data available for inactivation of protozoa with PAA. Barbee et al. (1999) noted that exposure to 0.2 % PAA for 12 min at 50°C was necessary to reach a > 1 log reduction of *Cryptosporidium parvum* whereas at room temperature these concentrations did not result in significant inactivation within 20 min.

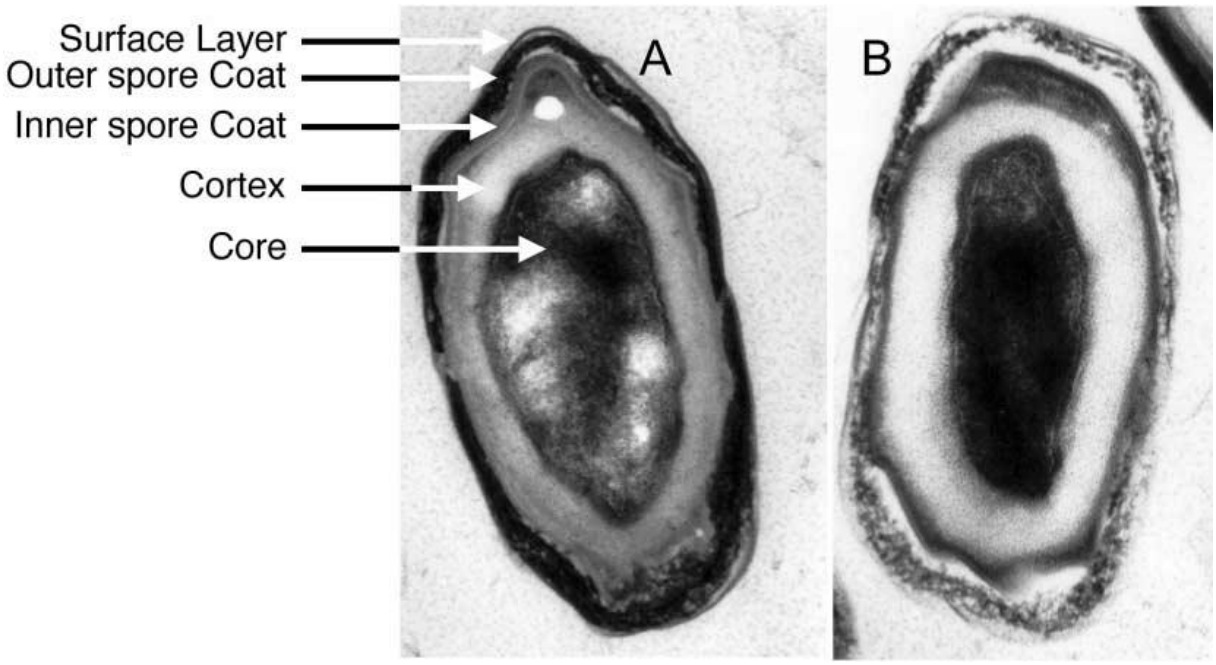

Figure 1.6. *Bacillus subtilis* spore before (A) and after (B) O_3 treatment (10 mg/L, 22°C, 1 min) (adapted from Khadre & Yousef, 2001)

Table 1.5. CT[a] values of H_2O_2 disinfection in oxidant demand free water or drinking water

Reference	Microorganism	Initial microbial load (CFU/ml)	CT[a] values (mg.min/l)			
			1 log	2 log	3 log	4 log
Bacteria						
Yoshpe-Purer & Eylan, 1986	*E. coli* (room T, pH 6,5)	10^2		32400		
Yoshpe-Purer & Eylan, 1986	*E. coli* (room T, pH 6,5)	10^6		32400		
USEPA, 1997	*E. coli* (24 C°, pH 7, 30 ppb Ag^+)				2640	
Yoshpe-Purer & Eylan, 1986	*Salmonella typhi* (room T, pH 6,5)	10^2		1800-2700		
Yoshpe-Purer & Eylan, 1986	*Salmonella typhi* (room T, pH 6,5)	10^6		3600-9000		
Yoshpe-Purer & Eylan, 1986	*Staphylococcus aureus* (room T, pH 6,5)	10^2		1350-2700		
Yoshpe-Purer & Eylan, 1986	*Staphylococcus aureus* (room T, pH 6,5)	10^6		16200		
Toledo et al., 1973	*Staphylococus aureus* (24 °C, pH 7)					285000[b]
Toledo et al., 1973	*Bacillus subtilis* variant *globigii* (24°C, pH 7)		1995000	2565000	3135000	
Toledo et al., 1973	*Bacillus coagulans* (24°C, pH 7)		1425000	1995000	2565000	
Toledo et al., 1973	*Bacillus stearothermphilus* (24°C, pH 7)		1140000	1425000		
Toledo et al., 1973	*Clostridium* spp. (24°C, pH 7)		285000	570000	855000	
Viruses						
Lund, 1963	poliovirus (Saukett strain)			1080000		
Mentel & Schmidt, 1973	rhinovirus types 1A,1B,7			360000		
Mentel & Schmidt, 1973	rhinovirus types 1A,1B,7			120000		
USEPA, 1997	MS2 phage (24 °C, pH 7, 100 ppb Ag^+)					36000
Protozoa						
Barbee et al., 1999	*Cryptosporidium parvum* (24°C, pH 7)			60000	600000[c]	
Weir et al., 2002	*Cryptosporidium parvum* (room T, pH 7,4)					240000

[a]concentration x contact time, [b] > 6log reduction. [c] > 3 log reduction

Table 1.6. CT values of chlorine disinfection in oxidant demand free water or drinking water

Reference	Microorganism	CT values (mg.min/l)				
		1 log	2 log	3 log	4 log	> 4 log
Bacteria						
Young & Setlow, 2003	*Bacillus subtilis* (20°C,pH 7)			400		
Virto et al., 2005	*Bacillus subtilis* (vegetative) (20°C, pH7)		1.8			
Lee et al., 2010	*E. coli* (4°C, pH 8,5)		0.13	0.20	0.25	
LeChevallier et al., 1988; Doull et al., 1980; Hoff,1986	*E. coli* (5°C, pH 6)		0.034-0.04			
Doull et al., 1980	*E.coli* (5°C, pH 10)		0.92			
Kott et al., 1975	*E. coli* (10°C, pH 6)		0.10			
Kott et al., 1975	*E. coli* (10°C, pH 10)		0.38			
Lee et al., 2010	*Mycobacterium fortuitem* (4°C, pH 8,5)		36		278	
Lee et al., 2010	*Mycobacterium fortuitem* (20°C, pH 7)		15		100	
Luh & Marinas, 2007	*Mycobacterium avium*			551-1152[a]		
Virto et al., 2005	*L. monocytogenes* (20°C, pH 7)		1.6			
Viruses						
Cromeans et al., 2010	adenovirus type 2,5,40,41 (5°C, pH 7)		0.01-0.02	0.02-0.06	0.04-0.15	
Baxter et al., 2007;Cromeans et al., 2010	adenovirus type 2,5,40,41 (5°C, pH 8)		0.02-0.04	0.04-0.12	0.03-0.27	
Shin & Sobsey, 2008	MS2 phage (5°C, pH 6)		1.7	2.5	3.3	
Cromeans et al., 2010	coxsackievirus B3 (5°C, pH 7)		0.97	1.4	2.9	
Cromeans et al., 2010	coxsackievirus B3 (5°C, pH 8)		0.65	1.1	1.7	
Cromeans et al., 2010	echovirus 1 (5°C, pH 7)		0.96	1.3	1.5	
Cromeans et al., 2010	echovirus 1 (5°C, pH 8)		0.99	1.3	1.6	
Cromeans et al., 2010	murine norovirus (5°C, pH 7)		<0.02	<0.02	<0.07	
Shin & Sobsey, 2008	norovirus (Norwalk virus) (5°C, pH 6)		2.9	4.3	5.8	

Shin & Sobsey, 2008	poliovirus 1 (5°C, pH 6)		15	22	30	
Doull et al., 1980	poliovirus 1 (5°C, pH 6)		1.1-2.1			
Doull et al., 1980	poliovirus 1 (5°C, pH 10)		11			
Doull et al., 1980	poliovirus 1 (15°C, pH 6)		1			
Doull et al., 1980	poliovirus 1 (15°C, pH 10)		3.5			
USEPA, 1991	viruses (5°C, pH 6)					22
USEPA, 1991	viruses (5°C, pH 8)					48
USEPA, 1991	viruses (20°C, pH 6)					8
USEPA, 1991	viruses (20°C, pH 8)					19
Protozoa						
Gyürék et al., 1997	*Cryptosporidium parvum* (22°C, pH 6)	2200				
Korich et al., 1990	*Cryptosporidium parvum* (25°C, pH 7)		7200[b]			
USEPA, 1991; Gyürék et al., 1997	*Giardia cysts* (5°C, pH 6)		31-44	61-87	92-131	
USEPA, 1991	*Giardia cysts* (5°C, pH 8)		67-96	135-192	202-288	
USEPA, 1991	*Giardia cysts* (20°C, pH 6)		11-16	23-33	34-49	
USEPA, 1991	*Giardia cysts* (20°C, pH 8)		23-36	51-72	76-108	

[a] dependent on strain, [b] > 2 log reduction

Table 1.7. CT values of ClO_2 disinfection in oxidant demand free water or drinking water

Reference	Microorganism	CT values (mg.min/l)			
		1 log	2 log	3 log	4 log
Bacteria					
Hoff 1986	*E. coli* (5°C, pH 6-7)		0.4-0.75		
LeChevallier et al., 1988	*E. coli* (20°C, pH 6.5)		0.18		
LeChevallier et al., 1988	*E. coli* (25°C, pH 7)		0.28		
Vicuna-Reyes et al., 2008	*Mycobacterium avium* (5°C, pH 6)	10	24	32	
Vicuna-Reyes et al., 2008	*Mycobacterium avium* (20°C, pH 6)	3	4.8	6.8	
Hosni et al., 2009	*Bacillus globigii* (20°C, pH 8)	52-72	114-160		
Viruses					
Thurston-Enriquez et al., 2005a	adenovirus type 40 (5°C, pH 6)				0.77-1.5
Thurston-Enriquez et al., 2005a	adenovirus type 40 (5°C, pH 8)				0.8-1.6
Thurston-Enriquez et al., 2005a	adenovirus type 40 (15°C, pH 6)				0.49-0.74
Thurston-Enriquez et al., 2005a	adenovirus type 40 (15°C, pH 8)				<0.12
Lim et al., 2010a	MS2 phage (5°C, pH 7)		0.079	0.21	0.42
Lim et al., 2010a	MS2 phage (20°C, pH 7)		0.021	0.071	0.14
Thurston-Enriquez et al., 2005a	feline calicivirus (5°C, pH 6)				20-30
Thurston-Enriquez et al., 2005a	feline calicivirus (5°C, pH 8)				4.2-6.7
Thurston-Enriquez et al., 2005a	feline calicivirus (15°C, pH 6)				>0.68
Thurston-Enriquez et al., 2005a	feline falicivirus (15°C, pH 8)				<0.18
Lim et al., 2010a	murine norovirus (5°C, pH 7)		0.071	0.15	0.25
Lim et al., 2010a	murine norovirus (20°C, pH 7)		< 0.001	0.055	
Hoff,1986	poliovirus type 1 (5°C, pH 6-7)		0.2-6.7		
Hoff ,1986	rotavirus (5°C, pH 6-7)		0.2-2.1		

USEPA, 1999a	viruses (5°C, pH 6-9)		5.6	17	33
USEPA, 1999a	viruses (25°C, pH 6-9)		1.4	4.3	8.4
Protozoa					
Peeters et al., 1989; Li & Finch, 1998; Li et al., 2001a	*Cryptosporidium parvum* (22-25°C, pH 7)	13-79	111-140	180	
Li et al., 2001a	*Cryptosporidium parvum* (5°C, pH 6-11)	221	442		
Hoff,1986	*Giardia muris* cysts (5°C, pH 6-7)		7.2-18.5		
USEPA, 1999a	*Giardia* spp. *cysts*(5°C, pH 6-9)	8.7	17	26	
USEPA, 1999a	*Giardia* spp. *cysts* (25°C, pH 6-9)	3.7	7.3	11	

Table 1.8. CT values of O_3 disinfection in oxidant demand free water or drinking water

References	Microorganism	CT values (mg.min/l) 1 log	2 log	3 log	4 log	5 log	6 log
Bacteria							
von Gunten, 2003b	*E coli* 20°C	0.01	0.02	0.02	0.03	0.04	0.05
von Gunten, 2003b	*B. subtilis* spores 20°C	0.17	0.34	0.52	0.69	0.86	1.0
John et.al., 2005	*E. intestinalis* spores 5°C	0.46 – 0.58	0.59 – 0.84	1.1 – 1.4			
Viruses							
Thurston-Enriquez et al., 2005b	feline calicivirus 5°C		0.03	0.25	0.30		1.2
Thurston-Enriquez et al., 2005b	adenovirus 40 5°C		0.12 – 0.15	0.30	0.60		
von Gunten, 2003b	rotavirus 20°C	0.01	0.03	0.04	0.05	0.07	0.08
Wickramanayake et.al., 1984a	poliovirus 1 5°C		0.22				
Wickramanayake et.al., 1984a; USEPA, 1999a	poliovirus 1 20°C		0.18	0.4	0.52		
USEPA, 1999a	poliovirus 1 25°C		0.16	0.26	0.32		
Protozoa							
Lawrence et al., 2008	*G. lamblia* 1°C	0.97		2.9			
USEPA, 1999a; Lawrence et al., 2008	*G. lamblia* 10°C	0.48	0.94	1.4			
USEPA, 1999a; Lawrence et al., 2008	*G. lamblia* 20°C	0.24	0.48	0.72			
von Gunten, 2003b	*G. lamblia* 25°C	0.03	0.07	0.10	0.14	0.17	0.21
von Gunten, 2003b	*G. lamblia* cysts 22°C	0.08	0.17	0.25	0.33	0.42	0.50
von Gunten, 2003b	*G. muris* cysts 25°C	0.06	0.13	0.19	0.26	0.32	0.39
Rennecker et.al., 1999	*C. parvum* oocysts 0.5°C	33	58	83	121	146	158
LeChevallier & Au, 2004	*C. parvum* oocysts 1°C	12	40	62			

Rennecker et.al., 1999	*C. parvum* oocysts 10°C	9.9	18	25	33	40	48
LeChevallier & Au, 2004	*C. parvum* oocysts 13°C	4	11	22			
Rennecker et.al., 1999	*C. parvum* oocysts 15°C	5.4	9.6	14	18	22	26
von Gunten, 2003b	*C. parvum* oocysts 20°C	0.60	1.2	1.8	2.4	3.0	3.6
Rennecker et.al., 1999	*C. parvum* oocysts 20°C	3.1	5.4	7.7	10	12	15
Rennecker et.al., 1999	*C. parvum* oocysts 25°C	1.7	3.1	4.4	5.8	7.1	7.8
Rennecker et.al., 1999	*C. parvum* oocysts 30°C	1.0	1.8	2.6	3.4	4.1	4.9

1.5.1.4. viruses

Viral inactivation can occur due to loss of infectivity (i.e. the epitopes on the capsid are altered) or damage to the genome (i.e. loss of replication ability).

Poliovirus, inactivated with ClO_2 or chlorine (no plaque forming units observed), could infect target cells, yet not replicate due to damage in RNA (Alvarez & O'Brien, 1982; O'Brien & Newman, 1979). RNA damage as cause of Poliovirus inactivation with chlorine was also confirmed by Nuanualsuwan & Cliver (2003b), without mentioning any loss of infectivity. Inactivation of Hepatitis A with ClO_2 occurred both due to loss of infectivity as well as genomic damage (Li et al., 2004b). For chlorine, damage to the genome of Hepatitis A preceded the loss of infectivity (Li et al., 2002). However, in another study capsid alteration (loss of infectivity) was the primary target for chlorination of Hepatitis A and feline calicivirus (Nuanualsuwan & Cliver, 2003a).

Inactivation of bacteriophage f2 with O_3 occurred due to loss of infectivity (Kim et al., 1980). RNA damage was the cause of Poliovirus inactivation with O_3. Capsid damage occurred but, initially, did not result in loss of infectivity (Roy et al., 1981). Also, Shin & Sobsey (2003) observed good correlation between on the one hand polymerase chain reaction results of the viral genome of Poliovirus and MS2 phage after O_3 treatment and on the other hand plaque forming assays, suggesting viral RNA as a primary target for inactivation. It appears both loss of infectivity and of replication ability are causes for inactivation of viruses with chlorine, ClO_2 and O_3, and the relevance of what type of damage occurs first is disputable.

Viruses are quite susceptible towards chlorine, PAA, ClO_2, and O_3, and less so to H_2O_2, although considerable variability in resistance to these chemical oxidants exists among virus types (Tables 1.5-1.8) (Baldry et al. 1991; Koivunen & Heinonen-Tanski, 2005b; Lazarova et al., 1998). Again, little is known about the virucidal action of PAA in oxidant demand free conditions. PAA was equally effective as chlorine to inactivate MS2 phage in synthetic wastewater (Koivunen & Heinonen-Tanski, 2005a).

1.5.2. Physical disinfection techniques

1.5.2.1. Ultraviolet irradiation

UV absorption by microorganisms is intended to be specific. At high UV dose UV light is absorbed by proteins in outer cell membranes leading to disruption of these membranes and subsequent death through cell leakage of protoplasm. However, the ability to replicate can be

disrupted at much lower UV doses through absorption of UV photons by the genome (Bolton & Cotton, 2011). When irradiation of microorganisms with UV-C light occurs, only proteins and nucleotides absorb significant amounts of UV light (Figure 1.7). The germicidal UV range, i.e. absorbance of UV photons by DNA, can range from 230 to 300 nm, with a peak near 260 nm (Mofidi et al., 2001). The major UV damage is due to formation of pyrimidine-pyrimidine photoproducts. Formed pyrimidine dimers cause errors when the nucleic acids are replicated or when transcription is executed. This leads to unviable microorganisms or mutant microorganisms unable to replicate further, and microorganisms rich in thymine tend to be more sensitive to UV irradiation (USEPA,1996, 1999b; Wang et al., 2006a). As photons in the germicidal UV range have insufficient energy to ionize atoms and molecules, UV-C light treatments are non-ionizing (Gomez-Lopez, 2012).

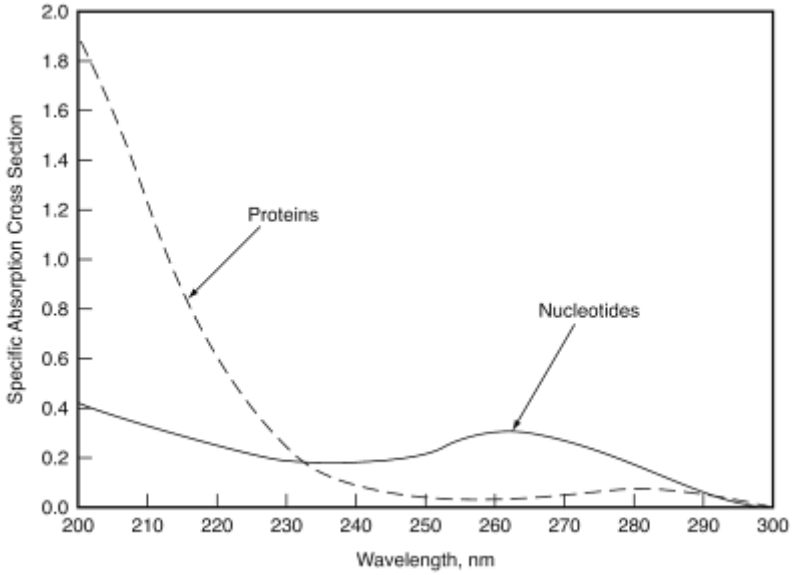

Figure 1.7. Absorption of UV-C light for proteins and nucleotides within a microbial cell with a composition of 70% proteins, 20% nucleotides, and 10% miscellaneous compounds such as lipids (adapted from Bolton & Cotton, 2011).

Damage by UV irradiation is not necessarily permanent as microorganisms have developed mechanisms to repair photochemical damage, which can increase the UV dose needed to achieve a certain level of inactivation in bacteria and protozoa (Knudson, 1985; Sommer et al., 2000). Dark repair is a slow mechanism that does not require light, whereas a faster and more important repair mechanism exists that necessitates visible light, i.e. photoreactivation. This process is catalyzed by photolyase, an enzyme that absorbs photons to split pyrimidine dimers (Gomez-Lopez, 2012). Concerning viruses, genome repair seems to be of little

significance (Heinen et al., 2006). Temperature, UV dose, exposure to visible light prior or during UV irradiation, type of microorganism and type of UV lamp can influence photoreactivation/dark repair (Table 1.9). Although *Cryptosporidium* has the capability to repair damage by UV, oocysts could not recuperate their infectious nature and *in vivo* reactivation of *Giardia* has only been observed at low MP UV exposure (Belosevic et al., 2001; Betancourt & Rose, 2004).

Table 1.9. Observations concerning photoreactivation/dark repair

Reference	Microorganism	Remark
Morita et al., 2002	*Cryptosporidium parvum*	no *in vivo* reactivation
Linden et al., 2002	*Giardia lamblia*	no *in vivo* reactivation
Craik et al., 2000	*Giardia muris*	no *in vivo* reactivation
Belosevic et al., 2001	*Giardia muris*	*in vivo* reactivation at low doses with MP lamp (5-25 mJ/cm²)
Hallmich & Gehr, 2010	*E. coli*	photoreactivation of LP lamps > MP lamps (below 10 mJ/cm²), similar above 40 mJ/cm²
Hallmich & Gehr, 2010	fecal coliforms	3 h delay before exposure to visible light (400-800 nm) almost eliminated photoreactivation
Hallmich & Gehr, 2010	fecal coliforms	exposure to visible light prior or simultaneously to UV irradiation decreased photoreactivation
Kohli & Gupta, 2003; Lage et al., 2000	*E. coli*	exposure to visible light increased resistance to UV disinfection
Locas et al., 2008	*E. coli*	higher photoreactivation at 25°C compared to 4°C

Bacterial spores and adenoviruses are much more resistant to UV than vegetative bacteria and (oo)cysts of *Cryptosporidium* and *Giardia*. The high susceptibility of the chlorine resistant *Cryptosporidium* to UV has increased the usefulness of UV technology for drinking water disinfection. Hijnen et al. (2006) calculated the inactivation kinetics of pathogenic and indicator microorganisms, based on a number of collimated beam tests of drinking water (Table 1.10).

Table 1.10. Required UV dose for inactivation in drinking water (Adapted from Hijnen et al., 2006)

Microorganism	Required UV dose (mJ/cm²) 1 log	2 log	3 log	4 log
bacterial spores				
Bacillus subtilis[a]	56	111	167	222
Clostridium perfringens[a]	45	95	145	_[b]
vegetative bacteria				
Campylobacter jejuni[d]	3	7	10	14
E. coli[a]	5	9	14	18
E. coli O157[d]	5	9	14	19
Legionella pneumophila[d]	8	15	23	30
Salmonella typhi[a]	6	12	17	51
Shigella dysenteriae[d]	3	5	8	11
Shigella sonnei[d]	6	13	19	26
Sterptococcus fecalis[a]	9	16	23	30
Vibrio cholerae[d]	2	4	7	9
Yersinia enterocolitica[d]	3	7	10	13
viruses				
adenovirus type 40	56	111	167	_[b]
adenovirus type 2,15,40,41	42	83	125	167
calicivirus bovine	5	11	16	21
calicivirus canine	10	21	31	41
calicivirus feline	9	19	28	38
coxsackie virus B5	8	17	25	34
hepatitis A virus	6	11	17	22
poliovirus type 1	7	15	22	30
rotavirus SA-11	10	20	29	39
protozoa				
Acanthamoeba[c]	40	71	119	167
Cryptosporidium[c]	3	6	12	_[e]
Giardia[c]	2	5	11	_[e]

[a] Environmental spp.; [b] maximum inactivation concentration < 4log; [c] No correction for environmental spp. (research needed); [d] corrected for environmental spp.; [e] No value due to tailing

1.5.2.2. Membrane filtration

Membrane filtration can provide virtually absolute barriers to pathogens, provided the pathogens are larger than the nominal pore size (i.e. average pore size), although certain characteristics of the microorganisms and water matrix and imperfections of membranes and unit seals can influence the efficiency of this barrier to a certain degree (Table 1.11) (Buckley & Hurt, 1996; Delebecque et al., 2006; Sadr Ghayeni et al., 1999; Hirani et al., 2010; Langlet et al., 2009; Madaeni, 1999; Madaeni et al., 1995; Reith & Birkenhead, 1998, Van der Bruggen & Vandecasteele, 2003; Wang et al., 2006a; Wijmans & Baker, 1995). A summary of studies on retention efficiency of microorganisms is presented in Table 1.12.

Table 1.11. Factors influencing removal efficiency of membrane filtration

Factors enabling passage of microorganisms with average size greater than the nominal pore size
-presence of abnormally large pores
-presence of ultramicrobacteria
-the transmission can increase with increasing pressure (increasing flux)/ high operating pressure can lead to deformation and cell volume reduction
-imperfections of the membrane (cracks) and unit seals
Factors inhibiting passage of microorganism of smaller size than the membrane pores
-association with particles or microorganisms (viruses with bacteria) or aggregates of microorganisms of same species/virus strain
-pore blockage by particles or bacteria
-electrostatic repulsion and adsorption can lead to retention of microorganisms smaller than the nominal pore size

Table 1.12. Microbial retention efficiency of membrane processes

Reference	Membrane	Nominal pore size	Efficiency
Ohtani et al., 2000	MF	0.1-3 µm	start of *Pseudomonas solanasearum* removal at 1 µm, >7 log reduction at 0.1 µm
Madaeni et al., 1995	MF	0.22 µm	1.3-2.4 log removal of poliovirus, depending on time, pressure, presence of *E. coli*
Hsu & Yeh, 2003	MF	0.1 µm	complete interception of *Giardia* and *Cryptosporidium* oocysts
Langlet et al., 2009	MF	0.1 µm	1.8 log removal of MS2 phage (adsorption)
Lonigro et al., 2006	UF	0.03 µm	3.6 log removal of *Giardia* cysts (transmission due to imperfect sealing), total removal after maintenance
Cabassud et al., 2001	UF	0.01 µm	total removal of bacteria and viruses
Langlet et al., 2009	UF	0.01 µm (MWCO 100 kDa)	>6 log removal of MS2 phage
Di Zio et al., 2004	UF	50 kDa MWCO	total removal of total coliforms and fecal coliforms
Madaeni et al., 1995	UF	30 kD	complete removal of poliovirus
Oron et al., 2008	UF	20 kDA MWCO	2.6-3.4 log of indigenous MS2 phage
Oron et al., 2008	UF	20 kDA MWCO	5 log reduction of fecal coliforms
Van der Bruggen & Vandecasteele, 2003	NF	300-250 Da MWCO	4-6 log units of MS2 phage
Van der Bruggen & Vandecasteele, 2003	NF	200 Da MWCO	6 log reduction of coliphage Q beta, 7 log poliomyelitis virus
Castro et al., 2008	NF		total removal of total and fecal coliforms and almost all somatic coliphages
Cooper & Straube, 1979	RO		7.3 log reduction of poliovirus, 5 log of coliphages
Regunathan et al., 1983	RO		virtually complete removal of total coliforms with RO-carbon adsorptive devise (less than 1/100 mL; starting from 10^4-10^7/100 mL)
Gerba et al., 1997	RO		>6 log reduction of bacteria, >4 log of viruses, > 3 log of *Cryptosporidium* oocysts; portable drinking water treatment system

1.5.2.3. Ultrasound

The ability of US to disinfect is based on the generation of acoustic cavitations (Shimizu et al., 2010). Disinfection by US comprises a physical factor (high temperature, pressure), a mechanical factor (shear stress caused by implosion) and a chemical factor (generation of HO• radicals, H_2O_2 and other oxidants by pyrolysis due to the high local temperatures). Through these mechanisms, US can produce membrane impairment and cell lysis and also damage DNA, organelles and cause functional and biochemical changes (Antoniadis, 2007; Dadjour et al., 2006; Joyce et al., 2003; Madge & Jensen, 2002; Mahamuni & Adewuyi, 2010; Mason et al., 2003; Shimizu et al., 2010). It is generally observed that the mechanical factor is most crucial for microbial inactivation. Although hot zones can inactivate some bacteria, they are very localized and therefore have limited influence (Furuta et al., 2004; Hulsmans et al., 2010; Mason et al., 2003; Piyasena et al., 2003).

Microbial inactivation efficiency increases by adding titanium dioxide (TiO_2) (sonocatalytic disinfection) or synergetic effects occur (Dadjour et al.,2005, 2006; Drakopoulou et al., 2009; Shimizu et al., 2010). The formation rate of cavitation bubbles is enhanced by the presence of heterogeneous particles by providing additional nuclei, thus causing increased formation of hot spots, leading to enhanced HO• radical generation. The presence of inert particles cannot fully explain the increased radical formation, as inert particles like Al_2O_3 showed significantly less oxidizing power than TiO_2. The production of excited positive holes on the TiO_2 surface (produced thermally by the hot spots), which react with water, increases HO• radical formation (Drakopoulou et al, 2009; Shimizu et al., 2010). Besides the chemical factor, the physical and mechanical factors are also enhanced, because of increased cavitation bubble formation and subsequent implosion. Addition of radical scavengers in US/TiO_2 experiments lowered inactivation, illustrating the significance of HO• radicals in US/TiO_2 disinfection treatment (Dadjour et al.,2005, 2006; Shimizu et al., 2010).

Disinfection efficiency of US towards bacteria can be ranked as following: gram-negative > gram-positive > bacterial spores. Little research has been conducted on the resistance of viruses and protozoa, though it is known that yeasts and protozoa show relatively high resistance to US compared to gram-negative bacteria. The resistance is presumably based on cell wall strength (Blume & Neis, 2004; Drakopoulou et al., 2009; Furuta et al., 2004; Gogate et al., 2007). Feline herpesvirus type 1 (enveloped) was significantly inactivated while feline calicivirus (non-enveloped) was not. The viral capsid is less easily destroyed than the viral

envelope, which is analogous to the higher resistance of non-enveloped viruses to environmental stress (Scherba et al., 1991). A summary of studies on the disinfection efficiency of US is presented in Table 1.13.

1.5.3. Influence of the state of the microorganism on disinfection efficiency

The **growth conditions** and **growth stage** of microorganisms can influence the disinfection efficiency. When grown under quasi natural aquatic conditions, when the specific growth rate is lower, or when under nutrient limitation or even starvation, the resistance of bacteria against chemical disinfectants and UV increases (Berg et al., 1982; Berney et al., 2006; Cromeans et al., 2010; Doull et al., 1980; Hijnen et al., 2006; Hoff & Akin, 1986; Koivunen, 2007; Lee & Frank, 1990; Lisle et al., 1999; Luh & Marinas, 2007; Taylor et al., 2000). Overall, the difference in UV resistance between environmental and lab-cultured microorganisms is more explicit for bacterial spores and vegetative bacteria than for viruses (Hijnen et al., 2006; Li et al., 2009). Gogate (2007) described cell size, cell shape, and development stage as some microorganism status related factors influencing disinfection efficiency with US. Larger cells of a species (dividing cells, diploid cells in yeast life cycle) experience greater tensile stresses in velocity gradients from cavitation (i.e. more sensitivity to US) than smaller cells. Also, it was suggested that compact bacteria were more resistant to cavitational effects than more elongated bacteria (Thacker et al., 1973).

Exposure to chemical disinfectants can induce **aggregation** of microorganisms. Aggregation of *E. coli* was observed after exposure to chlorine or O_3 (Arana et al., 1999) and of *Bacillus subtilis* when exposed to ClO_2 (Barbeau et al., 2005). Aggregates of *Mycobacteria*, bacteria, viruses and protozoa show increased resistance against chemical disinfectants (Barbeau et al, 2005; Bohrerova & Linden, 2006; Dietrich et al., 2007; Koivunen, 2007; USEPA, 1999a).

Biofilms primarily consist of microbial cells and exopolymeric substances. The ability of biofilm formation is almost ubiquitous among bacteria (Van Houdt & Michiels, 2010). Biofilms can consist of a collection of microorganisms including bacteria, protozoa, fungi, algae, and amoebae (Jang et al., 2006). It is considered to be the prevailing microbial life-cycle in most environments and renders the inhabitants more resistant to nutritional and oxidative stresses, desiccation and UV light exposure (Kim & Wei, 2012). In general, biofilm associated microorganisms are 2-3 orders of magnitude more resistant to disinfectants than planktonic ones (Simões et al., 2010). As biofilms can consist of multiple species, the biofilms can augment the resistance of a microorganism to water disinfection, as was for

example shown for a binary species biofilm of *Salmonella* Typhimurium in association with *Pseudomonas fluorescens*, which enhanced resistance of the former to chlorine (Leriche & Carpentier, 1995). Factors that determine the efficiency of disinfectants against biofilms compared to bacteria in suspended state include: disinfectant diffusion capacity, reaction with exopolymeric substances, biofilm density and age, and fluid flow conditions (Gagnon et al., 2005; Jang et al., 2006; Vaid et al., 2010; Van Houdt & Michiels, 2010). This means that the most efficient disinfectant against planktonic cells in a certain case does not necessarily exhibit the best performance in preventing and destroying biofilms. For example, while chlorine is much more efficient in reducing free roaming heterotrophic bacteria than monochloramine, the relative efficiency of monochloramine to kill biofilm associated heterotrophic bacteria compared to suspended ones is much higher than that of chlorine, because the former does not readily react with exopolymeric substances allowing better penetration in the biofilm (LeChevallier et al., 1988).

1.6. INFLUENCE OF PHYSICOCHEMICAL WATER QUALITY ON WATER DISINFECTION EFFICIENCY

1.6.1. Particulate and dissolved organic and inorganic matter

Particulate matter in water can decrease UV disinfection by absorbing or scattering UV light. In addition, when the reaction of the particles with chemical oxidants is significant, it can pose a disinfectant demand. Particle association of microorganisms describes the attachment to or entrapment within particles. Through particle association, diffusion resistance to chemical disinfectants can occur, and microorganisms can be shielded from UV (Dietrich et al., 2007; Li et al., 2009; Mamane & Linden, 2006). The influence of particle association on UV inactivation efficiency in wastewater depends on the size of the microorganism and the size of the particles. Particles less than 2 µm are large enough for protecting viruses and particles larger than 10 µm for protecting coliforms from UV. Protozoan (oo)cysts are larger in size, so effective shielding is less probable. Furthermore, protozoan parasites are in a dormant state and less likely to attach to particles (Li et al., 2009). Particle association significantly lowered inactivation of viruses, vegetative bacteria, and bacterial spores with UV (Havelaar et al., 1987; Mamane-Gravetz & Linden, 2004; Örmeci & Linden, 2002; Qualls et al., 1983; Templeton et al., 2003). Particle associated coliforms in primary sewage effluent were better protected from chlorine disinfection when associated with particles > 7 µm compared to particles < 7 µm (Berman et al., 1988). When comparing

inactivation of particle associated and non-associated coliforms in waste water, both UV and chlorine effectively inactivated non-associated coliforms, whereas chlorine was more efficient than UV for inactivation of particle associated coliforms, with contact time being the most important factor (Örmeci & Linden, 2002).

As described in section 1.3, chemical oxidants react with certain **molecular species in water**, and soluble forms of organics, metals (e.g. iron) and some anions (e.g. nitrate, sulfites) absorb UV irradiation, as such lowering the UV dose absorbed by the microbial targets (Bolton et al., 2001; USEPA, 2006). Surface waters and post-harvest waters have a certain load of organic and inorganic molecules that pose a disinfectant demand on chemical oxidants. As such, the effective exposure of microorganisms to chemical disinfectants is lower than the applied dose resulting in a detrimental effect on the disinfection efficiency. Due to their characteristic reaction kinetics with water matrix constituents, the disinfectant demand differs among chemical oxidants. All chemical oxidants applied for water disinfection show some immediate disinfectant demand which is characterized by a very rapid disinfectant decomposition. This is due to presence of certain reduced metals, inorganics, and organics with high affinity for the respective disinfectant. After this immediate demand a slower disinfectant decomposition occurs. Decrease in disinfectant residual is further attributed to volatilization and reaction with water, and pH and T conditions (Dell'Erba et al., 2004; Falsanisi et al., 2006; Janex et al., 2000; Veschetti et al., 2003; Xu et al., 2002). The extent of disinfectant demand depends on the water matrix and the disinfectant. This behavior has mostly been assessed in wastewater as it is more severe than in drinking water disinfection because of the higher physicochemical load. Although exact numbers of oxidant demand have little relevance as they depend on the application and the fact that real life water matrices are complex, they do provide qualitative information concerning the behavior of chemical oxidants in physicochemical loaded waters. Reactivity with water matrix constituents (and as such disinfectant demand) seems to increase in the following order: PAA < ClO_2 << chlorine and O_3 (Collivignarelli et al., 2000; Dell'Erba et al., 2004; Gehr et al., 2003; Gordon & Rosenblatt, 2005; Koivonen & Heinonen-Tanski, 2005a; Veschetti et al., 2003). Concerning H_2O_2, relatively little is known, although Koivonen & Heinonen-Tanski (2005a) and Veschetti et al. (2003) observed a lower decomposition of H_2O_2 than of chlorine in municipal wastewater. However, as H_2O_2 can be enzymatically removed by catalase, the presence of this enzyme (in a still active form) has impact on the H_2O_2 stability.

Table 1.13. Microbial inactivation efficiency of US

Reference	Microorganism	Power (P) in kiloWatt	Volume (L)	intensity(I) in kW/L	time (min)	Specific acoustic energy Es (kJ/L)	W/cm²	Frequency (kHz)	Reduction (log)
Bacteria									
Phull et al., 1997	*E. coli*				15		15	38	0.7
Hua &	*E. coli*	0.08	0.30	0.27	60	960		20	1.8
Thompson,	*E. coli*	0.09	0.30	0.28	60	1020		20	2.5
2000	*E. coli*	0.14	0.30	0.47	60	1680		20	2.7
Antoniadis et al., 2007	*E. coli*	0.15	0.10	1.50	30	2700		80	5
Dadjour et al.,	*E. coli*	0.20			30			39	0.06
2005	*E. coli* (0,5 g/l TiO2)	0.20			30			39	1.7
Madge & Jensen, 2002	fecal coliforms (wastewater)			0.70	6			20	4
Drakopoulou,	fecal coliforms	0.30	0.20	1.50	60	5400		24	3.9
2009	total coliforms	0.30	0.20	1.50	60	5400		24	3.3
	total coliforms (5 g/l TiO_2)	0.30	0.20	1.50	60	5400		24	3.9
	fecal coliforms (5g/l TiO_2)	0.30	0.20	1.50	60	5400		24	5.4
	fecal streptococci	0.30	0.20	1.50	60	5400		24	0.9
	fecal streptococci (5 g/l TiO_2)	0.30	0.20	1.50	60	5400		24	0.6
	Pseudomonas spp.	0.30	0.20	1.50	60	5400		24	3.2
	Pseudomonas spp. (5 g/l TiO2)	0.30	0.20	1.50	60	5400		24	4.4
Scherba et al.,	*Pseudomonas aeruginosa*				30		3	26	0.55
1991	*Stapylococcus aureus*				30		3	26	0.24

Dadjour et al.,	*Legionella pneumphila*	0.30	5.80	0.05	30	93		36	0.09
2006	*Legionella pneumphila* (0,2 g/l TiO2)	0.30	5.80	0.05	30	93		36	1.22
Shimizu et al.,	*Legionella*	0.20			30			36	0.09
2010	*Legionella* (1 g/l TiO2)	0.20			30			36	1.5
Mason et al., 2003	bacteria (viable plate count)				20		0.6	38	0.29
Scherba et al., 1991	*Bacillus subtilis*				30		3	26	0.62
Joyce et al.,	*Bacillus subtilis*	0.05	0.20	0.24	15	216		20	0.05
2003	*Bacillus subtilis*	0.05	0.15	0.32	15	288		20	0.4
	Bacillus subtilis	0.05	0.10	0.48	15	432		20	0.7
Drakopoulou,	*Clostridium perfringens*	0.30	0.20	1.50	60	5400		24	0.7
2009	*Clostridium perfringens* (5 g/l TiO2)	0.30	0.20	1.50	60	5400		24	0.9
Viruses									
Scherba et al.,	feline herpesvirus type 1				60		3	26	affected
1991	feline calicivirus				60		3	26	not affected

Fouling on the lamp sleeves reduces the transmittance of UV light through the sleeve into the water and fouling on the monitoring windows affects measured UV intensity and dose monitoring. Hardness (as $CaCO_3$), alkalinity, temperature, ion concentration, ORP, and pH all influence the rate of fouling. Fouling can occur because of photochemical reactions, particle deposits and compounds for which solubility decreases as temperature increases (e.g. $CaCO_3$, $CaSO_4$, $MgCO_3$, $MgSO_4$, $FePO_4$, $FeCO_3$, $Al_2(SO_4)_3$). MP lamps foul faster than LP because of the higher operating temperatures (USEPA, 2006). Mass accumulation at the membrane surface is a natural consequence of separation. Fouling/scaling is a crucial factor in operating membrane filtration processes. Membrane fouling is caused by particulate deposition (suspended matter, colloids), microbial adhesion and growth (biofilm formation) or adsorption of organic matter, which occurs in all membrane filtration processes. Scaling is the deposition of salts, when exceeding solubility due to high concentrations at the membrane surface and this occurs in NF and RO (Bacchin & Aimar, 2005; Boerlage, 2001; Sadr Ghayeni et al., 1998; Madaeni, 1999). Smaller particles may contribute more to fouling than larger particles as was shown for RO, presumably because larger particles experience higher velocities and shear force at the membrane. Pore blockage is generally higher in membranes with higher pore size, allowing more particle deposits (Ohtani et al., 2000; Winfield, 1979). Adsorbed organics may provide nutrients for microorganisms, promoting biofilm growth on membranes (Wang et al., 2006a).

The effects of organic matter and suspended solids on **US** efficiency are not straight-forward. On the one hand organic matter present in the water can react with generated HO• radicals and particles can shield microorganisms from shear forces. On the other hand, suspended solids may provide additional nucleation sites, thus enhancing disinfection (Antoniadis et al., 2007; Drakopoulou et al., 2009; Madge & Jensen, 2002). Antoniadis et al. (2007) observed negative influence of high COD (1200 mg/l) on disinfection. Other studies observed no significant influence of particulate and dissolved matter on US disinfection efficiency (Drakopoulou et al., 2009; Madge & Jenson, 2002). As in other processes which rely (partially) on radicals, scavengers have detrimental effects on HO• radical action and consequently on US inactivation efficiency (Antoniadis et al., 2007; Shimizu et al., 2010). Presence of scavengers may be of greater influence in US/TiO_2 disinfection as radicals play a significant and greater role than in stand-alone US and Madge & Jensen (2002) observed no influence of alkalinity (presence of scavengers) on US efficiency.

1.6.2. Temperature

In general temperature tends to increase the disinfection rate of **chemical oxidants**. However, temperature also increases the reaction rate with water matrix constituents (Corona-Vasquez et al., 2002; Luh & Marinas, 2007; Raffellini et al., 2011; Vicuna-Reyes et al., 2008; Zanetti et al., 2007). Also, the solubility of chlorine gas, ClO_2 and O_3 in water and the formation of HOCl due to reaction with H_2O decrease with increasing temperature. As such, O_3 disinfection is often preferable at lower temperatures, requiring lower O_3 doses (Collivignarelli et al., 2000; Doull et al., 1980; LeChevallier & Au, 2004; White, 2010). Temperature can influence the chlorine generation by electrochlorination. Slightly decreased chlorine formation occurred above 40 °C because of cathodic reduction of OCl^-. Also chlorate formation occurred above 40°C (Khelifa et al., 2004). Therefore, a higher temperature does not necessarily equal a better disinfection with chemical oxidants.

Minimal effect of temperature on **UV** irradiation efficiency has been observed in the range 5-35 °C for bacterial, viral and protozoan inactivation (Severin et al., 1983; Malley, 2000; USEPA, 1996, 2006). Viscosity of water decreases with increased temperature, leading to higher flux through **membranes** at constant transmembrane pressure. Algae growth, potentially causing problems for membrane operation, is enhanced by higher water temperature (USEPA, 2005). Raising the ambient temperature leads to easier bubble formation in **US** treatment, but the cavitation bubbles contain more vapor, which reduces the intensity of cavitation by cushioning the implosion (Thompson & Doraiswamy, 1999). However below 60°C, the power output is hardly affected. Also, US increases the temperature and attempts to control the temperature result in decreased disinfection as part of the US inactivation of bacteria can be attributed to exposure to generated heat (Madge & Jensen, 2002; Raso et al., 1999).

1.6.3. pH

As HOCl has a higher disinfection efficiency than OCl^- and a pKa around 7.5, chlorine is more efficient at acidic pH. However, pH values below 5 result in increased corrosion and formation of chlorine gas (Parish et al., 2003; White, 2010).

Generally, the microbial inactivation with ClO_2 is relatively stable in a wide pH range (pH 3 to 9) with possibly a slight increase in efficiency with increasing pH (Alvarez & O'Brien, 1982; Ayyildiz et al., 2010; Chang et al., 2000; Clark et al., 2003; Huang et al., 1997; Li et al., 2001a; Ruffell et al., 2000; Thurston-Enriquez et al., 2005a; Vicuna-Reyes et al., 2008).

H_2O_2 has a higher antimicrobial effect at acidic pH on bacteria and bacterial spores (Cords et al., 2005; Raffellini et al., 2008, 2011). Also, the H_2O_2 decomposition rate is reduced at lower pH, especially below pH 3 (Ortiz et al., 2000; Watts et al., 1999).

The pK_a of PAA is 8.2 and therefore the disinfection efficacy is reduced at higher pH values as the undissociated PAA is the biocidal form. PAA hydrolysis is negligible in the pH range 5.5 to 8.2 and generally lower pH values somewhat improve efficiency in that range (Kitis, 2004; Wagner et al., 2002; Zanetti et al., 2007).

As a higher formation of HO• radicals occurs during O_3 treatment at alkaline pH, a possible difference in inactivation efficiency can be expected depending on the relative contribution of both oxidants to the disinfection process. The expected ratio of the concentrations of HO• radicals and O_3 during ozonation of natural waters is typically in the range 10^{-7} – 10^{-9} (Audenaert, 2012). Considering the inactivation rate of *Cryptosporidium parvum* during ozonation ($kO_3 = 7 .10^2$ $M^{-1}.s^{-1}$), the inactivation rate of *Cryptosporidium parvum* with HO• radicals would have to be about 7.10^{10} $M^{-1}.s^{-1}$ for an equal contribution of both O_3 and HO• radicals to the disinfection process. Because this rate constant is about a factor of 10 higher than typically observed for HO• radical reactions, von Gunten (2003b) concludes that the contribution of HO• radicals to the disinfection process is fairly limited. It needs to be noted that in other waters than natural waters to produce drinking water the HO• to O_3 ratio can be different or change during the ozonation process, and depend on the relative composition of water matrix constituents of the influent (Audenaert, 2012). For *Cryptosporidium parvum* and some bacterial spores, HO• radicals could theoretically play some role in O_3 based AOPs (HO• to O_3 ratio of 10^{-6} can be expected), but as the reaction rate of O_3 with bacteria and viruses are 2 to 3 orders of magnitude higher than for *Cryptosporidium parvum*, it is most likely that even in O_3 based AOPs the effect of HO• radicals on disinfection will be insignificant (von Gunten et al., 2003b). O_3 disinfection is generally more efficient in slightly acidic pH (Harakeh & Butler, 1984; Lim et al., 2010b; Zuma et al., 2009) or relatively independent of pH in the range pH 6 to 9 (Domingue et al., 1988; Gyurek et al., 1999; Li et al., 2001b; Rennecker et al., 2001; USEPA, 1999a), as has been observed for viruses, bacteria, and protozoa. Some studies contradicted these results and noted the presence of shorter shoulders when inactivating *Bacillus subtilis* spores at alkaline pH, both in oxidant demand free water, in water containing humic acids and in river water, attributed to the HO• radical action (section 1.4.1.2) (Cho et al., 2003; Larson & Marinas, 2003), and higher disinfection

efficiency of *Giardia muris* cysts at alkaline pH (Wickramanayake, 1984b). However, the latter study observed a lower disinfection efficiency of *Naegleria gruberi* cysts at alkaline pH.

During electrochlorination, similar pH conditions as with chlorination are desired. At pH above 9, only OCl^- is present and the formation of HO• radicals is favored (Kerwick et al., 2005a). If chloride was absent and mainly HO• radicals were produced, pH showed no influence on efficiency in the range pH 5 to 9 (Cong et al., 2008). Alkaline pH in the vicinity of the cathode leads to precipitation of $CaCO_3$ and $Mg(OH)_2$ and when electrogenerating H_2O_2 in seawater, lower pH values reduced cathode fouling (Da Pozzo et al., 2008; Martinez-Huitle & Brillas, 2008).

UV Dose-response of microbial inactivation is independent of pH from pH 6 to 9 (USEPA, 1996; Malley, 2000). The pH effects on MS2 phage disinfection efficiency have been attributed to pH-induced clumping of the microorganisms (Malley et al., 1996).

As pH influences charges, pH alters electrostatic interaction between particle and membrane. Favorable conditions for adsorption can thus be created (Madaeni, 1999). Certain membrane materials must be operated in a certain pH range, e.g. cellulose acetate (and derivatives) in the pH range 4 to 8 (USEPA, 2005). High pH favors formation of $CaCO_3$ and $Mg(OH)_2$ scales (Boerlage, 2001).

Inactivation by US is not affected by pH in the range pH 6 to 8 (Madge & Jensen, 2002). Low pH ($pH \approx 2.5$) may increase sensitivity to US, comparable to a lower heat resistance at low pH (Salleh-Mack & Roberts, 2007).

1.7. DISINFECTION KINETICS

Disinfection kinetics are based on the disinfectant dose (chemical, irradiation, or US power consumption) and contact time. Several models have been constructed to describe the inactivation kinetics of microorganisms. As such, relative weights can be given to disinfectant dose and contact time in order to explain their relative influence on inactivation. Also, initial shoulders (or lag phases), and tailing-off (rapid inactivation followed by a decrease in disinfection rate) have been accounted for. Shoulders are attributed to inadequate mixing, delays in diffusion of disinfectant, or multiple targets on the microorganism that need to be affected. Tailing-off behavior results from the presence of subpopulations with varying resistance, a distributed inactivation resistance within the population of the microbial species, clumping of microorganisms, particle association, or disinfectant consumption (Gyürek &

Finch, 1998; Madge & Jensen, 2006). As the disinfectant concentration can change due to consumption, disinfectant decay needs to be accounted for in kinetic models. Gyürek & Finch (1998) compiled a list of models describing microbial inactivation kinetics.

Disinfectant residual and contact time are not always the parameters that explain microbial inactivation. As O_3 is highly reactive with the water matrix and microorganisms, the disinfection in physicochemical loaded waters often occurs without presence of O_3 residual as the O_3 is rapidly consumed. As such, the disinfection can often be estimated by the O_3 dose transferred to the water (Hunt & Marinas, 1999; Janex et al., 2000; Xu et al., 2002). The higher the immediate O_3 demand, the higher the O_3 dose that is added to satisfy the demand. As such, the microorganisms are exposed to a higher O_3 dose and a higher level of inactivation is reached (Xu et al., 2002). A similar disinfectant demand versus inactivation trend was observed for chlorine disinfection of grey water although no significance could be attributed to this trend due to large standard deviations (Winward et al., 2008). This behavior can be explained by the fact that microorganisms also pose a disinfectant demand and as such are inactivated while the demand is met (Xu et al., 2002).

Water disinfection technologies show vastly different kinetic behaviors towards inactivation of microorganisms, dependent on the inherent disinfection efficiency as well as the influence of physicochemical quality of the water matrix on the disinfectant concentration.

1.8. ALTERNATIVE DISINFECTION TECHNIQUES

There are other technologies that have been and are being studied at present and which potentially could become mainstream technologies for water disinfection purposes.

1.8.1. Disinfection by metals

Because of their antimicrobial properties, particular metals have been employed as disinfectants since ancient times (Lemire et al., 2013). Cu and Ag induce cell lysis and death by attaching to the negatively charged bacterial cell surface, disrupting cell wall permeability, blocking cell respiration, and causing extra- and intracellular protein denaturation (Lin et al., 1998; Feng et al., 2000; Huang et al., 2008; Agnihotri et al., 2013). Feng et al. (2000) observed that exposure of *E. coli* and *Staphylococcus aureus* cells to Ag^+ led to transformation of the DNA into a condensed form, leading to loss of replication ability.

Today, water disinfection using metals has been researched in multiple forms (Al-Hakami et al., 2013). Metals can be used alone or combined, such as Fe, Cu, or Ag and this in the solid phase (You et al., 2005), or in ionic form (Meyer, 2001; Martinez et al., 2004; Jiang et al., 2007). Metals can be incorporated in a solid matrix, such as in zeolites (Rivera-Garza et al., 2000; Hrenovic et al., 2012), ceramic materials (Kim et al., 2004), metal nanoparticles (Choi et al., 2008) and coating of metal nanoparticles on surfaces such as plastic membranes (Mecha & Pillay, 2014).

Upon decrease of Ag particle size from µm to nm level, properties such as diffusivity, mechanical strength, and chemical reactivity are improved. Adsorption of Ag nanoparticles to the cell surface alters the membrane properties. Ag nanoparticles degrade lipopolysaccharide molecules and form pits in the membrane, increasing membrane permeability and penetration of Ag nanoparticles through membranes, followed by release of Ag^+ which results in DNA damage (Sondi & Salopek-Sondi, 2004; Li et al., 2008). ZnO nanoparticles exhibit antimicrobial activity against a broad spectrum of bacteria. Disorganization of bacterial membranes due to contact with ZnO nanoparticles has been observed, and there is indication of the production of reactive oxygen species, even under ordinary room light (Sawai, 2003; Adams et al., 2006; Huang et al., 2008; Jones et al., 2008; Li et al., 2008; Shi et al., 2013).

Combined with UV at 254 nm, Ag^+ enhanced photodimerization in viral genomes and resulted in synergetic viral inactivation (Rahn et al., 1973; Butkus et al., 2004). More recently, it has been shown that UV-A and visible light irradiation, both exhibiting virtually no antimicrobial activity by itself, enhanced the inactivation of *E. coli* and MS2 phage by Ag^+ disinfection. The photochemical reaction of Ag^+ with thiol groups in proteins of the microorganisms was suggested as inactivation mechanism (Kim et al., 2008). Zhao et al. (2013) observed 2 requirements for the antimicrobial effect of Ag nanoparticles combined with UV-A against *E. coli*: uptake of nanoparticles (particle size < 0.1 µm for effective uptake) inside the cells, followed by release of Ag^+ from the Ag nanoparticles due to oxidation of the nanoparticles by UV-A. ZnO nanoparticles were more toxic towards *E. coli* and *Bacillus subtilis* when the particles were preilluminated with UV-A or UV-B than with visible light, and the photo-dissolution of ZnO nanoparticles increased with UV preillumination, explaining the observed disinfection effect of the ZnO nanoparticles (Kim & An, 2012).

1.8.2. Combination of disinfection technologies

All studied individual techniques have their pros and cons. Some techniques are economically unviable for disinfection as stand-alone technique, but can enhance performance of other techniques if used in sequence or combined, serving as pre/posttreatment or inducing radical formation. In the last decade, much research has been conducted on the efficiency and possible synergy of combined disinfection techniques. Synergy implies that less chemicals or energy are needed to achieve a certain disinfection efficiency (Caretti & Lubello, 2003; Alkan et al., 2007). Because of the simultaneous execution of different disinfection mechanisms when combining certain disinfection techniques, a wider range of microorganisms can be attacked (Caretti & Lubello, 2003; Koivunen & Heinonen-Tanski, 2005a; Jung et al., 2008).

1.8.2.1. Sequential or combined use of disinfection technologies

Promising results have been reported if **chemical oxidants were combined or subsequently used**. Exposure to one oxidant can lead to easier penetration/increased damage by another oxidant (Li et al., 2004c; Zhang et al., 2007). Sequential use of O_3 with monochloramine/free chlorine (Biswas et al.,2003, 2005; Cho et al., 2006; Koivunen, 2007), combined or sequential use of chlorine and monochloramine (Kouame & Haas, 1991; Zhang et al., 2007), and (regardless of the order) sequential ClO_2 and free chlorine (Cho et al., 2006), all resulted in synergetic effects.

Combining UV with chlorine or ClO_2 has also been studied. Cho et al. (2006) reported no synergy in sequential use of UV and chlorine but Lotierzo et al. (2003) did and also with sequential UV and ClO_2, the latter independently of the disinfectants order.

US, as stand-alone technique, is not efficient at the moment due to the high energy demand for disinfection (Madge & Jensen, 2002; Joyce et al., 2003; Gogate, 2007; Drakopoulou et al., 2009). Combining US with other disinfection treatments (combination or sequence) has shown improved results compared to the individual techniques. Pretreatment with US can reduce particle size, thus reducing the masking of microorganisms and it can also scatter clumps of microorganisms, making them more available for UV or chemical disinfectants. Furthermore, US can inactivate microorganisms to some degree, or weaken cell walls, making them more susceptible to other treatments. Simultaneous operation can enhance mass transfer of chemical disinfectants and as such improve contact of the chemical with the microorganism (Gogate, 2007). Beneficial effects of combining US with other techniques include shorter contact times, lower needed concentration of the disinfectant or lower electrical power input. UV lamps showed no fouling through the mechanical cleaning effect of US cavitation

(Naddeo et al., 2009). Combinations of US with chlorine, electrolysis, UV, H_2O_2 and O_3 all have shown increased inactivation efficiencies compared to the individual treatments (Phull et al., 1997; Blume & Neis, 2004; Duckhouse et al., 2004; Gogate, 2007).

1.8.2.2. Advanced oxidation processes

AOPs involve the generation and reaction of HO• radicals. AOPs have been widely studied for oxidizing organic matter (Caretti & Lobello, 2003; Meunier et al., 2006; Bandy, 2009; Sarathy & Mohseni, 2010). HO• radical attack is very reactive yet not very selective, which can be an advantage for oxidation of pollutants with a high stability or low biodegradability. However, this is not always an advantage for water disinfection in high organic loaded waters (Blanco-Galvez et al., 2006). AOPs include O_3/UV, H_2O_2/UV, PAA/UV, TiO_2/UV, Fenton reaction ($H_2O_2/Fe^{2+ \text{ and } 3+}$), and TiO_2/US. Recently more attention has been given to those AOPs that can function through solar irradiation, i.e. light with a wavelength > 300 nm (sun light/TiO_2 and photo-Fenton) due to reduced costs (Blanco-Galvez et al., 2006; De la Hoz et al., 2009; Malato et al., 2009).

For disinfection purposes **UV has been used for photolysis of H_2O_2, PAA or O_3**, thus generating free radicals (Bianchini et al. 2002; Koivunen & Heinonen-Tanski, 2005a; Jung et al. 2008). Another disinfection mechanism of these combined techniques has been suggested: the multiple damage mechanism, meaning UV targets nucleic acids while the chemical disinfectant attacks cellular structures and enzymatic systems (Koivunen & Heinonen-Tanski, 2005a). H_2O_2 is unfeasible as stand-alone disinfection technique due to the low inactivation potential, although efficiency can somewhat be enhanced if H_2O_2 is combined with $Fe^{2+ \text{ and } 3+}$, Ag^+, or $Cu^{+\text{and}2+}$ (Pedahzur et al., 1997, 2000; Batterman et al., 2000; Tofant et al., 2006; de Velasquez et al., 2008; Toté et al., 2009). Combined with UV, the effectiveness of H_2O_2 is questionable. In some cases shorter treatment times were needed compared to stand-alone UV but no significant synergy was observed (Alkan et al., 2007; Labas et al., 2009), whereas Koivunen & Heinonen-Tanski, (2005a) observed antagonistic effects. By contrast, experiments on combined use of UV with O_3 or PAA overall resulted in promising results. Enhanced efficiency or synergy was observed with O_3/UV (Meunier et al., 2006; Oh et al. 2007; Jung et al., 2008; Mishchuk et al. 2008) and PAA/UV (Lubello et al., 2002; Caretti & Lubello, 2003; Koivunen & Heinonen-Tanski, 2005a). An advantage of all three techniques is the decreased re-growth potential compared to the stand-alone techniques (Alkan et al., 2007; Martin & Gehr, 2007; Yasar et al., 2007). No harmful by-products were formed with

H_2O_2/UV treatment (Kruithof et al., 2002, 2007) and O_3/UV showed decreased bromate formation compared to stand-alone O_3 treatment, due to the lower O_3 doses (Collivignarelli & Sorlini, 2004; Meunier et al., 2006).

O_3 can also be combined with H_2O_2 as an AOP **(peroxone)**. This combination increases HO• radical formation, which is the fundamental difference with ozonation, which relies heavily on direct O_3 oxidation. The net result is that for oxidation purposes, peroxone is often superior to stand-alone O_3 (Wolfe et al., 1989a, 1989b; USEPA, 1999a; Wu et al., 2008; Can & Cakir, 2010). For disinfection purposes however, this is generally not the case. Some studies indicated that for inactivation of bacteria and viruses, peroxone was less efficient (Wolfe et al., 1989a, Can & Cakir, 2010) or equally efficient (Wolfe et al., 1989b; Hall & Sobsey, 1993; Sommer et al., 2004) as stand-alone O_3. Superior disinfection of peroxone was observed when disinfecting *Clostridium perfringens* (Lanao et al., 2008). The molar ratio of H_2O_2/O_3 dictates the disinfection efficiency. An optimal molar ratio for disinfection of 0.3 was found (USEPA, 1999a). Wolfe et al. (1989a) observed that the bactericidal efficiency of peroxone decreased drastically if the molar ratio was increased from 0.5 to 0.8 but not at lower ratios. These results suggest that presence of O_3 residual is the determining factor in peroxone disinfection and that peroxone in itself is generally not that interesting for disinfection purposes.

The **Fenton reaction** works through the decomposition of H_2O_2 by $Fe^{2+ \text{ or } 3+}$, Fe^{2+} being regenerated through reaction with H_2O_2, resulting in HO• radical production (reaction 11 and 12) (Rincon & Pulgarin, 2006). The process is optimal at pH 3 and above pH 4 Fe^{3+} precipitates as iron hydroxide (Neyens et al., 2002).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + OH^- \quad (11)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+ \quad (12)$$

When disinfecting *E. coli* cells by the Fenton reaction, substantial leakage of intracellular materials was observed and the damage was attributed to chemical products with a high oxidizing power (such as HO• radicals) (Diao et al., 2004). Fenton reaction rates are increased by UV-Vis irradiation, i.e. the photo-Fenton reaction. The irradiation of Fe^{3+} regenerates Fe^{2+} photochemically, generating an additional HO• radical compared to the Fenton process, with light sensitivity of the system up to 600 nm. As such the amount of usable photons and the depth of light penetration of usable photons is higher than with solar disinfection (SODIS) (Blanco-Galvez et al., 2006; Rincon & Pulgarin, 2006; Spuhler et al., 2010).

Heterogenous photocatalysis functions by inducing a series of oxidative and reductive reactions at a photocatalyst surface (the semiconductor TiO_2 has generally been shown to be the most active) by photon energy (wavelength < 400 nm) (Chong et al., 2010). Although the exact identities of the involved reactive oxygen species remain unclear, the HO• radical plays an important role in photocatalysis (wavelength in the range 300 – 400 nm) with TiO_2 (Cho et al., 2002; Yu et al., 2005), and it was observed to be the primary disinfectant for inactivation of *E. coli* (Cho et al., 2004). Photo-activated TiO_2 damages the cell membrane, as such promoting intracellular leakage as was shown for vegetative bacteria (Saito et al., 1992), and induces lipid peroxidation (Sunada et al., 1998; Maness et al., 1999; Blanco-Galvez et al., 2006). The increasing permeability of the cell allows the TiO_2 particles to enter the cell and attack intracellular structures and enzyme systems (Huang et al., 2000; Sunada et al., 2003). Using a lamp with emission between 300 and 800 nm the disinfection rate of *E. coli* in deionized and natural water followed the following order: TiO_2/H_2O_2/UV-Vis > TiO_2/UV-Vis > Fe^{3+}/H_2O_2/UV-Vis > Fe^{3+}/UV-Vis ≈ H_2O_2/ UV-Vis > UV-Vis (Rincon & Pulgarin, 2006). In general, the main technical issue that obstructs commercialization of photocatalytic water treatments is the post-recovery of the catalyst particles after water treatment (Chong et al., 2010).

The contribution of HO• radicals to the disinfection efficiency differs among described AOPs. This can be explained by i) the relative efficiency of the disinfection techniques in the combination of disinfection techniques compared to the disinfection efficiency of HO• radicals, and ii) the relative amount of HO• radicals produced. Peroxone in general has no added effect on disinfection efficiency compared to ozonation. This can be explained by the ratio of HO• radicals to O_3 as described in section 1.6.3. On the contrary, in the disinfection process with the Fenton reaction and with heterogeneous photocatalysis HO• radicals play a significant role. H_2O_2 is a relatively slow water disinfectant (section 1.5.1). As such, it is reasonable to assume that the contribution of HO• radicals (produced by the Fenton reaction) will be relatively higher than in the peroxone process (where the rapid water disinfectant O_3 is involved, section 1.5.1 and section 1.6.3). Heterogeneous photocatalysis with UV exposure in the range 300 – 400 nm will have little to no contribution of the UV exposure to the overall disinfection (contrary to e.g. highly lethal UV irradiation at 254 nm, section 1.5.2). Therefore it is understandable that the contribution of HO• radicals to the heterogeneous photocatalytic disinfection process is significant. To illustrate, Cho et al. (2004) found that > 100 min were needed for a 2 log *E. coli* reduction in oxidant demand free conditions with heterogeneous

photocatalysis (pH 7, 20°C, 2 g/L TiO_2, $7.9.10^{-6}$ $Einstein.L^{-1}.s^{-1}$, UV in the range 300-400 nm). Considering the production of HO• radicals in this process, the CT value to inactivate 2 log *E. coli* with the produced HO• radicals was 5.10^{-10} M.min. To compare, the CT value to inactivate 2 log *E. coli* with O_3 (at 20°C and pH 7) is 2.10^{-7} M.min (von Gunten, 2003b). In the case of photocatalytic disinfection, there was no inactivation in the absence of TiO_2, so there was no contribution of the UV light to the inactivation (Cho et al., 2004). However, in O_3 based AOPs, such as peroxone, where HO• to O_3 ratios of 10^{-6} can be expected (von Gunten, 2003b), based on the given CT values for HO• radicals and O_3, the HO• radicals would not significantly contribute to the inactivation of *E. coli*.

1.9. INTEGRATION OF DISINFECTION TECHNIQUES IN THE WATER TREATMENT SYSTEM IN PRE- AND POSTHARVEST PRACTICES AND THE USE OF PRETREATMENTS.

Surface water contains particles and organics originating from urban and industrial sources (Delpla et al., 2009), as well as from biota. Organics can be introduced by biota growing in water bodies by excretion or decay of these organisms but also by degradation of terrestrial vegetation (Volk et al., 2002). Considerable fluctuations in turbidity and organic matter may occur through seasonal changes that influence precipitation and surface runoff, imposing an additional challenge for treating surface water (Shrestha & Kazama, 2007; Massé et al., 2011). During processing, wash water accumulates sugars, starches, other organic materials and residual pesticides, and thus the process water may contain considerable amounts of COD (Tarver, 2008). The degree of accumulation of suspended solids and organic matter in postharvest washing operations, and thus the magnitude of influence on the disinfection efficiency, depends on the type of crop, the product to water ratio, the degree of water recycling, and whether it concerns whole produce or fresh-cut produce (Humphries & Fleming, 1996; Luo, 2007; Selma et al., 2008a, 2008b). If the produce is pre-washed before transport to the processing area, field soil on the produce and plant exudates released from harvest cuts are removed to some degree, which will reduce the disinfectant demand of the water (Suslow, 2000a).

To achieve sufficient microbial abatements in highly turbid/organics loaded waters without having to apply excessive amounts of chemical disinfectants/energy consumption, avoid a high DBPs production, or to prevent compromising normal operation (e.g. excessive

membrane fouling), implementing a physicochemical treatment might prolong water recirculation in postharvest practices or facilitate water disinfection of water intended for pre- and postharvest practices (Collivignarelli et al., 2000). For example, Suslow, (2004b) states that filtration is essential for any O_3 use in recirculated water systems such as flumes, hydrocoolers or for rinsing, because otherwise the build-up of suspended solids would be a too big interference to achieve proper disinfection. Besides the disinfection context, eventually water constituents like BOD, suspended solids, phosphorous and nitrogen must be removed to acceptable levels from the polluted process water before discharge in surface waters. Processors may choose to implement their own wastewater treatment systems which can be a considerable investment, or treatment can be done by wastewater treatment plants, which can translate in costly sewage treatment levies in case of highly loaded wastewaters (Casani et al., 2005; Tarver, 2008).

There is a great amount of available technologies that are used or experimented with to remove COD and turbidity in water treatment including membrane processes, rapid and slow sand filtration, biodegradation (including sequencing batch reactors, biological aerated filters, membrane bioreactors), oxidation and AOPs, activated carbon, electrochemical oxidation, electrocoagulation, and dissolved air flotation (Jung et al., 2008; Hyun & Lee, 2009; Yoo & Hsieh, 2010; Alvarez et al., 2011; Awang et al., 2011; Chen et al., 2011; Esparza-Soto et al., 2011; Gurel & Buyukgungor, 2011; Mahvi et al., 2011; da Silva et al., 2013; Prazeres et al., 2013).

1.10. CONCLUDING REMARKS

Outbreaks due to pathogens related to fresh produce have been detected and irrigation water is a possible source of contamination. Microbial contamination can be spread among crops during post-harvest washing. Therefore, water disinfection can be useful in both pre- and postharvest practices. Microbial inactivation kinetics of a range of microorganism types have been fairly well established for chlorine, ClO_2, O_3, H_2O_2 and UV. On the other hand, microbial inactivation studies for PAA in oxidant demand free conditions are lacking. Because PAA is promoted as an alternative to free chlorine for disinfecting fresh produce wash water, this should be studied in more detail for both relevant pathogenic and spoilage microorganisms. The production of DBPs due to chemical oxidants has been studied for drinking and wastewater conditions. In the case of H_2O_2 however, knowledge concerning the oxidation products that are formed is limited. Combinations of disinfection techniques

(including AOPs) have recently been studied quite extensively. Some questions remain concerning the relative contribution of the disinfectants in the combination towards the disinfection process, e.g. the contribution of HO• radicals in these disinfection processes. Better understanding of these processes could elucidate whether and where specific combinations of water disinfectants could be applied.

CHAPTER 2

ORGANIC ACID BASED SANITIZERS AND FREE CHLORINE TO IMPROVE THE MICROBIAL QUALITY AND SHELF-LIFE OF SUGAR SNAPS

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2. ORGANIC ACID BASED SANITIZERS AND FREE CHLORINE TO IMPROVE THE MICROBIAL QUALITY AND SHELF-LIFE OF SUGAR SNAPS

2.1. ABSTRACT

A screening in a sugar snaps packaging company showed a converged build-up of APC (ca. 6.5 log CFU/100 mL), Y&M, and LAB (both ca. 4.5 log CFU/100 mL) in the wash water in the absence of water sanitizer, and a low build-up of COD (30 ± 5 mg O_2/L) and turbidity (5.2 ± 1.1 NTU).

Decontamination experiments were performed in the lab with Purac FCC 80® (80% L(+) LA), two other commercial water sanitizers based on organic acids (NATRApHASE-ABAV®, and NATRApHASE-FVS®) and chlorine to evaluate their performance in reduction of the sugar snaps microbial load as well as their functionality as disinfectant of the wash water to avoid cross-contamination.

An additional 1 log reduction of APC on the sugar snaps was achieved with LA in the range 0.8 to 1.6 %, ABAV 0.5 %, and free chlorine 200 mg/L when compared to a water wash, while no significant difference in the numbers of Y&M was obtained when washing in sanitizer compared to water. There was no significant influence of the studied concentration and contact time on decontamination efficiency. Treatment with LA 0.8% resulted in a lower APC contamination on the sugar snaps than on the untreated and water washed samples for 10 days. Chlorine 200 mg/L was the only treatment able to maintain the Y&M load lower than the untreated samples throughout the entire storage duration. The use of water sanitizers could not extend the sensorial shelf-life. Microbial loads were not indicative/predictive for visual microbial spoilage (shelf-life limiting factor), whereas maturity and amount of damage at the calyx end of the pods were.

The APC wash water contamination (5.2 log CFU/100 mL) was reduced significantly by chlorine 20 to 200 mg/L (to 1.4 log CFU/100 mL), ABAV 0.5 to 1.5 % (to 2.7 log CFU/100 mL), FVS 0.5 % (to 2.7 log CFU/100 mL) and LA 0.8 to 1.6% (to 3.4 log CFU/100 mL). Only the use of chlorine enabled the reduction of the Y&M wash water contamination significantly (from 3.4 to 1.4 log CFU/100 mL). The low physicochemical build-up of the sugar snaps wash water during the industrial washing process makes free chlorine attractive as water disinfectant to prevent bacterial and fungal cross-contamination, whereas the sanitizers based on organic acids are not, due to their weak water disinfection efficiency.

2.2. INTRODUCTION

Most grown sugar snaps (*Pisum sativum* var. *macrocarpon*) in the world are produced for local markets. In the last decades however, there has been a rise in the production of non-traditional export crops, including sugar snaps. Industrialized countries import large quantities of sugar snaps from tropical developing countries (such as Kenya and Guatemala), in order to have a year round supply and because of the high labor costs involved with picking (Humphrey et al., 2004; Messiaen et al., 2004). The main spoilage microorganisms on beans and peas are *Pythium butleri*, the fungal plant pathogens *Rhizoctonia solani*, *Sclerotinia* spp, and *Botrytis cinerea* and the pectinolytic bacterium *Erwinia carotovora* that breaks down the pectic substances of the middle lamella, with consequential loss of mechanical protection and rigidity (Walker et al., 1998; Tournas, 2005; Brummell, 2006). The production of acid or antimicrobial compounds by native microbial flora may interfere with the colonization, survival and proliferation of foodborne pathogens (a.o. *Salmonella* spp., pathogenic *E. coli*, *L. monocytogenes*) (Liao and Fett, 2001; Johnston et al., 2009; Shi et al., 2009; Teplitski et al., 2011). On the other hand, the chances of bacterial pathogen proliferation on and internalization in fresh produce are improved by the disruptive actions of certain fungal and bacterial spoilage microorganisms on the plant tissues (Ryser et al., 2009; Critzer and Doyle, 2010; Brandl & Sundin, 2013). Sugar snaps from Guatemala, which can be consumed either raw or cooked, have been the suspected vector of a *Shigella dysenteriae* outbreak in Sweden (May-June 2009) (Lofdahl et al., 2009) and a second *Cyclospora cayetanensis* outbreak (June 2009) was also reported in Sweden, with sugar snaps from Kenya as the suspected source (Insulander et al., 2010).

Organic acids applied at relatively low concentrations exhibit inhibitory effects on microbial growth and are used to preserve acid foods and beverages. At higher concentrations organic acids can be used as decontaminants of food products such as fruits and vegetables and meat carcasses to improve food safety and quality (Virto et al., 2006). Organic acids are weak acids, and therefore they exist in a pH dependent equilibrium between the dissociated and undissociated states. The uncharged, undissociated acid can diffuse across the plasma membrane of microorganisms. Inside the cell the organic acid deprotonates, causing a pH drop and accumulation of toxic anions. As such, membranes can be disrupted, the proton motive force dissipated, essential metabolic reactions inhibited, and the intracellular pH homeostasis stressed (Brul and Coote, 1999; Capozzi et al., 2009).

Chlorine is the most used water disinfectant in fresh produce washing processes because of the low cost, the proven ability to rapidly inactivate suspended bacteria, and the minimal impact on the nutritional and sensorial fresh produce quality. Drawbacks of chlorination are the possibility of chlorine gas generation in the working environment when incorrectly applied (i.e. below pH 5 and excessive dosing), the rapid decomposition in the presence of organic matter, and most notoriously the possibility of creating harmful DBPs in the wash water (Section 1.4.1).

After transport by airplane or container ship, sugar snaps are washed for rehydration and removal of materials from the pod surface. The objective of this chapter was to evaluate the use of water sanitizers for the reduction of the sugar snaps microbial load and extension of shelf-life as well as their functionality as disinfectant of the wash water to prevent cross-contamination. An on-site screening of an industrial sugar snaps washing process in the absence of water sanitizers was performed in a packaging company to observe the evolution of microbial and physicochemical parameters in function of processing time. In a second step, lab-scale experiments were performed with commercial formulations based on organic acids as produce and wash water sanitizers, and with chlorine as reference method.

2.3. MATERIALS & METHODS

2.3.1. The sugar snaps

The sugar snaps used in the experiments originated from Peru and Guatemala. They were transported to the packaging company by container ship during 19 to 22 days at 3 ± 1 °C in modified atmospheric packaging (O_2 < 10%, CO_2 > 0.5%, Xtend®, StePac, Israel). Experiments were performed on 5 different batches (1 from Peru for the evaluation of the washing process in the packaging company and 4 for the decontamination experiments, of which 3 from Peru and 1 from Guatemala) that were sampled at different dates in the period October – December 2012. The acquired sugar snaps for the decontamination trials were as they were delivered to the packaging company, i.e. unwashed in crates of 4.5 kg.

The state of the pod at the calyx end, the amount of mechanical damage on the sugar snaps and the size of the seeds in the pod, were compared among batches at reception, in order to be able to observe the impact of these characteristics on microbial number, growth, and the onset of visual microbial spoilage. Seed size is an indicator of maturity status. In the immature state, seeds do not fill the hull, in the mature state they fill the hull without deforming it, and in the overmature state they deform the hull (Basterrechea and Hicks, 1991).

2.3.2. Evaluation of the washing process in the packaging company

The packaging company applied a bubble washer of 750 L volume with a replenishing rate of 400 L/h. 1000 kg of sugar snaps were washed in 188 minutes, air dried, screened with machine vision to remove pods showing excessive browning, and packaged in 300 g consumer units (Figure 2.1). At several time points during the washing process, samples of both sugar snaps before and after washing (after 0, 18, 54, 96, and 188 minutes) and of the wash water (after 0, 10, 18, 30, 54, 96, 120, 188 minutes) were taken. Samples of the wash water were taken in the beginning, middle and end of the washing bath at each time point (Figure 2.1). Also, samples of the tap water were taken at the point of entrance in the washing bath. Temperature, pH, and conductivity (all with HQ40d meter, Hach Lange, Belgium) of the wash water were measured at the packaging company. The residence time of the sugar snaps in the washing bath was measured (n=12) by labeling individual sugar snaps with fluorescent tape and timing the period from entrance till exit from the washing bath. The samples were transported under refrigerated conditions to the lab for further analysis. Alkalinity, turbidity, COD of the wash water were determined. Water samples were also analyzed for APC, Y&M, and LAB. Sugar snaps, collected before and after washing, were analyzed for moisture content and water activity (a_w). Samples of the sugar snaps (250 g) were stored in plastic bags for 22 days at 5 ± 1 °C under normal atmospheric conditions and periodically sampled (after 0, 6, 10, 15, and 22 days) for APC, Y&M, and LAB and judged for onset of visual microbial spoilage (i.e. fungal rot, bacterial slime formation).

2.3.3. Evaluation of water sanitizers to improve shelf-life of sugar snaps and maintain wash water quality

Sodium hypochlorite (28.4 g/L NaOCl, La Croix, Belgium), acetic acid (Sigma-Aldrich, Belgium), Purac FFC 80® (80% L(+) LA, Purac, The Netherlands), Natraphase-ABAV® (fine powder containing natural acids, Natural Biotechnology, Belgium) or Natraphase-FVS® (blend of EU and FDA food approved organic acids and vitamins, Natural Biotechnology, Belgium) were used as water disinfectants. The experimental disinfectant concentration - contact time settings are shown in Table 2.1.

For Purac FFC 80, the added concentration is expressed as active compound, i.e. L(+) LA. For chlorine, the pH was adjusted to 6.5 using HCl (1 mol/L). Each experiment (i.e. disinfectant; concentration; contact time) was executed on three different batches in order to

incorporate possible influence of variation in microbiology and physical and physiological differences among sugar snaps in different batches.

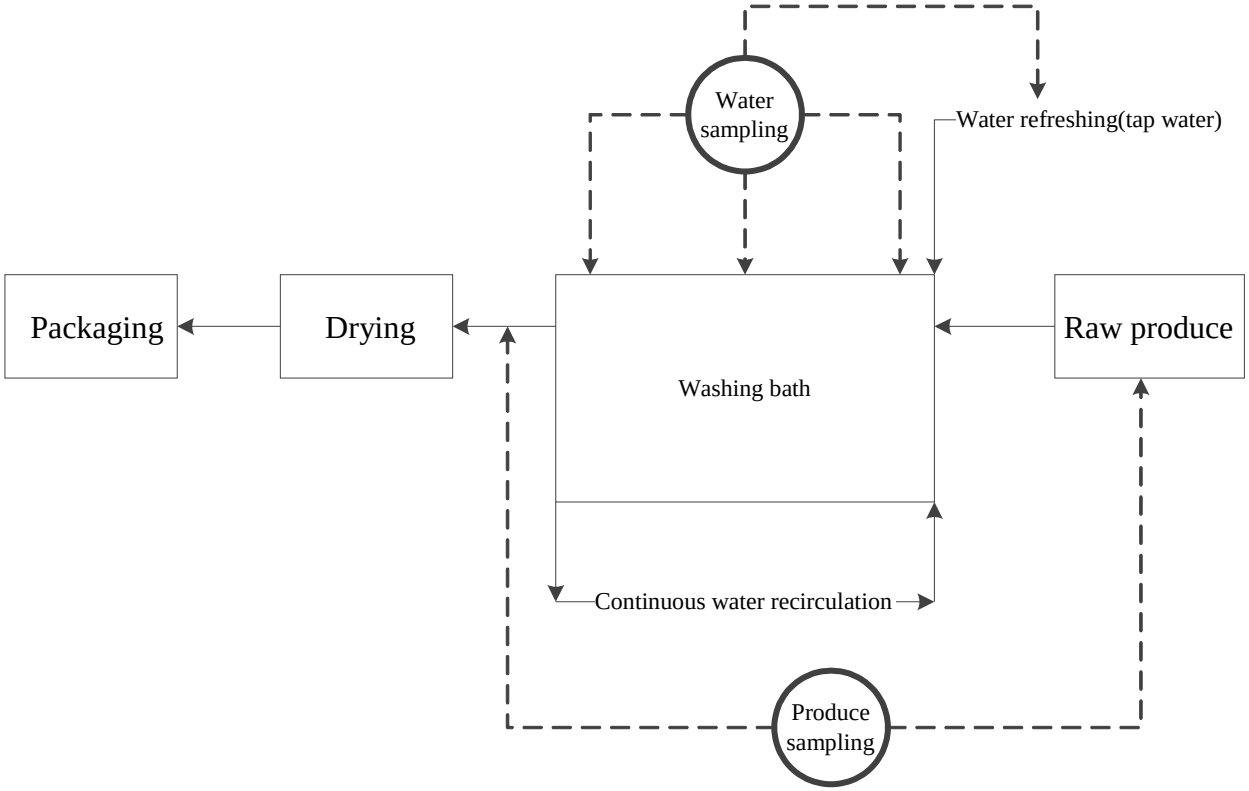

Figure 2.1. Scheme of industrial sugar snaps washing process and sampling locations in the process

Portions of 250 g of sugar snaps were washed by mechanical agitation in 4 L of tap water (5 ± 1 °C) with added water disinfectant. After washing, water samples were immediately quenched with $Na_2S_2O_3$ (0.1 mol/L) for quenching sodium hypochlorite, or phosphate buffer (pH 7.5) for Purac FCC 80, acetic acid, ABAV and FVS. Microbial analyses (APC, Y&M, LAB) were performed on the water samples. The sugar snaps were rinsed (0.1 L/kg.s for 10 s) with tap water. At the highest exposure conditions in the experiment (i.e. highest contact time and disinfectant concentration), samples of washed sugar snaps were either rinsed or not rinsed to observe the effect of residual disinfectant on discoloration, off-odors, damage and texture loss. ABAV samples were never rinsed and FVS samples were always rinsed because these patented formulations were recommended to be used respectively with or without a final rinsing step by the manufacturers. All samples were dried with sterile absorbent paper, and subsequently, samples were screened for discoloration, and sugar snaps showing browning

were discarded. The samples were stored in plastic bags at 5 ± 1 °C for 22 days under normal atmospheric conditions and periodically sampled (after 0, 6, 10, 15, and 22 days) for microbial analyses (APC, Y&M, LAB), and at the same time monitored for the presence of visual microbial decay, discoloration, off-odors, damage and texture loss due to the decontamination treatments.

2.3.4. Physicochemical parameters

Alkalinity was determined with acid titration, turbidity with a turbidimeter (HI98703; HANNA Instruments; Belgium), COD according to the small-scale sealed-tube method (LCI 400; Hach Lange; Belgium), absorbance at UV 254 nm with a UV-Visible (UV-Vis) spectrophotometer (UV1601, Shimadzu, Belgium) and quartz cuvettes with a 1-cm path length (Hellma, Belgium) after filtration through a 0.45 µm polytetrafluorethylene filter (Macherey-Nagel, Belgium). a_w of the sugar snaps was measured with a dew point water activity meter (AquaLab Series 4:4TE, Decagon Devices, The Netherlands). Moisture content of the sugar snaps was determined through homogenization of 5 g of sample (T18 Basic Ultra-Turrax, IKA, Germany) and drying in an air circulation oven of 105 °C for 3 h. Free chlorine was measured with the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method (Eaton et al., 2005).

2.3.5. Microbial analyses

The sugar snaps samples were prepared by weighing 25 g of sugar snaps in a sterile stomacher bag with full-surface filter (0.5 mm pore size) (VWR, Belgium), which was homogenized in 225 mL peptone water (Oxoid, Belgium) for 1 min. Sugar snaps were analyzed for APC, Y&M, and LAB. APC was enumerated with plate count agar (Oxoid, Belgium) using the pouring plate method (incubation at 22 °C, 5 days). Y&M were enumerated with Rose Bengal Chloramphenicol agar (Oxoid, Belgium) containing 150 mg/L chloramphenicol and using the spreading plate method (incubation at 22 °C, 5 days). Membrane filtration through cellulose nitrate filters of 0.45 µm (Sartorius, Germany) was used to lower the detection limit of Y&M to 1 log CFU/g, by filtering 1 mL of sugar snaps/peptone water mix. LAB were enumerated with MRS (De Man, Rogosa, Sharpe) agar (Oxoid, Belgium), containing 1.4 g/L sorbic acid and with a final pH of 5.7, adjusted with NaOH (1 mol/L), using the pouring plate method with an additional cover layer of agar (incubation at 22 °C, 5 days). The water samples were analyzed for the same microorganisms, using the same enumeration methods. In addition, membrane filtration of 10 or 100 mL water

was used to lower the detection limit for microbial enumeration to respectively 1 or 0 log CFU/100 mL.

2.3.6. Statistics

Data analysis was performed with SPSS statistics 21. Influence of disinfectant type, concentration and contact time was assessed with one-way ANOVA, or Brown-Forsythe when equal variance could not be assumed. Group comparison was done with post-hoc tests (Tukey or Games-Howell) when all relations among groups were of interest. However, when only certain relations were of interest, i.e. a significant reduction of the wash water contamination, or a significantly lower contamination on the sugar snaps compared to water washed or untreated sugar snaps, simple contrast analysis was performed. A level of significance $p \leq 0.05$ was chosen for all statistical analyses. Noted deviations on measurements represent standard deviations.

2.4. RESULTS

2.4.1. Evaluation of the washing process in the packaging company

The average residence time of the sugar snaps in the washing bath was 26 ± 15 s. The residence time values were clustered (Figure 2.2). During sampling, it was observed that these clustered residence times occurred due to recirculation patterns in the washing bath. The microbial contamination in the wash water increased till about 25 min of exploitation after which the wash water contamination remained relatively stable (Figure 2.3).

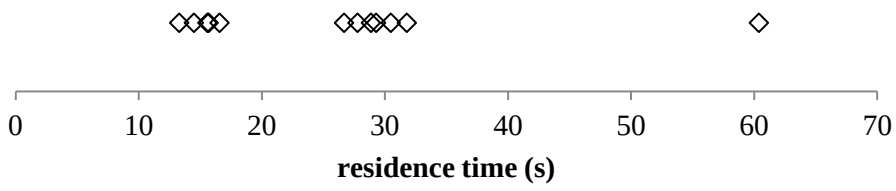

Figure 2.2. Residence time of sugar snaps in the washing bath during the industrial washing process (n=12)

For APC and Y&M, the microbial contamination of the municipal water used in the washing bath immediately at the tap was significantly lower than the water samples taken in the washing bath immediately before the start of the washing process, indicating the presence of

some microbial contamination on the washing equipment prior to the start of operation. The microbial load on the sugar snaps before washing (3.0 ± 0.8, 2.7 ± 0.4, 2.3 ± 0.5 log CFU/g for APC, Y&M, and LAB respectively) was not significantly different from that on the washed sugar snaps (3.5 ± 0.4, 2.6 ± 0.4, 2.2 ± 0.8 log CFU/g for APC, Y&M, and LAB respectively). The microbial contamination on the washed sugar snaps did not change significantly in function of exploitation time. The COD and the turbidity of the wash water were significantly correlated (spearman's rho = 0.668; p = 0.005). The peak values (at 20 – 25 min) of turbidity and COD coincided with a slow water refreshing rate and long sugar snaps retention times in the washing bath, both due to start-up of the packaging apparatus leading to overall slower than optimal operation. Both COD and turbidity became relatively stable from about an hour of exploitation until the end of operation: COD of 30 ± 5 mg O_2/L and turbidity of 5.2 ± 1.1 NTU (Figure 2.4). The temperature was 7.7 ± 0.7 °C, the pH 8.0 ± 0.1, the conductivity 425 ± 4 µS/cm, and the alkalinity 3.05 ± 0.02 mmol/L bicarbonate. All these parameters did not significantly change in function of processing time. The washing process increased the water content of the sugar snaps (from 81.6 ± 3.5 % to 86.0 ± 1.8 %, p = 0.304), though not significantly. Also, the a_w increased significantly (from 0.986 ± 0.001 to 0.990 ± 0.001, p = 0.004).

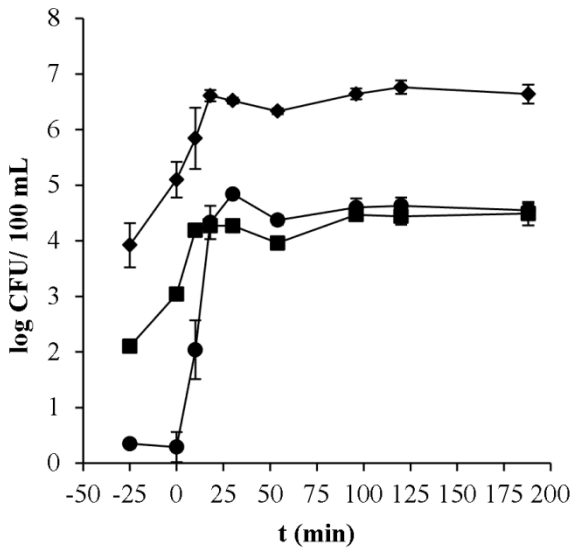

Figure 2.3. Microbial contamination in the washing bath during the industrial washing of sugar snaps (APC (♦), Y&M (■), LAB (●)). The data points at t = -25 min show the microbial quality of the used tap water. Error bars denote standard deviation (n=3)

Table 2.1. The experimental concentration-time settings used in the decontamination experiments (n=3)

Disinfectant	Used settings	Settings that did not impact the sensorial quality	Rinsing
Chlorine	20,50,125,200 mg/L 30,60,180 s pH 6.5 (HCl)	all were usable	not necessary, no impact on product
Acetic acid	0.8,1.2,1.6 % 30,60 s	all settings caused defects, also generation of noxious vapors from the washing bath	rinsing removed off-odors but not the other issues
LA	0.32,0.8,1.2,1.6 % 30,60,180 s	0.32 % (30-180 s) 0.8 % (30-60 s) 1.2-1.6 % (30 s)	necessary
ABAV	0.1,0.5,1.5 % 30, 60, 180 s	0.1 % (30-180 s) 0.5 % (30 s)	were never rinsed[a]
FVS	0.04,0.1,0.5 % 30, 60, 180 s	0.04-0.1 % (30-180 s) 0.5 % (30 s)	were always rinsed[a]
Water	30,60,180 s		

[a] The recommendations of the manufacturer were followed.

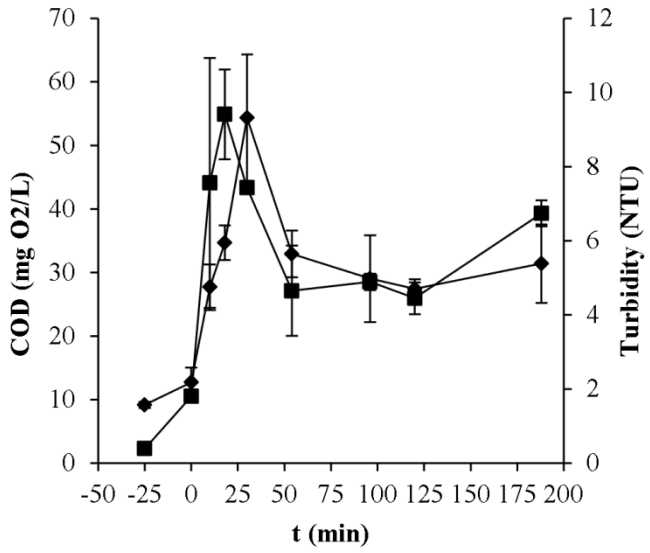

Figure 2.4. COD (♦) and turbidity (■) in the washing bath during sugar snaps washing. The data points at t = -25 min show the COD/turbidity of the used tap water. Error bars denote standard deviation (n=3)

2.4.2. Microbial and visual quality of untreated sugar snaps at reception and during storage

The variation of APC and LAB among different batches of sugar snaps at reception and during storage was more pronounced than for the Y&M contamination (Figure 2.5).

The onset of visual microbial decay was not directly related to the overall microbial contamination degree of the sugar snaps. As the LAB were below or close to the detection limit (1 log CFU/g) in some batches, it was hard to make statistical claims concerning disinfection of LAB and therefore no such conclusions were made. The initial seed size, and to a lesser degree the integrity of the calyx end, seemed to have an impact on the onset of visual microbial decay (Table 2.2).

Violation of calyx end integrity became most apparent through brown discoloration and loss of firmness. The visual microbial decay manifested itself in the pod tissue towards the calyx end, and on major mechanical wounds. Except for batch 1 where all samples and most individual sugar snaps within samples showed signs of microbial decay, the onset of microbial decay in the other batches was mostly only visual on 1 sugar snap within a decaying

sample (comprising ca. 20-50 remaining sugar snaps dependent on the storage time), which at a later date could become visible on one or more other sugar snaps.

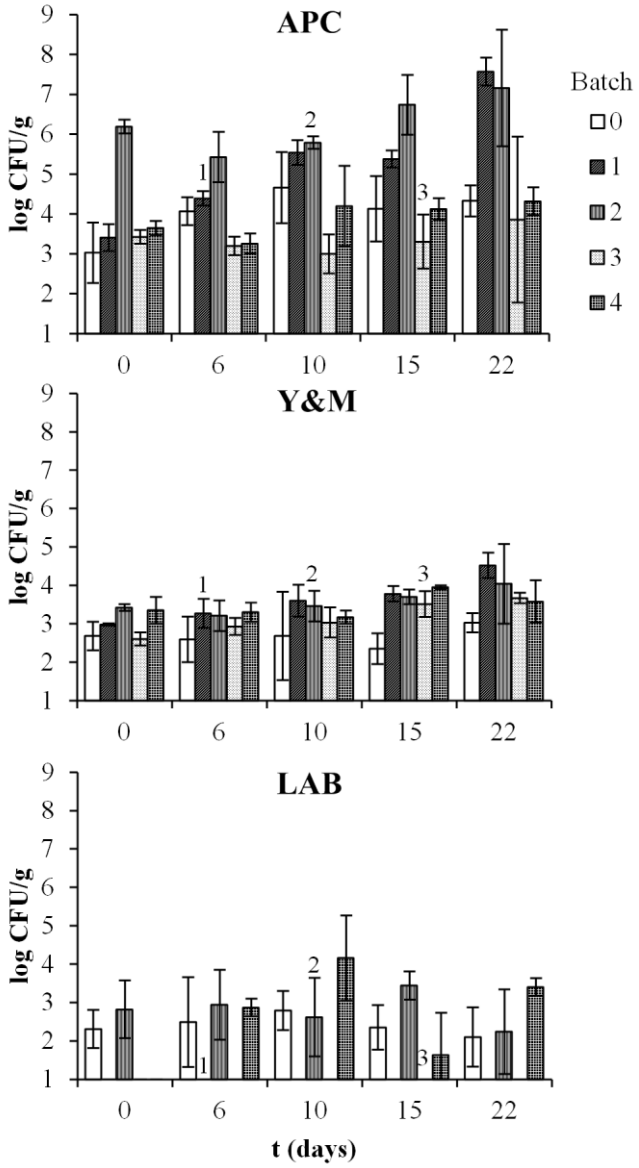

Figure 2.5. Microbial load of the untreated sugar snaps in function of experimental batch and storage time. Numbers in the graphs indicate the batch on which visual microbial decay was observed at that storage time. Error bars denote standard deviation (n=3)

Table 2.2. Comparison of visual characteristics of different batches untreated sugar snaps (n=3)

Batch	Maturity (seed size)	Damage to calyx end	Other mechanical damage	Visual microbial decay (days storage)
0	I-M[a]	+[b]	++	/[c]
1	OM	+++	+	6 (3/3)[d]
2	M	+	++	10 (1/3)
3	M	++	++	15 (1/3), 22 (2/3)
4	I	+	+++	/

[a] I : immature, M : mature, OM : overmature, [b] Number of '+' expresses relative severity of characteristic: + (<10 % of sugar snaps were damaged); ++ (20-40 % of sugar snaps were damaged); +++ (> 50 % of sugar snaps were damaged), [c] No visual microbial decay observed in 22 days of storage, [d] fraction of samples that showed decay.

2.4.3. Evaluation of water sanitizers to improve shelf-life of sugar snaps

Certain of the tested disinfectant settings caused damage to the sugar snaps, i.e. brown discoloration and formation of irregularities on the pod surface, more specifically, pit formation. In addition, acetic acid caused off-odors (Table 2.1). The data of the settings that caused damage to the product were not further incorporated in the shelf-life analyses. A water wash did significantly lower the concentration of Y&M (0.6 ± 0.4 log reduction; $p = 0.003$) on the sugar snaps, yet not of APC (0.5 ± 0.7 log reduction; $p = 0.059$) (Table 2.3).

However, it is important to not blindly accept the statistical analysis. Reductions of 0.5 log compared to the untreated samples are very low in microbiological terms, both from the point of food spoilage (no considerable impact on the regrowth) and of food safety (the human dose-response and the associated increase in risk) (FDA, 2001). Duration of washing had no influence on reduction efficiency of any of the washing treatments, including a water wash, and these values were pooled to increase sample sizes for statistical analysis. LA in the range 0.8 to 1.6 % (1.4 ± 0.5 log reduction), ABAV 0.5 % (1.6 ± 0.2 log reduction), and free chlorine 200 mg/L (1.4 ± 0.5 log reduction) caused a significantly higher reduction of APC than a water wash (Table 2.3).

Table 2.3. Microbial log reduction on sugar snaps during storage

		Storage time (day)				
		0	6	10	15	22
APC	Untreated (n=12)	0.0±0.2	0.0±0.3	0.0±0.5	0.0±0.5	0.0±0.8
	Water (n=12)	0.5±0.7	-0.4±0.7	0.3±0.5	0.0±0.8	0.5±0.9
	ABAV 0.1 % (n=9)	0.6±0.8	-0.2±1.0	0.6±0.7	-0.2±1.0	-0.3±1.2
	ABAV 0.5 % (n=3)	1.6±0.1 [ab-c]	0.4±0.2	1.0±0.9	0.4±0.3	2.0±0.6 [ab]
	Chlorine 20 mg/L (n=9)	1.2±0.5 [a]	0.4±0.8	1.0±0.4 [a]	1.0±0.4 [a]	0.3±0.9
	Chlorine 50 mg/L (n=9)	1.1±0.6 [a]	0.0±0.5	0.4±0.8	0.1±1.0	0.1±1.0
	Chlorine 125 mg/L (n=9)	1.1±0.3 [a]	0.0±0.2	0.4±1.1	0.5±0.2	1.1±0.8
	Chlorine 200 mg/L (n=9)	1.4±0.5 [ab]	0.5±0.8 [b]	0.7±0.7 [a]	0.3±0.8	0.7±1.2
	FVS 0.04 % (n=9)	0.7±0.8	-0.2±0.7	0.4±0.5	0.2±0.5	0.1±0.6
	FVS 0.1 % (n=9)	1.0±0.5 [a]	0.4±0.1	-0.1±0.9	0.4±0.6	1.3±1.2
	FVS 0.5 % (n=3)	0.6±0.8 [a]	1.1±0.4 [ab]	0.7±1.3	0.3±1.2	1.7±1.5 [a]
	LA 0.32 % (n=9)	0.9±0.4 [a]	0.1±0.7	1.0±0.5 [a]	0.6±0.4	0.6±0.5
	LA 0.8 % (n=6)	1.2±0.5 [ab]	0.6±1.1 [ab]	1.3±1.1 [ab]	0.1±1.6	0.4±1.1
	LA 1.2 % (n=3)	1.5±0.4 [ab]	0.2±0.9	0.5±1.6	0.7±0.5	1.4±0.3 [a]
	LA 1.6 % (n=3)	1.8±0.2 [ab]	0.2±0.3	0.1±0.2	1.2±1.2 [ab]	1.1±0.6
Y&M	Untreated	0.0±0.2	0.0±0.3	0.0±0.3	0.0±0.2	0.0±0.5
	Water	0.6±0.4 [a]	0.0±0.5	0.2±0.3	0.5±0.3	0.6±0.6 [a]
	ABAV 0.1 %	0.8±0.2 [a]	0.1±0.4	-0.2±0.3	0.4±0.7 [a]	0.2±0.5
	ABAV 0.5 %	0.6±0.4	0.1±0.1	0.1±0.1	0.3±0.1	0.4±0.2
	Chlorine 20 mg/L	1.2±0.7 [a]	0.3±0.5	0.6±0.7 [a]	1.1±0.6 [ab]	0.5±0.6 [a]
	Chlorine 50 mg/L	0.8±0.7 [a]	0.2±0.4	0.3±0.5	0.7±0.5 [a]	0.7±0.6 [a]
	Chlorine 125 mg/L	0.9±0.2 [a]	0.3±0.3	0.4±0.6	0.7±0.6 [a]	0.9±0.4 [a]
	Chlorine 200 mg/L	1.0±0.9 [a]	0.7±0.4 [ab]	0.5±0.4 [a]	0.9±0.6 [a]	0.6±0.4 [a]
	FVS 0.04 %	0.8±0.6 [a]	0.0±0.3	-0.4±0.3	0.6±0.1 [a]	0.3±0.2
	FVS 0.1 %	0.3±0.2	0.0±0.3	0.3±0.3	0.4±0.2	0.2±0.5
	FVS 0.5 %	0.2±0.3	0.4±0.3	0.2±0.2	0.3±0.2	-0.2±0.3
	LA 0.32 %	0.8±0.6 [a]	-0.1±0.4	-0.1±0.3	0.4±0.3	0.0±0.6
	LA 0.8 %	0.5±0.5	0.1±0.6	0.0±0.5	0.2±0.5	0.3±0.5
	LA 1.2 %	0.7±0.3 [a]	-0.2±0.4	-0.3±0.9	0.3±0.5	0.4±0.2
	LA 1.6 %	0.6±0.2	-0.1±0.4	-0.5±0.4	0.1±0.3	0.6±0.4 [a]

[a] denotes a significant microbial reduction compared to the untreated samples, [b] denotes a significant microbial reduction compared to a water wash.,[c]for APC and Y&M separately.

In the studied concentration ranges, there was no relation between concentration and decontamination efficiency of APC for chlorine ($p = 0.648$) and FVS ($p = 0.759$) and some, yet no significant relation for ABAV ($p = 0.069$) and LA ($p = 0.057$). None of the decontamination treatments removed Y&M significantly more effective from the sugar snaps

than a water wash. Free chlorine (range 20 to 200 mg/L) had the highest reduction of Y&M (on average 1.0 ± 0.7 log reduction).

None of the treatments maintained the APC contamination lower than the untreated and water washed samples for the whole storage duration (Table 2.3). Treatment with LA 0.8% resulted in a lower APC contamination on the sugar snaps than on the untreated or water washed samples for 10 days. Chlorine 200 mg/L was the only treatment able to maintain the Y&M load lower than the untreated samples throughout the entire storage duration. For the other treatments, any significance in microbial reduction on the sugar snaps was lost in less than 10 days of storage.

Visual microbial decay occurred more or equally rapid on untreated than treated (including water washed) sugar snap samples (Table 2.4). Disinfection concentration had no effect on delaying the occurrence of visual microbial decay. In batches 1 and 2, the samples which showed microbial decay had a more rapid growth of APC than the other samples, and overall high counts were reached during storage (Figure 2.6).

Batch 3 showed similar visual microbial decay as batches 1 and 2, but a relatively high proportion of batch 3 was Y&M, and no differences in counts between decayed and other samples were observed for Y&M or APC. Batch 4 also had a high relative abundance of Y&M, yet none of the samples of batch 4 showed any visual microbial decay during storage. The visual decay manifested itself in the same way as with the untreated samples. There was no significant difference in disinfection efficiency of the disinfectants between the different treated batches, despite the differences in initial microbial load as well as microbial growth during storage (Figure 2.6).

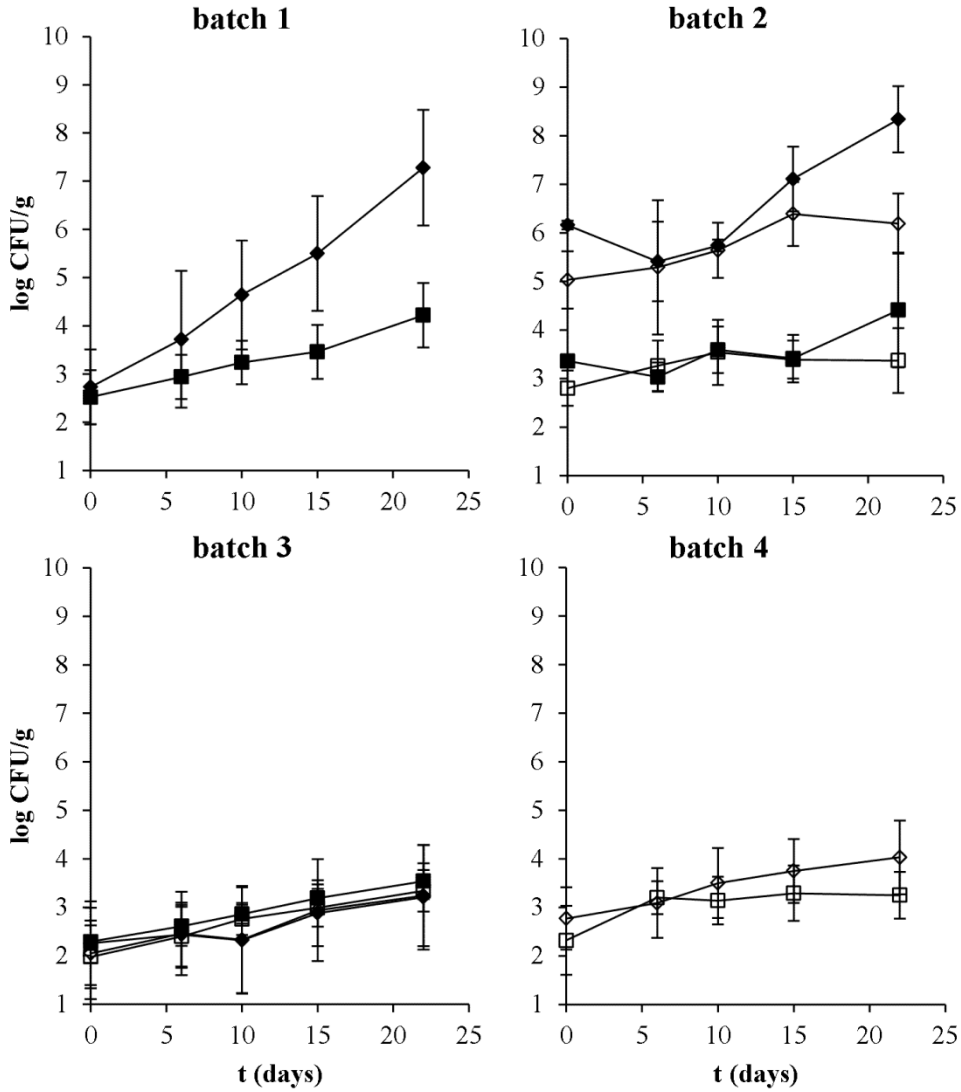

Figure 2.6. Microbial load in function of storage time for both samples that showed visual microbial decay during storage (APC (♦), Y&M (■)) and those that did not (APC (◊), Y&M (□)). Batches consisted of 36 to 48 samples, error bars denote standard deviation

Table 2.4. Overview of sugar snaps samples that showed visual microbial decay in the different batches in function of storage time

	Batch 1				Batch 2				Batch 3				Batch 4			
	day				day				day				day			
	6	10	15	22	6	10	15	22	6	10	15	22	6	10	15	22
Untreated	3/3[a]					1/3					1/3	2/3				0/3
Water	3/3							0/3				0/3				0/3
Chlorine	12/12						1/12					0/4				0/8
LA	12/12							0/12				2/8				0/4
ABAV				/[b]				0/9			3/9	7/9				0/9
FVS				/				0/9			2/9	6/9				0/9

[a]fraction of samples that showed visual microbial decay, [b]was not executed in that batch.

2.4.4. Evaluation of water sanitizers to maintain the wash water quality

Washing sugar snaps for up to 3 min had only minimal influence on the physicochemical water quality: turbidity increased from 0.41 ± 0.05 to 1.16 ± 0.71 NTU and absorbance at UV 254 nm (0.45 µm filtered) from 0.020 ± 0.003 to 0.047 ± 0.015. The pH value of the washing solutions did not change significantly after 3 min washing, the free chlorine concentration diminished 1.46 ± 0.08 mg/L when adding 20 mg/L free chlorine and no significant changes were observed when washing with 200 mg/L free chlorine for 3 min. The initial microbial load of the used tap water was 3.6 ± 1.0 log CFU/100 mL APC and 0.5 ± 0.6 log CFU/100 mL Y&M. The degree of microbial contamination transferred from the sugar snaps to the water during washing in water was independent of both washing time and experimental batch. The washing time had no significant influence on the water disinfectant efficiency to lower the wash water contamination in any of the washing setups, so these values were pooled to increase sample sizes for statistical analysis. On the other hand, the disinfectant concentration had a significant influence on the water disinfectant efficiency of APC and Y&M for ABAV and FVS, although for chlorine (20 to 200 mg/L) and LA (0.32 – 1.6 %) this was not the case (Figure 2.7).

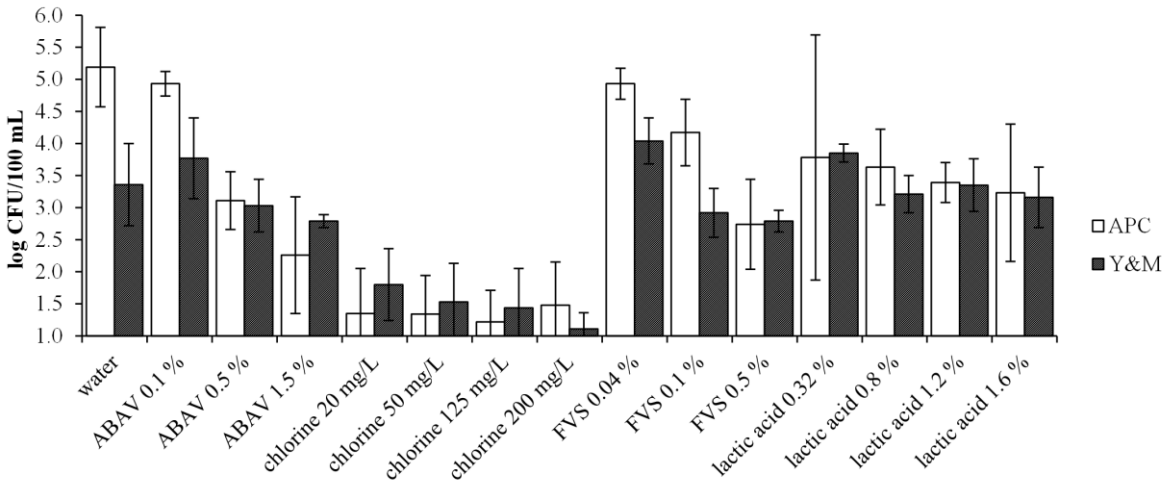

Figure 2.7. Microbial wash water contamination during sugar snaps decontamination experiments. Error bars denote standard deviation (n=9)

The APC wash water contamination (5.2 ± 0.6 log CFU/100 mL) was significantly reduced by chlorine 20 to 200 mg/L (to 1.4 ± 0.6 log CFU/100 mL), ABAV 0.5 to 1.5 % (to 2.7 ± 0.8 log CFU/100 mL), FVS 0.5 % (to 2.7 ± 0.7 log CFU/100 mL) and LA 0.8 to 1.6% (to 3.4 ± 0.8 log CFU/100 mL), whereas the Y&M wash water contamination (3.4 ± 0.6 log CFU/100 mL) was only reduced significantly by chlorine 20 to 200 mg/L (to 1.4 ± 0.5 log CFU/100 mL). Y&M were more resistant to water disinfection with organic acids than APC. For chlorine, both Y&M and APC were reduced to similar numbers and one could argue that the remaining microorganisms were mostly Y&M.

LA in the range 0.32 to 1.6% reduced APC in the water and on the sugar snaps with on average 1.3 ± 0.6 and 1.3 ± 0.5 log respectively and Y&M with on average 0.1 ± 0.4 and 0.6 ± 0.4 respectively. For the commercial sanitizers based on organic acids, ABAV and FVS, the same pattern was observed, i.e. no significant difference between disinfection efficiency of sugar snaps and water disinfection efficiency, which contrasts the much better performance of free chlorine to inactivate microorganisms in the suspended state (Wilcoxon signed rank test; $p > 0.05$ for LA, FVS, and ABAV, $p < 10E\text{-}5$ for chlorine).

2.5. DISCUSSION

2.5.1. Spoilage of sugar snaps during storage in relation to microbial counts, physical damage and the physiological status of the sugar snaps

Visual microbial spoilage was the limiting factor of shelf-life. Nonetheless, APC and Y&M numbers were ineffective to indicate this visual microbial spoilage, as a large variation in microbial counts of the sugar snaps was found. The heterogeneity among microbial contamination of individual sugar snaps, and the scarcity of sugar snaps that actually show signs of microbial spoilage make it unlikely to pinpoint excessive microbial growth through sampling. Also, APC and Y&M generally do not provide information about individual species and the growth of specific spoilage microorganisms might be masked by these broad-spectrum microbial analyses (Gram et al., 2002). Nonetheless, identifying and measuring the specific spoilage microorganisms would not solve the problem of variation in microbial counts within a batch as observed in this chapter. Although some studies observed that the level of total microbial counts or specific spoilage microorganisms in fresh produce were not related to the product quality and shelf-life (Bennik et al., 1998; Gimenez et al., 2003; Ragaert et al., 2007;Allende et al., 2008), others have shown a good correlation between sensorial shelf-life and microbial numbers, such as Chen et al. (2010) (correlation with aerobic mesophilic and psychrotrophic bacteria and Y&M on fresh-cut asparagus lettuce) and Jacxsens et al. (2003) (correlation with yeasts and LAB on mixed bell peppers and grated celeriac). The type of microbial spoilage and sensorial quality deterioration depends on the type of fresh produce (Jacxsens et al., 2003). It is plausible that increased understanding of the specific spoilage microorganisms, spoilage mechanisms, and produced metabolites in a certain type of fresh produce, will enable a better prediction of shelf-life through microbial measurements, not considering the microbial variability issues observed in this chapter.

Characteristics explaining physical damage and the physiological status of the sugar snaps, i.e. the maturity of the sugar snaps and the integrity of the pod at the calyx end, were more predictive towards the visual shelf-life of the sugar snaps. This illustrates that harvesting at the right stage of maturity, avoiding damage during harvesting and more thorough visual selection before processing could lead to an end product with longer shelf-life. Fungal spoilage usually originates from latent infections established in the field or wound infections during harvesting and handling (Terry and Joyce, 2004). *Pseudomonas* spp. and *Erwinia* spp. that colonize plant surfaces adhere preferentially in the natural depression of stomata or in the intercellular junction, or cracks or crevices formed through damage, after which diverse

biofilms can arise, composed of gram-negative and gram-positive bacteria, yeasts and filamentous fungi (Carmichael et al., 1998). The weakening natural defense mechanisms of overmature or mechanically damaged sugar snaps and the loss of structural integrity at the calyx ends, potentially leading to increased solute leakage, improve the growth conditions of phytopathogens (El ghaouth et al., 1992; Nunes et al., 2010). Regardless the observed microbial related issues of overmature sugar snaps, sugar snaps should always be harvested before physiological maturity is achieved. Otherwise, excessive sugar to starch conversion results in loss of sweetness and crispness with advancing maturity, becoming tougher and fibrous (Basterrechea and Hicks, 1991; Sams, 1999).

2.5.2. Water disinfection and produce decontamination with free chlorine and organic acid formulations and their impact on the shelf-life of sugar snaps

Chlorine was confirmed to be an efficient, fast acting water disinfectant against vegetative bacteria as observed in previous studies (Lee et al., 2010; Luo et al., 2011). Chlorine also effectively removed Y&M from the wash water, but did not significantly enhance the Y&M reduction on the sugar snaps compared to a water wash. Pereira et al. (2013) reported fungi to be more resistant to chlorination in drinking water than bacteria and viruses, but less resistant than *Cryptosporidium* oocysts. Beuchat et al. (1998) suggested a large abundance of chlorine resistant cell types among fungi. Contrary to its efficiency to remove suspended, vegetative microorganisms, chlorine is much less efficient as fresh produce decontaminant, a behavior shared among the chemical water disinfectants chlorine, ClO_2, and O_3 and observed in a myriad of studies. Although gaseous chlorine, ClO_2, H_2O_2 and O_3 have a higher diffusion capacity than when dissolved in water and have a higher potential for decontaminating injured and other hard to reach produce surfaces, gaseous disinfectants do not solve the problems of microbial internalization in fresh produce (Gomez-Lopez et al., 2008). Han et al. (2001) observed an increased reduction of spot inoculated *L. monocytogenes* of 3 log reduction when applying ClO_2 as gas treatment (3 mg/L, 10 min, 20°C) compared to an aqueous ClO_2 treatment (3 mg/L, 10 min, 20°C) of both uninjured and injured green peppers. However, Hadjok et al. (2008), who used vacuum infiltration in order to achieve internalization of inoculated *Salmonella* Montevideo in fresh-cut iceberg lettuce, observed that gas exposure of the produce to 1.5% H_2O_2 at 50°C resulted in 2 log reduction on the lettuce, whereas only 0.5 log of the internalized *Salmonell*a Montevideo was inactivated.

The behavior of weak organic acids is fundamentally different from chemical oxidants such as chlorine as weak organic acids are not compromised as severely when inactivation of

microorganisms is needed in the presence of organics in the water or food matrix, or exopolymeric substances in biofilms. Where chlorine is decomposed through reaction with organic matter, the loss of the 'active substance' of weak organic acids is synonymous with deprotonation, and buffer capacity in the vicinity of the produce surface, as well as alkalinity of the water (both inorganic and organic such as from anions of organic acids with pKa ≥ 4) could theoretically pose a disinfection barrier (Hemond, 1990). However, the alkalinity did not change significantly during the 3 hour washing trials in the packaging company. Unless considerable amounts of buffering substances are introduced during washing, efficiency of organic acids will not be severely influenced by the water matrix during produce washing operations. However, weak organic acids in general are inefficient water disinfectants, and the results in this chapter show that, given the contact times used, the efficacy to inactivate microorganisms in suspended state is not better than the reduction of microorganisms on sugar snaps surfaces. Virto et al. (2006) modeled the inactivation of *L. monocytogenes* and *E. coli* in function of concentration of citric or LA, temperature and contact time. To achieve a 3 log reduction in sterile distilled water at 5°C and with 1.6 % LA (the most severe LA settings applied in this chapter) would take 25 min and 35 min of contact time for *E. coli* and *L. monocytogenes* respectively. In this chapter, the short contact times applied (range 30 to 180 s), in combination with the experimental variability, masked the water disinfection kinetics of LA. For comparison of LA and free chlorine as water disinfectants, according to Chick-Watson kinetics, it would take 10 s for 1 mg/L free chlorine at pH 6.5 in oxidant demand free buffer to reduce *E. coli* O157 by 3 log (section 3.4.1.2; Table 3.2). LA, FVS, and ABAV failed at effectively reducing Y&M, both on the produce and in the wash water. The resistance of spoilage fungi to organic acids is related to the membrane ATPase activity and pH homeostatic mechanisms such as acid anion efflux pumps (Brul and Coote, 1999; Smits and Brul, 2005).

Within the studied parameter ranges, contact time had little influence on the decontamination effectiveness of the sugar snaps decontamination treatments. The same was observed for concentration, except for ABAV and LA which showed increased (yet not significant) reduction of APC with increased concentration. However, for the disinfectants based on organic acids, exposure of sugar snaps to the disinfectant could lead to damage, and in that regard concentration and contact time had a significant influence. On that note, the high variation in sugar snaps residence times in the washing bath during the full-scale washing process could lead to excessive exposure of a fraction of the sugar snaps when disinfectants

based on organic acids are used and could result in that fraction being damaged and unfit for selling. The decontamination behavior in function of time and concentration and observed in numerous studies can be described as the following: the microbial load can initially be reduced quite effectively with limited exposure (concentration x contact time), after which increased exposure is less successful in achieving further microbial reduction (Ayhan et al., 1998; Beuchat et al., 1998; Akbas and Olmez, 2007b; Mahmoud et al., 2008; Olmez and Akbas, 2009; Chen and Zhu, 2011). This again can be explained by the state/location of the microorganisms on fresh produce, comprising of microorganisms that are easily, hard, or virtually impossible to inactivate with water disinfectants. Easily removable microorganisms that are vulnerable against the respective disinfectant require relatively little exposure, whereas those which reside in thick biofilms and stomata, require a much higher exposure. The severity of the exposure might be limited by produce damage or engineering issues such as long duration of produce washing steps, or might be virtually futile in the case of internalized microorganisms. The lack of influence of concentration on decontamination efficiency observed in this chapter can be explained by i) working in a concentration range in which all concentrations inactivated the easily reachable microorganisms, ii) the lack of disinfection efficiency of a certain disinfectant to remove hard to reach microorganisms, iii) the masking of the possible influence of concentration on decontamination efficiency of difficult to remove microorganisms due to the high variability in microbial counts, and iv) the presence of recalcitrant internalized microorganisms. The lack of influence of contact time on decontamination efficiency could be explained by i) working in a too small range of contact times to observe differences, ii) decontamination kinetics of disinfectants (suppose that contact times > 30 s would result in no further significant inactivation), and iii) interference of high variability in microbial counts. Some studies (Ayhan et al., 1998; Beuchat et al., 1998; Akbas and Olmez, 2007b; Olmez and Akbas, 2009) show a more severe limitation of further fresh produce decontamination (and as such less influence of concentration and contact time beyond the initial effective decontamination stage) than others (Mahmoud et al., 2008; Chen and Zhu, 2011) in which further increase in exposure resulted in a more successful further microbial reduction. Different inactivation behaviors can be due to several causes: i) fresh produce type and whole *versus* fresh-cut produce, ii) inoculation method or naturally present microflora, iii) the microorganism type, iv) (related to i, ii, and iii) the relative abundance of easily reachable, hard to reach, and infiltrated microorganisms, v) characteristics of the disinfectant (inherent disinfection potential and disinfection kinetics, liquid or gas form), and

vi) the applied experimental conditions and execution such as the created turbulence during the washing process.

Except for treatment with LA 0.8 % or chlorine 200 mg/L, gained reductions of the other treatments compared to the untreated sugar snaps were lost in less than 10 days of storage. Microbial regrowth can potentially occur quickly after decontamination due to reduction of competition (Delaquis et al., 1999; Ragaert et al., 2007; Gomez-Lopez et al., 2008). In this chapter, free chlorine, LA, and ABAV were more effective than a water wash for reduction of APC but not of Y&M on the sugar snaps. Comparison of organic acids and chlorine as fresh produce decontaminants to reduce spoilage microorganisms has also been studied on rocket leaves (Martinez-Sanchez et al., 2006), fresh-cut iceberg lettuce (Akbas and Olmez, 2007a; Allende et al., 2008), fresh-cut escarole (Allende et al., 2008) and fresh-cut cilantro (Allende et al., 2009). Based on those studies, there is no clear, discernible pattern as to whether Y&M are less efficiently removed from fresh produce than mesophilic or psychrotrophic counts, whether LA or citric acid are more/less efficient than chlorine to remove fungal or bacterial microorganisms from fresh produce, and whether these disinfectants improve the shelf-life of the produce.

The consequences of slow water disinfection kinetics by organic acids, as confirmed in this chapter, are that organic acids cannot be used to control cross-contamination, which Lopez-Galvez et al. (2009) demonstrated for *E. coli* transfer from inoculated to non-inoculated fresh-cut iceberg lettuce during washing with 2 % Purac or 0.5 % Citrox®. Therefore, it seems that organic acids are not suitable for washing applications of fresh produce, although there might be potential for their use in decontamination applications through spraying or electrostatic spraying on fresh produce (Ganesh et al., 2010, 2012), as such bypassing the low water disinfection efficiency by using a method without water immersion. This especially has potential when applied as a warm/hot spray, as research on *E. coli* and *L. monocytogenes* suspended in water (4 °C *VS* 20 °C *VS* 40 °C) as well as *E. coli* O157:H7 inoculated on baby spinach (22 °C *VS* 40 °C) has shown that the disinfection efficiency of LA is significantly enhanced by increased temperature (Virto et al., 2006; Huang and Chen, 2011).

Washing of whole produce such as sugar snaps, introduces exudates in the wash water (most probably from wounded surfaces) to a much lesser extent than washing of fresh-cut produce. As such, the transfer of organic materials depends in greater part on foreign organics and particles present on the sugar snaps. The converged COD values (30 ± 5 mg O_2/L) in this

chapter were relatively low compared to the converged COD measured in two fresh-cut leafy vegetable companies, COD 465 ± 2 and 1405 ± 57 mg O_2/L (Section 3.4.1.2). Therefore, considering the high microbial build-up during washing, the inability of the tested water sanitizers to prolong the shelf-life, the absence of detrimental effects of chlorine on the sensorial quality of the sugar snaps, the high performance of free chlorine as a water disinfectant, and the low physicochemical load of the sugar snaps wash water which would minimize the DBPs generation, maintaining a free chlorine residual seems to be a suitable strategy to avoid cross-contamination of vegetative bacteria and fungi in the washing process of sugar snaps.

2.6. CONCLUDING REMARKS

- Organic acid based sanitizers and chlorine were used to decontaminate sugar snaps
- Sanitizers failed to extend shelf-life of sugar snaps (visual microbial spoilage)
- Maturity of the sugar snaps and damage at calyx end of pods predicted visual microbial spoilage
- Low physicochemical and high microbial build-up occurred in water during full-scale sugar snaps washing
- Chlorine effectively removed bacterial and fungal wash water contamination, organic acids did not

CHAPTER 3

THE USE OF CHLORINE AND PERACETIC ACID AS A FRESH-CUT LETTUCE WASH WATER DISINFECTANT INCLUDING THE IMPACT OF PHYSICOCHEMICAL WATER QUALITY

Redrafted from

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3. THE USE OF CHLORINE AND PERACETIC ACID AS A FRESH-CUT LETTUCE WASH WATER DISINFECTANT INCLUDING THE IMPACT OF PHYSICOCHEMICAL WATER QUALITY

3.1. ABSTRACT

Free chlorine and PAA+LA were assessed as reconditioning agents. SPW, a watery suspension of cut butterhead lettuce or iceberg lettuce, was used for the experiments. In the process water recycling experiments, disinfectant was added to SPW inoculated with *E. coli* O157 (6 log CFU/mL). Regression models were constructed based on the inactivation data and validated in IPW obtained from leafy vegetables processing companies. The stability of free chlorine and as such the *E. coli* O157 inactivation with chlorine were influenced by the COD whereas for PAA the UV_{254}(F) was the best indicator for PAA decay. *L. monocytogenes* was more resistant to chlorine reconditioning in SPW than *Salmonella* spp. and *E. coli* O157. Both microbial inactivation rate and disinfectant decay rate were considerably higher in the case of chlorine compared to PAA+LA. The disinfection efficiency of PAA+LA increased with decreasing pH.

Furthermore, chlorine and PAA+LA were assessed as wash water disinfectants in a dynamic washing process with continuous influx of *E. coli* O157 in the washing tank. The wash water contamination was estimated based on *E. coli* O157 inactivation rate constants that were determined in low oxidant demand conditions, as the model assumed that knowledge of the disinfectant residual was sufficient to estimate the microbial contamination, regardless the physicochemical load. For PAA+LA and for free chlorine in low COD conditions, the model was accurate. In high COD conditions, a lower microbial wash water contamination was observed which could not be predicted by the model, most likely because the influence of disinfectant demand was not incorporated into the model.

Only minor amounts of TTHMs were formed in the water during process water recycling with chlorine. TTHMs accumulated to higher concentrations in the water during the wash water disinfection experiments and reached 124.5 ± 13.4 µg/L after 1 hour execution of the washing process in COD 1000 mg O_2/L. However, no TTHMs were found on the fresh-cut lettuce after rinsing.

3.2. INTRODUCTION

Chlorine is the most used water disinfectant in general, and also specifically in the fresh-cut produce industry for wash, spray, or flume waters, due to the low cost, the reliable availability, the good effectiveness against suspended vegetative bacteria and some enteric viruses, and due to the minimal impact on the nutritional and sensorial quality of the produce (Parish et al., 2003; Nou & Luo, 2010; Tomas-Callejas et al., 2012a). However, the efficiency of chlorine as a sanitizer for lettuce decontamination is generally limited to 1-2 log reductions, even at high chlorine concentrations (Sapers, 2001; Luo et al., 2011; Tirpanalan et al., 2011). The common applied free chlorine concentrations in washing processes are in the range of 50 to 200 mg/L. Contact times of 1 to 2 min and pH values between 6 and 7.5 assure the presence of chlorine in the hypochlorous acid form and yet minimize corrosion of equipment (Parish et al., 2003; Tirpanalan et al., 2011). The use of high chlorine concentrations may cause the generation of chlorine gas in the production facilities as well as lead to the production of excessive amounts of harmful DBPs in the water (Nieuwenhuijsen et al., 1999; Hua & Reckhow, 2007; Goslan et al., 2009; Legay et al., 2010; Nou & Luo, 2010; chapter 1). PAA has been suggested as an alternative wash water disinfectant for chlorine. It has been intensively studied for use in wastewater disinfection because of its stability in the presence of organic matter and does not produce harmful DBPs (Stampi et al., 2001; Santoro et al., 2007; chapter 1). These properties make it attractive for use in fruit and vegetable washing processes, it has been studied as disinfectant for washing a variety of fruits and vegetables (Gomez-Lopez et al., 2012), it has been commercialized in combination with LA for washing salad as Fresh Rinse ® (Grace Ho et al., 2011).

Washing produce is a potential pathway for spreading contamination among crops (Holvoet et al., 2012) and the risk of cross-contamination is not removed by using large quantities of water (Lopez-Galvez et al., 2009). Chemical oxidants, including chlorine and PAA, are much more effective for inactivation of bacterial pathogens in wash water than for removal of these pathogens from fresh produce (Sapers, 2001; Gil et al., 2009). In addition, once cross-contamination has occurred, rewashing the newly infected lettuce in free chlorine solutions proves unable to completely remove the newly attached *E. coli* O157, even shortly after the contamination event (Lopez-Galvez et al., 2009, 2010a; Luo et al., 2011).

In this chapter, both process water recycling and process wash water disinfection were studied for the water disinfectants free chlorine and PAA+LA and prediction models for *E. coli* O157 inactivation were made.

3.3. MATERIALS & METHODS

3.3.1. Experimental setup modeling

For the **process water recycling or reconditioning**, inactivation models were calibrated in SPW with controlled physicochemical parameters and inoculated with *E. coli* O157 (Figure 3.1). Both statistical and kinetic models were considered. Repetition of the experiments in IPW was executed for generation of validation data in order to assess the validity of the constructed models.

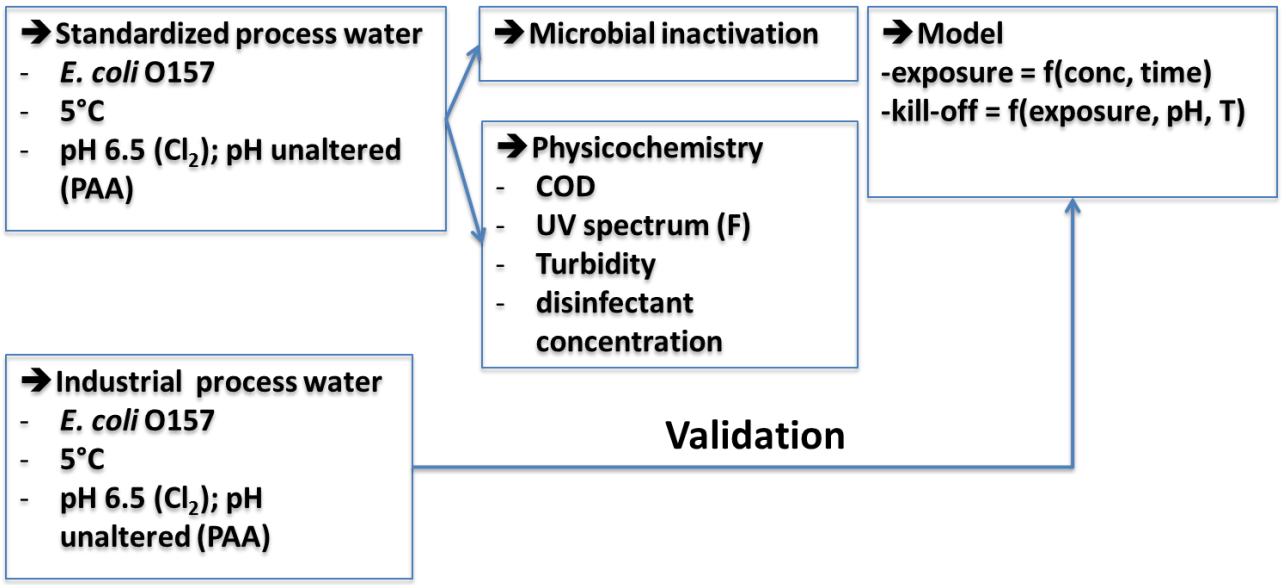


Figure 3.1. Overview of experimental setup reconditioning

For the **Process wash water disinfection**, a dynamic leafy vegetables process water was simulated. Two slightly different setups were used for the experiments concerning chlorine and PAA+LA (Figure 3.2). In the case of chlorine, a continuous washing process of batches of inoculated lettuce was executed, whereas for PAA+LA the contamination was introduced by continuous in- and outflow of inoculated SPW. Also, in the case of chlorine, an initial COD was chosen, whereas for PAA+LA, the experiment was initiated with tap water, after which a COD build-up occurred due to continuous introduction of SPW. Semi-mechanistic models were constructed based on *E. coli* O157 inactivation constants and experimental, operational data (water refreshing rate, contamination inflow, disinfectant residual concentration). Models were validated with measured *E. coli* O157 wash water contamination values.

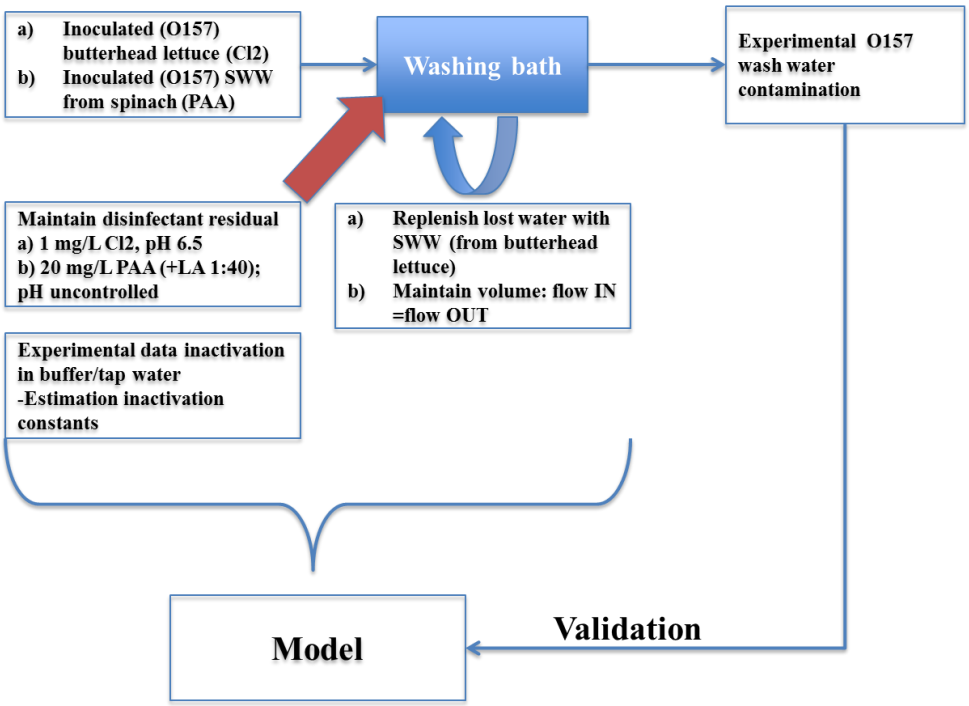


Figure 3.2. Overview of experimental setup for Process Wash Water Disinfection; a) setup for chlorine, b) setup for PAA+LA

3.3.2. Materials & methods: free chlorine

3.3.2.1. Physicochemical measurements

COD, turbidity, UV_{254}, and free chlorine were measured as described in section 2.3.4. Total chlorine was measured with the DPD method for total chlorine (Eaton et al., 2005). NH_4^+ was measured with LCK304 from Hach Lange (Germany), and Fe^{2+} and Mn^{2+} with Inductive Coupled Plasma emission spectrometry (Vista-MPX, Varian).

3.3.2.2. Standardized process water

Butterhead lettuce (*Lactuca sativa)* was purchased from a local market in Belgium and transported under refrigerated conditions to the lab for further handling. After discarding the outer leaves, 67 g of lettuce was put in a stomacher bag with full-surface filter (0.5 mm pore size), 200 mL of tap water was added, and the mixture was homogenized for 2 min. The COD of this suspension was determined, and subsequently the suspension was diluted with tap water to obtain SPW containing the opted COD (500, 800 or 1500 mg O_2/L). Before executing the disinfection experiments the physicochemical parameters of this SPW were measured.

3.3.2.3. Collection of industrial process water

IPW from two fresh-cut produce companies was collected after 2 h of operation in sterile recipients and transported under refrigerated conditions to the lab, where it was stored at 4°C for maximum 24 hours. In company 1 tap water was used as water source during washing of salad mix and iceberg lettuce. Company 2 utilized bore hole water for processing butterhead, iceberg, endive and radicchio.

3.3.2.4. Bacterial inoculation

Two attenuated (non-verotoxin producing) nalidixic acid resistant *E. coli* O157 strains (LFMFP 662 and 679) were used. The strains were grown at 37°C for 24 h in Brain Heart Infusion (Oxoid, United Kingdom) containing 50 µg/mL nalidixic acid (Sigma-Aldrich, Belgium). LFMFP 662 is a nalidixic acid-resistant version of the strain CECT 5947 provided by the Hibro Group from the University of Cordoba (Spain), while LFMFP 679 is a nalidixic acid-resistant version of the strain MB3885 provided by the Technology and Food Science Unit from ILVO (Belgium). Two gentamicin resistant *Salmonella* strains were used: a *Salmonella* Thompson strain (LFMFP 687) which is a clinical isolate that was linked to an outbreak from cilantro (Brandl et al., 2005) and a *Salmonella* Typhimurium (LFMFP 690) strain, which is a natural streptomycin resistant mutant of *Salmonella* Typhimurium SL1344 (Kroupitski et al., 2009). The strains were grown in Tryptone Soya Broth containing 15 µg/mL gentamicin (Sigma-Aldrich, Belgium) for 24 h at 37°C. Three *L. monocytogenes* strains, isolated from green and red peppers (LFMFP 207, 233, 680) were used. The strains were grown in Brain Heart Infusion for 24 h at 37°C. For each species, a cocktail was made by combining volumes of individual strains. Cocktails were centrifuged at 4°C, 1800 g for 10 min. The pellets were washed twice in phosphate buffer (pH 7), with intermittent centrifugation, and subsequently resuspended in phosphate buffer.

3.3.2.5. Inactivation experiments

Buffered experiment

Chlorine solutions were made, containing 0.2, 0.3, 0.4, and 0.5 mg/L free chlorine by diluting a chlorine stock solution (28.4 g/L NaOCl/ La Croix, Belgium) in phosphate buffer pH 6.5 and maintained at a temperature of 5°C. *E. coli* O157 was added to 100 mL of these solutions to obtain 6 log CFU/mL. The solutions were continuously stirred and microbial samples were taken after 15, 30, and 60 s contact time. Samples were immediately quenched using $Na_2S_2O_3$ (0.1 M). *E. coli* O157 was enumerated by the pour plating method on Chromocult Coliform-agar (Merck, Belgium) containing 50 µg/mL nalidixic acid. To extend the limit of quantification (LOQ) , *E. coli* O157 was

also enumerated using membrane filtration **(**ISO, 2000) in which the use of tergitol 7 was replaced by Coliform Chromocult-agar (Merck, Germany) containing 50 µg/mL nalidixic acid, to increase the selectivity of the analysis.

Reconditioning experiment

The pH of the SPW was adjusted to pH 6.5 using HCl (1 M). The pathogens' cocktails were added to the SPW up to concentrations of 6 log CFU/mL. The SPW was continuously stirred during the experiment. The disinfection was executed at 5°C in volumes of 100 mL. The necessary amount of chlorine was added in 1 pulse. SPW of approximately COD 500, 800, and 1500 mg O_2/L was exposed to chlorine concentrations of 20, 35, 50 and 75 mg/L. Samples from the fresh-cut produce companies were chlorinated with 20, 35 and 50 mg/L chlorine. All experiments were executed in triplicate. Samples for free chlorine measurement were taken simultaneously with the microbial samples and immediately analyzed. Microbial samples were quenched using $Na_2S_2O_3$ (0.1 M). *E. coli* O157 enumeration was done as described for the buffered experiment, except the membrane filtration was not executed. Specifically for the inactivation data used in modeling, the LOQ was lowered to 0.5 log CFU/mL by pour plating 3 mL of the non-diluted sample over three plates. Enumeration of *Salmonella* spp. and *L. monocytogenes* was executed with the spread plating method (streaking 100 µL of serial diluted sample) on XLD (Oxoid, United Kingdom), containing 15 µg/mL gentamicin, and Brilliance Listeria Agar (Oxoid, United Kingdom) respectively.

Wash water experiment

Cut lettuce leaves were inoculated overnight with *E. coli* O157 to obtain ca. 4.0 log CFU/g, and subsequently washed. The washing process consisted of washing portions of 50 g of lettuce for 1 minute in a washing bath (volume 4 L) below 7°C by manual stirring. The experiment was done in duplicate. The washing process was continued for 1 hour by consecutively passing 50 g portions through the same washing bath. Each portion of lettuce was rinsed with tap water (product-to-water ratio of 1 kg : 1 L) after washing. The pH of the washing water was reduced to 6.5 with HCl (1 M) and free chlorine was continuously added with a pump to maintain 1 mg/L of free chlorine in the water. The level of free chlorine was measured each minute and the flow of the pump adapted accordingly. The experiment was performed in i) tap water and ii) in SPW, the latter with COD values of approximately 500 or 1000 mg O_2/L. The water loss resulting from the lettuce batches exiting the washing bath was measured and after each batch the washing bath was refilled with water of the respective COD value to maintain 4 L in the washing bath throughout the duration of the trial. Water samples for microbial enumeration were taken. *E. coli* O157 was enumerated using

membrane filtration (ISO, 2000**)** in which the use of tergitol 7 was replaced by Coliform Chromocult-agar (Merck, Germany) containing 50 µg/mL nalidixic acid. The LOQ for experiments in tap water or SPW with COD 500 mg O_2/L was 1.7 log CFU/100 mL and for SPW with COD 1000 it was 2.0 log CFU/100 mL. The *E. coli* O157 enumeration on the lettuce was performed by weighing 10 g of lettuce in a stomacher bag, which was homogenized for 1 minute in 90 mL peptone water (Oxoid, United Kingdom). Chromocult Coliform-agar was used for enumeration of *E. coli* O157 (incubation at 37°C, 24 h)**.**

3.3.2.6. Disinfection by-products

The formation of TTHMs, i.e. chloroform, bromoform, dichlorobromomethane, and dibromochloromethane, was measured. Samples were taken after the reconditioning experiment, i.e. after 30 min contact time. In the washing bath experiment, a water sample was taken from the washing bath at the end of the 1 hour trials and from the final batch of lettuce during each trial. The TTHMs in both the water and on the lettuce were analyzed, using head space/gas chromatography/mass spectrometry as described by Lopez-Galvez et al. (2010b).

3.3.3. Materials & methods: peracetic acid + lactic acid

3.3.3.1. Physicochemical measurements

COD, turbidity, and UV_{254} measured as described in section 2.3.4. PAA concentration during water treatments was assessed by means of spectrophotometric measurements. Protocol used is a modification of the protocol used by Cavallini et al. (2012). Samples of 10 mL were taken from beakers containing 100 mL of SPW+PAA+LA at different time intervals and were disposed in test tubes containing 1 mL of catalase (320 mg/L) (Sigma-Aldrich, Belgium). Catalase was used to eliminate interference by H_2O_2 in the PAA measurement. From these tubes, 10 mL were taken and analyzed according to the total chlorine method (section 3.2.2.1) with absorbance measurement at 530 nm.

3.3.3.2. Preparation of standardized process water

For the reconditioning experiments, iceberg lettuce (*Lactuca sativa* L.) was purchased from a local wholesale market in Ghent (Belgium) and transported within 15 min to the laboratory, where it was kept at 4 °C before use. Water with high COD was prepared following the procedure described in Lopez-Galvez et al. (2012), and then was filtered through the filter of a stomacher bag (Seward, UK), in order to separate big solid particles. Afterwards, samples were taken to measure COD and water was kept at 4 °C before use (always the same day of preparation). Finally, SPW with different

levels of COD (500, 800, or 1500 mg/L) was prepared by mixing the adequate volume of high COD water with tap water.

For the process wash water disinfection experiments, SPW from spinach (*Spinacia olearacea L.*) was made as described by Gomez-Lopez et al., 2014.

3.3.3.3. Collection of industrial process water

Wash water from two fresh-cut produce companies was collected into sterile recipient containers and transported under refrigerated conditions to the laboratory, where it was stored at 4 °C for maximum of 24 h. At company 1, tap water was used as the water source during washing of sugarloaf, iceberg lettuce, endive and radicchio. Company 2 utilized borehole water for processing butterhead lettuce, iceberg lettuce, endive, and radicchio.

3.3.3.4. Bacterial inoculation

Two attenuated *E. coli* O157 strains (LFMFP 662, 679) were used in the PAA experiments, and were grown and purified as described in section 3.2.2.4.

3.3.3.5. Disinfection treatments

Disinfectant solutions consisted of a combination of PAA (Chriox 5, Christeyns NV, Belgium) and LA (Purac Biochem, The Netherlands). PAA-LA solutions were used in a mass ratio of 1:40 in all experiments.

Process Water Recycling or Reconditioning

The different SPW waters and the waters from fresh-cut produce companies were inoculated with the *E. coli* O157 cocktail to a level of approximately 6 log CFU/mL just before the beginning of the treatment. The SPW was continuously stirred during the experiment. Then, disinfectant solution was added to obtain the desired PAA and LA concentrations, and samples for microbiological analysis were taken periodically. All reconditioning experiments were performed in triplicate at 5 °C. To assess the influence of pH on *E. coli* O157 inactivation, inactivation in oxidant demand free buffer was executed in the same way as described for the reconditioning experiments. Buffer solutions at pH 6, pH 8.2 (consisting both of phosphate buffer 0.07 M) and pH 10.2 (carbonate buffer 0.1 M) were used to manipulate the acid dissociation of PAA to 99%, 50%, and 1% respectively.

Process Wash Water Disinfection

Disinfection experiments were performed using a pilot plant system that has been used as standard dynamic system in previous studies (Gómez-López et al., 2014) (Figure 3.3).

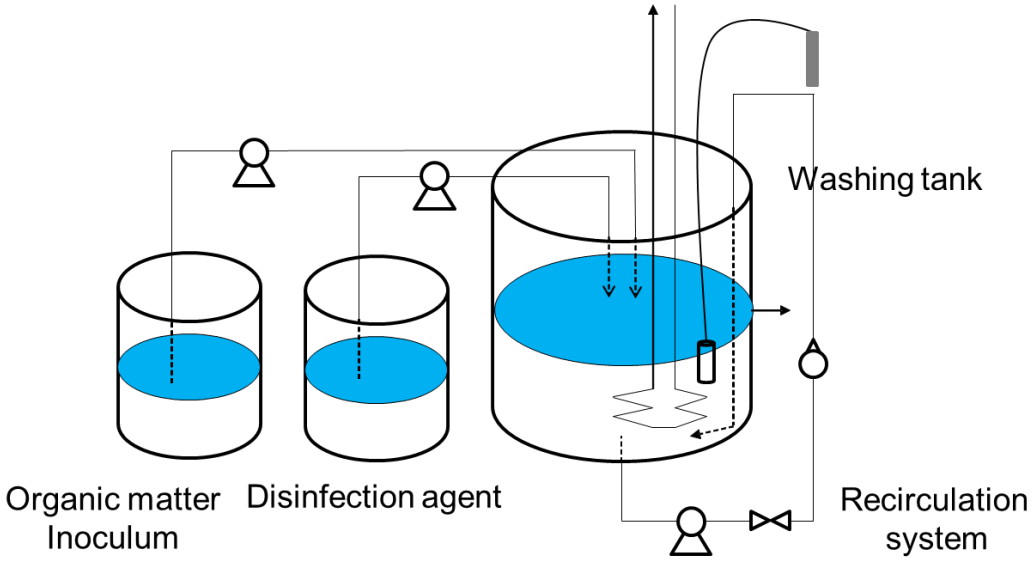


Figure 3.3. Pilot plant system used in the PAA wash water disinfection studies (adapted from Gomez-Lopez et al., 2014)

The system includes: reservoir polypropylene tank for inoculated concentrated process wash water, two peristaltic pumps for dosing, reservoir polypropylene tank for sodium hypochlorite, 30 L stainless steel tank (treatment tank), cooling system, centrifuge pump for water recirculation, control board, sensor for pH, redox potential and temperature, overflow valve, rotameter and flow control valve. Process temperature was controlled by the cooling system which pumped cold water through a stainless steel heat exchanger placed into the treatment tank. The water recirculation rate was adjusted by means of a valve to 750 L/h. Process wash water disinfection treatments were performed starting with clean potable water and applying a continuous increase of COD. Concentrated process water was diluted to a COD of 500 mg O_2/L with tap water and inoculated with the *E. coli* O157 cocktail in order to obtain ca. 6 log CFU/mL. The volume of water contained in the treatment tank was 6 L. An opening at the appropriate height served as a water exit to keep the volume constant against the continuous income of process water and PAA solution. Before starting, the treatment tank was filled with clean tap water. PAA and LA concentration in the washing tank was adjusted to the desired level by addition of PAA and LA from stock solutions. Inoculated process water was continuously dosed to the treatment tank at a flow rate of 7.7 L/h; simultaneously, a PAA+LA solution was dosed to the treatment tank at a flow rate necessary to

maintain a constant PAA residual (target value 19 mg/L). Water samples were collected every 3 minutes in order to monitor PAA and re-adjust PAA+LA solution flow, and at pre-set time intervals in order to determine COD and microbial evolution spread over 60 min.

3.3.3.6. Microbiological analyses

Changes in levels of *E. coli* O157 in water were measured at different time intervals. For reconditioning experiments samples of 1 mL were taken from treated water and transferred into tubes containing 8 mL of a neutralizing solution. Neutralizing solution contained phosphate buffer saline (Na_2HPO_4 1.2 g/L, NaH_2PO_4 0.22 g/L, NaCl 8.5 g/L) supplemented with sodium thiosulphate (1 g/L) (all reagents from Sigma-Aldrich, Belgium). After mixing by means of a vortex, 1 mL of catalase solution (500 mg/L) was added. Phosphate buffer saline was used to keep samples at neutral pH, sodium thiosulphate neutralized PAA, and catalase neutralized residual H_2O_2. Then, samples were diluted when needed, using neutralizing solution, and plated in Chromocult coliform agar+Nal (Merck, Germany) (50 µg/mL) for *E. coli* O157. Plates were incubated at 37 °C for 24 h before counting.

3.3.4. Statistics used for assessing the models

SPSS statistics 20 and Microsoft Excel were used for statistical analysis. The Kolmogorov-Smirnov test and Levene's test were used to assess normality and equality of variance ($P \geq 0.05$) respectively. Differences between treatments, i.e. influence of physicochemical parameters, disinfectant concentration or bacterial species, were determined with ANOVA. If normality or equality of variance could not be assumed, the Kruskal-Wallis or Brown-Forsythe tests were used respectively as alternative to ANOVA. If significant differences were found, the Tukey HSD or Games-Howell post-hoc tests were used at a significance level of $P \leq 0.05$ for further analysis if the group variances were equal or unequal respectively. Non-linear regression for fitting the log-linear model, Chick-Watson, and HOM model was executed with SPSS statistics 20 (sequential quadratic programming algorithm). The Geeraerd model was fitted by using the Excel add-on GinaFit v1.6 (generalized reduced gradient algorithm) (KULeuven). To investigate for possible local minima, the values of the parameters at which the search algorithm initiates were altered to observe whether different solutions were obtained (depending on the initial parameter values), which was not the case. Noted deviations on measurements represent standard deviations unless otherwise stated.

3.3.4.1. Process Water Recycling or Reconditioning

Multilinear regression was executed in JMP 10 (SAS Institute Inc.). In the case of PAA+LA, model selection was done based on variable significance level and ranked according to the Akaike

Information Criterion. For assessing the overall quantitative quality of the models, both the squared correlation coefficient (r^2) for predicted values versus measured values and the ratio of prediction to deviation (RPD) were used. The RPD is the ratio of the standard deviation of the measured log reduction values to the root mean square error (RMSE) of the predicted values. The RPD expresses the increase of prediction accuracy compared to using the mean log reduction value to predict all disinfection trials. A ratio larger than 2 is necessary for a decent calibration, whereas a ratio below 1.5 indicates insufficient prediction potential of the model (Karoui et al., 2007). In order to provide additional qualitative information on the origin of the error, the Theil's decomposition of the mean square error (MSE) was used. Decomposition of the MSE can be done as following:

$$\mathrm{MSE} = (\bar{y} - \bar{y}_m)^2 + \left(s'_{\bar{y}} - r s'_{\bar{y}_m}\right)^2 + (1 - r^2) s'^2_{\bar{y}_m} \quad (3.1)$$

where $\bar{y}_m$ = mean of measured data points

$\bar{y}$ = mean of modelled data points

r = the Pearson's coefficient of correlation

$$s'_{\bar{y}_m} = \sqrt{\frac{\sum_i (y_{m,i} - \bar{y}_m)^2}{N}} \quad (3.2)$$

$$s'_{\bar{y}} = \sqrt{\frac{\sum_i (y_i - \bar{y})^2}{N}} \quad (3.3)$$

Dividing equation 1 by the MSE allows a proportional representation of the three decomposed factors of the MSE (eq. 3.4 and 3.5).

$$1 = \frac{(\bar{y} - \bar{y}_m)^2}{\mathrm{MSE}} + \frac{\left(s'_{\bar{y}} - r s'_{\bar{y}_m}\right)^2}{\mathrm{MSE}} + \frac{(1 - r^2) s'^2_{\bar{y}_m}}{\mathrm{MSE}} \quad (3.4)$$

$$1 = U_m + U_r + U_d \quad (3.5)$$

U_m measures the proportion of the MSE related to bias in the prediction model. U_r represents the proportion of the MSE that is caused by deviation of the regression line between measured and predicted data points from the 45° perfect fit line. U_d represents random prediction errors that can't be reduced. Ideally, U_m and U_r would be zero, while U_d would equal the MSE.

To assess for nested models whether adding parameters to the model (i.e. increasing complexity) leads to a significantly better fitted model (with consideration of the difference in prediction error of

both models, the number of data points and the number of parameters of both models), the partial F-test was used (eq. 3.6):

$$F = \frac{(SSE_r - SSE_f)/SSE_f}{(f-r)/(N-f)} \quad (3.6)$$

where SSE_r = sum of square errors of prediction of the reduced model

SSEf = sum of square errors of prediction of the full model

N = number of data points

r = number of parameters in reduced model

f = number of parameters in full model

The resulting F-value is compared with the critical F-value for a given α (α = 0.05), which is given by $F_{f\text{-}r,N\text{-}f,1\text{-}\alpha}$ (Glatting et al., 2007).

3.3.4.2. Process Wash Water Disinfection in the Washing Tank

The models were constructed in @RISK (add-on Excel). Distributions were fitted to the measured parameters that were used for constructing the model. Monte Carlo simulation was used to select random samples from the input distributions as input for the model. As such, a set of output samples (*E. coli* O157 wash water contamination) were obtained, and distributions were fitted to these output samples. For assessing the overall quality of the time series models in this experiment, the Theil's inequality coefficient (TIC) was used (eq. 3.7). TIC values range from 0 to 1 and values below 0.3 indicate a decent agreement of the model with the experimental data (Audenaert et al., 2010).

$$\mathrm{TIC} = \frac{\sqrt{\sum_i (y_i - y_{m,i})^2}}{\sqrt{\sum_i y^2_i} + \sqrt{\sum_i y^2_{i,m}}} \quad (3.7)$$

As with the reconditioning experiments, Theil's decomposition of the MSE was used to further assess the prediction quality of these models.

3.4. RESULTS

3.4.1. Free chlorine

3.4.1.1. Inactivation of E. coli O157 in oxidant demand free buffer

E. coli O157 reduction in oxidant demand free buffer with chlorine can be seen in Table 3.1. *E. coli* O157 was highly vulnerable to chlorine disinfection. Three basic disinfection models (log-linear kinetics, Chick-Watson, and Hom) were fitted to the data concerning *E. coli* O157 reduction in oxidant demand free buffer (Table 3.2). The Chick-Watson model gave a value for n close to 1, meaning only a slightly higher weight was given to the free chlorine concentration than to the contact time. Not surprisingly, applying log-linear kinetics, i.e. log-linear as the concentration remained virtually constant in the chlorine demand free buffer, resulted in a similar RPD value, and the prediction quality of Chick-Watson model was not significantly better than that of the log-linear model (partial F-test p =0.595). For the Hom model the m value was smaller than 1, suggesting the presence of some tailing effect. The fact that *E. coli* O157 was still detectable (without enrichment) after 2 min in the cases of 0.2, 0.3 and 0.4 mg/L free chlorine allows the possibility of such a tailing effect. However, as these measurements were below the LOQ and the number of time points was limited, this could not be confirmed. The less complex Chick-Watson and log-linear models deviated somewhat from the perfect fit-line (Ur = 0.205 and 0.182 respectively). The added parameters in the Hom model were justified when comparing the log-linear model with the Hom model (partial F-test result p=0.005).

Table 3.1. Chlorine inactivation of *E. coli* O157 (log N/N_0) in oxidant demand free buffer (n=3)

	Contact time (min)			
Free chlorine (mg/L)	0.25	0.5	1	2
0.2	-0.9 ± 0.1	-1.6 ± 0.1	-3.4 ± 0.3	< - 6.3 [a]
0.3	-1.8 ± 0.6	-2.7 ± 0.3	-4.9 ± 0.2	< - 6.3 [a]
0.4	-1.9 ± 0.2	-2.7 ± 0.2	-5.2 ± 0.4	< - 6.3 [a]
0.5	-3.0 ± 0.2	-5.4 ± 0.2	< - 6.3 [a]	< - 6.3

[a] detectable by direct plating, yet below the limit of quantification.

3.4.1.2. Chlorine as reconditioning agent

The measurements of selected physicochemical parameters of the SPW can be seen in Table 3.3. The consumption of free chlorine in the SPW occurred rapidly, with presence of a high initial decay. Initial decay means a very rapid disinfectant decay (within the first min) that is not explained by the further (in time) observed disinfectant decay rate. It is determined as the difference between

the added disinfectant concentration and the concentration at which the fitted curve cuts the y-axis. At COD values of 800 and 1500 mg O_2/L, 75 mg/L of free chlorine was virtually completely consumed within 1 min (data not shown). Water with a COD 456 ± 1 mg O_2/L represents a best case scenario, i.e. water with the best possible physicochemical quality in this chapter. Even at this lower bound of organic load free chlorine was consumed rapidly (Figure 3.4). Within 2 min, 50 mg/L free chlorine was reduced to below 0.1 mg/L and an initial addition of 100 mg/L free chlorine resulted in a residual below 1 mg/L free chlorine after 5 min. Considerable total chlorine values formed due to free chlorine decomposition (data not shown), which consisted predominantly of organic chloramines, due to the low NH_4-N content.

Table 3.3. Physicochemical characteristics of the used process waters (n=3)

	SPW COD 500 mg O_2/L	SPW COD 800 mg O_2/L	SPW COD 1500 mg O_2/L	Company 1	Company 2
pH	7.6 ± 0.1	7.5 ± 0.1	7.6 ± 0.0	7.3 ± 0.0	7.2 ± 0.1
Turbidity (NTU)	66.4 ± 5.4	118 ± 21	228 ± 51	13.8 ± 0.9	72.6 ± 6.6
COD (mg O_2/L)	510 ± 20	772 ± 20	1430 ± 58	465 ± 2	1405 ± 57
UV_{254}	1.58 ± 0.05	2.40 ± 0.06	3.74 ± 0.39	0.36 ± 0.03	1.08 ± 0.05
UV_{254} (F)	0.82 ± 0.05	1.40 ± 0.11	2.12 ± 0.21	0.22 ± 0.02	0.70 ± 0.02
Fe (mg/L)	ND[a]	0.72 ± 0.07	ND	0.19 ± 0.01	0.22 ± 0.01
Mn (mg/L)	ND	0.070 ± 0.000	ND	0.013 ± 0.002	0.024 ± 0.003
NH_4-N (mg/L)	ND	0.45 ± 0.03	ND	1.13 ± 0.03	0.89 ± 0.05

[a] Not determined

The inactivation of *E. coli* O157 occurred during the first minute of contact tim e (Figure 3.5). This was due to the virtually complete free chlorine consumption within this first minute (Figure 3.4), except for the case of adding 75 mg/L in SPW of COD 500 mg/L, the latter resulting in reductions below the LOQ (Figure 3.6). No further significant inactivation occurred during the 30 min contact time (Figure 3.7). Therefore, when disinfecting *E. coli* O157 in SPW with the studied amounts of COD and added free chlorine concentrations, the time factor could be discarded if a contact time above 1 min was maintained.

Table 3.2. Prediction quality of kinetic disinfection models in chlorine demand free buffer

Model	k	n	m	r^2	U_m	U_r	U_d	RPD	Partial F-test (p value)
$\ln N/N_0 = -k.C.t$ (log-linear)	37.3 ± 1.8 [a]			0.82	0.059	0.182	0.759	2.11	
$\ln N/N_0 = -k.C^n.t$ (Chick-Watson)	41.6 ± 8.0	1.11 ±0.18		0.83	0.070	0.205	0.725	2.13	0.595 (versus log-linear)
$\ln N/N_0 = -k.C^n.t^m$ (Hom)	32.6 ± 5.3	0.97 ± 0.19	0.70 ± 0.08	0.87	0.001	0.003	0.996	2.78	0.002 (versus Chick-Watson) 0.005 (versus log-linear)

[a] standard error of mean

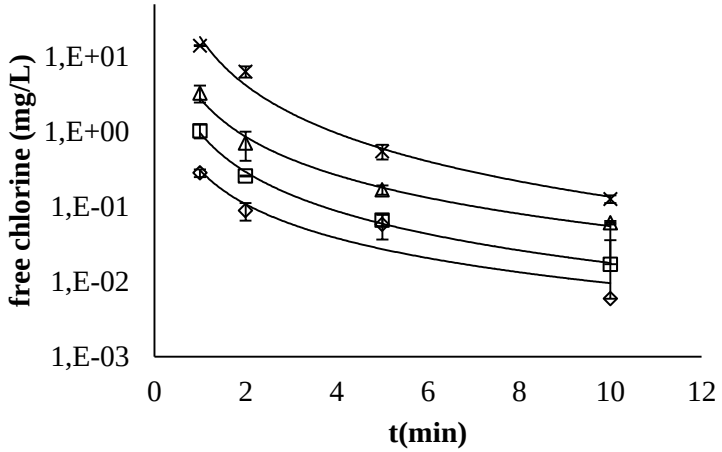


Figure 3.4. Free chlorine consumption in SPW with COD 456±1 mg O_2/L, (◊) 50 mg/L, (□) 60 mg/L, (Δ) 70 mg/L, (×) 100 mg/L chlorine (n=3)

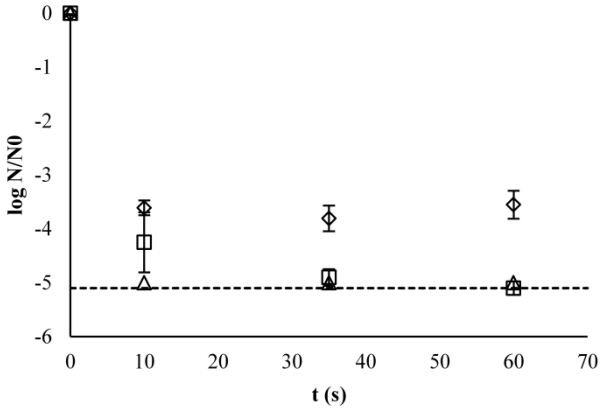


Figure 3.5. Inactivation of *E. coli* O157 in SPW of COD 500 mg O_2/L: (◊) 20 mg/L, (□) 35 mg/L, (Δ) 50 mg/L chlorine, (---) LOQ (n=3)

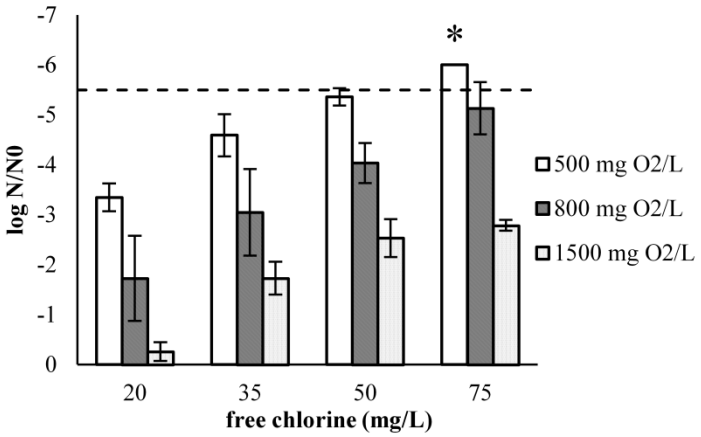


Figure 3.6. Inactivation of *E. coli* O157 in SPW using a 2 min contact time, (---) LOQ, (*) below the limit of detection (LOD) (n=3)

An overview of the inactivation experiments, using a default contact time of 2 min is shown in Figure 3.6. The inactivation was significantly influenced both by the added free chlorine and the COD of the SPW. When comparing the inactivation of *E. coli* O157 with other relevant bacterial pathogens it was observed that the *L. monocytogenes* strains were significantly more resistant ($p = 0.002$) to chlorination in SPW than the *Salmonella* and *E. coli* O157 strains (Figure 3.8). There was no significant difference in the inactivation of the latter two pathogens.

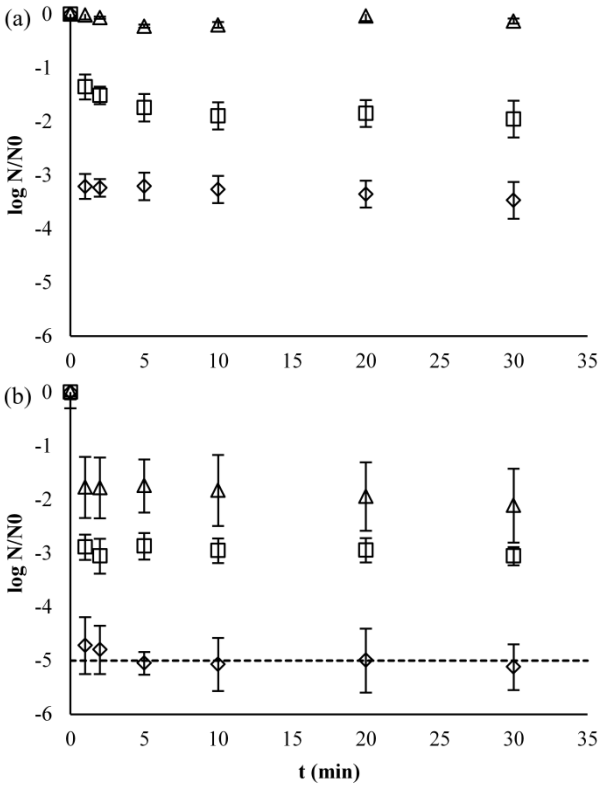


Figure 3.7. Inactivation of *E. coli* O157 with 20 mg/L free chlorine (a), and 35 mg/L free chlorine (b) in SPW containing (◊) COD 500 mg O_2/L, (□) 800 mg O_2/L (Δ) 1500 mg O_2/L, (---) LOQ (n=3)

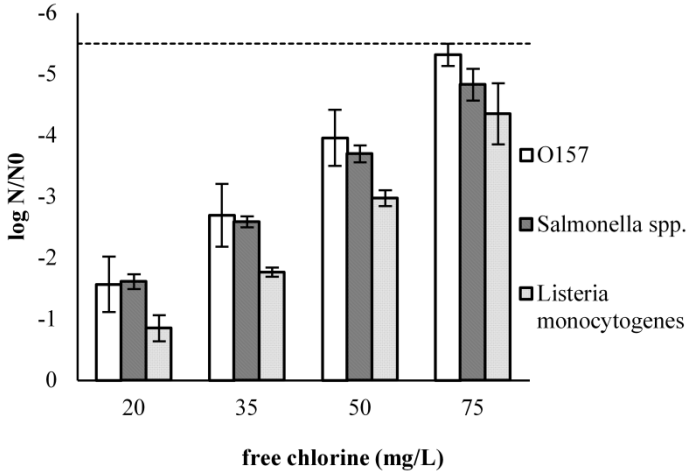


Figure 3.8. Inactivation of *E. coli* O157, *Salmonella* spp. and *L. monocytogenes* in SPW of COD 800 mg O_2/L (2 min contact time), (---) LOQ (n=3)

For the modeling, as the influence of contact time on inactivation was insignificant in the SPW, the contact time was fixed at 2 min. The data from the addition of 75 mg/L free chlorine were

discarded, due to the presence of inactivation values below the LOQ, and the remaining data were used for construction of the model. These disinfection data were divided into a calibration (n=27) and validation (n=13) set, while the data from the experiments in water from the fresh-cut produce companies (n=18) was used as additional external validation set. The linear, quadratic, and interaction terms were modeled with multilinear regression, and based on data from the SPW experiments (calibration set) (eq. 3.8), selecting variables that showed statistical significance.

$$\mathrm{Log}\left(\frac{N}{N_0}\right) = a + bX + cCl_2 + dX^2 + eCl_2{}^2 + fXCl_2 \qquad (3.8)$$

where N = CFU/mL after chlorination

N_0 = initial CFU/mL

a-f = parameters

X = physicochemical variable

Cl_2 = added free chlorine

All linear models were constructed. Concerning the integration of 2nd order terms in the model, in all cases only the square of the physicochemical parameter, e.g. COD², contributed significantly to the regression models (Table 3.4). In the SPW, the physicochemical parameters that negatively influenced the chlorination efficiency all correlated significantly ($p < 0.0005$) with each other. This suggests that all parameters would predict the inactivation efficiency with somewhat similar efficiencies, and indeed, all models predicted the validation set of SPW samples with RPD ≥ 2. Due to this correlation, a model incorporating all physicochemical parameters was redundant and did not have additional predictive value. In fact, when performing regression on all physicochemical parameters, the model was reduced to the linear model based on UV_{254} (F), excluding the other parameters. Concerning predicting actual process water, only the RPD values for the COD models were > 2, meaning all physicochemical parameters, except for COD, failed to predict the disinfection efficiency of chlorine in the actual process water from both fresh produce processing companies. A large systematic bias was observed for the models based on UV_{254}, UV_{254} (F), and turbidity (high U_m) values. The linear model based on COD predicted the disinfection efficiency better than the quadratic model. For the linear model based on COD, similar r^2 (0.86) and RPD (3.01) of calibration as of validation (Table 3.4) indicate that the model does not suffer from overfitting. The quadratic model had a considerable bias (U_m) and deviated from the perfect fit line (U_r), as well as a lower RPD value. Therefore, the quadratic model suffered from overfitting and the simpler linear model was more robust towards predicting the inactivation efficiency in the process water from the companies.

Table 3.4. Prediction quality of the reconditioning models based on different physicochemical parameters

					Calibration SPW	Validation SPW					Validation wash water companies				
Linear	Intercept	Free chlorine	X[a]	X²	r²	r²	U_m	U_r	U_d	RPD	r²	U_m	U_r	U_d	RPD
COD	-2.58±0.47[b]	-0.079±0.010	(27±3).10^{-4}		0.86	0.88	0.034	0.010	0.956	2.92	0.91	0.050	0.070	0.880	3.23
UV_{254}(F)	-3.00±0.48	-0.078±0.010	1.92±0.22		0.82	0.80	0.012	0.001	0.987	2.34	0.75	0.872	0.014	0.114	0.70
UV_{254}	-3.05±0.52	-0.079±0.011	1.11±0.14		0.82	0.81	0.004	4.10^{-5}	0.996	2.39	0.71	0.875	0.010	0.115	0.65
Turbidity	-2.38±0.56	-0.067±0.012	0.014±0.002		0.77	0.77	0.006	0.014	0.980	2.15	0.77	0.733	0.067	0.199	0.96
2^3 factorial design															
COD	-6.76±0.89	-0.087±0.008	0.013±0.002	(-5±1).10^{-6}	0.88	0.86	0.057	0.034	0.909	2.68	0.91	0.305	0.356	0.339	2.04
UV_{254}(F)	-5.36±0.92	-0.083±0.009	5.72±1.34	-1.24±0.43	0.83	0.79	0.027	0.030	0.943	2.21	0.91	0.981	$5.53.10^{-5}$	0.019	0.48
UV_{254}	-7.60±1.05	-0.081±0.008	4.81±0.80	-0.66±0.14	0.80	0.81	0.022	0.042	0.935	2.34	0.90	0.986	0.003	0.011	0.29
Turbidity	-4.50±0.60	-0.079±0.009	0.053±0.008	(-1.2±0.1).10^{-4}	0.87	0.85	0.023	0.011	0.966	2.65	0.93	0.974	0.002	0.024	0.51

[a] denotes physicochemical parameter, [b] standard error of mean

3.4.1.3. Chlorine as fresh-cut lettuce wash water disinfectant

Washing fresh-cut lettuce without the use of free chlorine caused a rapid microbial build-up in the wash water that reached 5.4 ± 0.4 log CFU/100 mL after 1 hour (Figure 3.9). Maintaining a residual of 1 mg/L free chlorine resulted in a wash water contamination that was maintained below 2.7, 2.5 and 2.5 log CFU/100 mL for tap water, SPW COD 500 and 1000 mg O_2/L respectively (Figure 3.9).

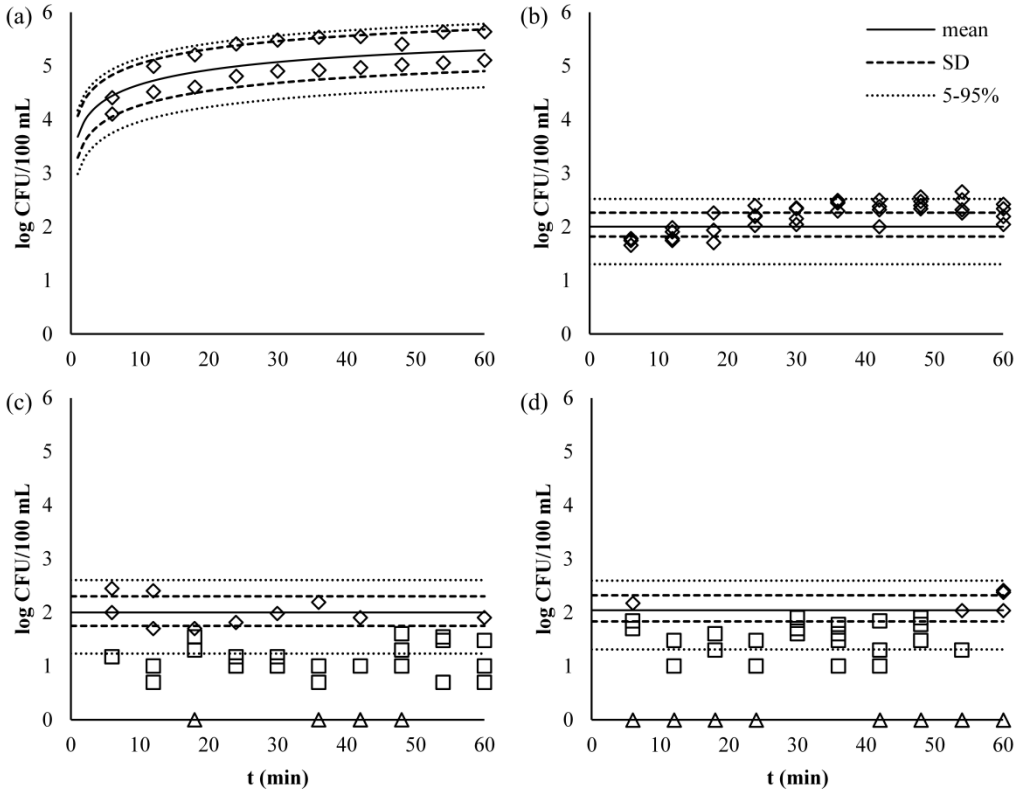


Figure 3.9. Measured and modeled *E. coli* O157 contamination in the washing bath during the fresh-cut lettuce washing process, without chlorine in tap water (a), and with 1.17 ± 0.26 mg/L free chlorine in tap water (b), 1.16 ± 0.33 mg/L free chlorine in COD 500 mg O_2/L (c), 1.09 ± 0.39 mg/L free chlorine in COD 1000 mg O_2/L (d), *E. coli* O157 measurements > LOQ (◊), > LOD and < LOQ (□), < LOD (Δ) (n=2)

The microbial contamination was significantly correlated with time (r^2 = 0.52; P < 0.0005) when chlorinating in tap water, whereas no correlation with time was present when chlorinating in SPW. When disinfecting wash water containing COD of 500 or 1000 mg O_2/L, a larger variation of *E. coli* O157 counts was observed compared to those in tap water and there were overall lower *E. coli* O157 counts. Greater variation in free chlorine concentrations occurred in the presence of higher amounts of COD (Levene's statistic = 0.021 for tap water *VS* COD 500 mg O_2/L and 0.046 for

COD 500 *VS* COD 1000 mg O_2/L) (Table 3.5). The reduction of *E. coli* O157 on the lettuce due to washing with water was 0.5±0.1 log CFU/g. Only in the case of washing with 1 mg/L free chlorine in COD 1000 mg O_2/L the reduction on the lettuce was significantly higher during the 1 hour duration of the experiment compared to the wash with only water (Table 3.5). In all cases, rinsing had no additional influence on pathogen removal.

Table 3.5. Measured chlorine consumption and DBPs production in the washing bath experiment (n=2)

	Tap water	COD 500 mg O_2/L	COD 1000 mg O_2/L
COD (mg O_2/L)	36 ± 13	500 ± 25	1017 ± 4
Free chlorine residual (mg/L)	1.17 ± 0.26	1.16 ± 0.33	1.09 ± 0.39
Free chlorine dose (mg/L/min)	0.33 ± 0.02	2.6 ± 0.2	6.6 ± 1.2
Chlorination breakpoint (mg/L)	1.9 ± 0.2	81.0 ± 14.4	245 ± 19
Cumulative dose (mg/L)	17.1 ± 2.1	236± 24	609 ± 59
Reduction of *E. coli* O157 on the lettuce (log CFU/g)	0.6 ± 0.2	0.6 ± 0.2	1.6 ± 0.2
TTHMs (water) (µg/L)	<6.3	27.8 ± 5.4	125 ± 13
Chloroform	<6.3	27.8 ± 5.4	111 ± 17
Bromodichloromethane	<6.3	<6.3	13.4 ± 2.9
TTHMs (lettuce) (µg/kg)	<6.3	<6.3	<6.3

A model to assess the *E. coli* O157 contamination in the wash water during fresh-cut lettuce washing was based on the following three assumptions: i) free chlorine is free to inactivate bacteria, or otherwise stated, knowledge of the residual free chlorine in the washing bath can be used to estimate the microbial kill-off regardless the physicochemical load, ii) rinsing is considered to be insignificant towards *E. coli* O157 removal and as such not considered in the model, and iii) the discrete experimental setup is interpreted as a continuous process where lettuce is continuously added instead of in intervals of 1 min. The following model was made for the *E. coli* O157

contamination in the washing bath, to quantify the change in microbial load with respect to time (eq. 3.9):

$$\frac{dN}{dt} = LR_1R_2 - kCl_2{}^nN - \frac{R_2V_L}{V}N \quad (3.9)$$

Where N = *E. coli* O157 load in the washing bath (CFU)

L = contamination of lettuce entering the washing bath (CFU/g)

R_1 = fraction of *E. coli* O157 transferred from the lettuce to the washing bath

R_2 = lettuce entering the washing bath per unit of time (g/s)

k = Chick-Watson inactivation rate constant for *E. coli* O157 inactivation in chlorine demand free buffer solution ($(mg^n)^{-1}.L.s^{-1}$)

n = Chick-Watson empirical constant

Cl_2 = free chlorine residual (mg/L) in the washing bath

V_L = water volume loss per g of lettuce (mL/g)

V = water volume of washing bath (mL)

Solving the differential equation with respect to the initial condition N(t=0) = 0 CFU yields in the absence and presence of free chlorine (eq. 3.10 and 3.11 respectively):

$$N(t) = \frac{LR1R2}{\frac{R2V_L}{V}}\left(1 - e^{\left(-\frac{R2V_L}{V}\right)t}\right) \quad (3.10)$$

$$N(t) = \frac{L.R1.R2.}{-kCl_2{}^n - \frac{R2V_L}{V}}\left(e^{\left(-kCl_2{}^n - \frac{R2V_L}{V}\right)t} - 1\right) \quad (3.11)$$

The contamination in 100 mL is than calculated as

$$N(t)_{100\,mL} = N(t)\frac{100}{V} \quad (3.12)$$

The model that predicted the contamination of the washing bath in the presence of free chlorine converged very rapidly to an equilibrium (Figure 3.10). Therefore, the output of the model could be considered constant (eq. 3.13).

$$\lim_{t\to\infty} N(t)_{100\,mL} = \left(\frac{LR1R2}{kCl_2{}^n + \frac{R2V_L}{V}}\right)\frac{100}{V} \quad (3.13)$$

The model made a good prediction for the contamination in function of time in the absence of free chlorine (TIC = 0.028; U_d = 0.93) but in the presence of 1.17± 0.26 mg/L free chlorine there was some bias (U_m =0.29). Nonetheless the TIC value was decent (0.077), indicating the overall error was quite low (Table 3.6 and Figure 3.9). The quality of the models of chlorination in 500 or 1000 mg/L COD was not assessed because of the large amount of data points with values below the LOQ (Figure 3.9). The difference in variability of the measured free chlorine residuals between washing in wash water with different COD values, had no significant impact on the model output which predicted similar outputs for water disinfection in tap water, SPW 500 and 1000 mg/L COD (2.0 ± 0.4 log CFU/100 mL).

Table 3.6. Prediction quality of the *E. coli* O157 contamination models in tap water

Free chlorine (mg/L)	r^2	U_m	U_r	U_d	TIC
0.0±0.0	0.58	0.02	0.05	0.93	0.028
1.17±0.26	0	0.29	0	0.71	0.077

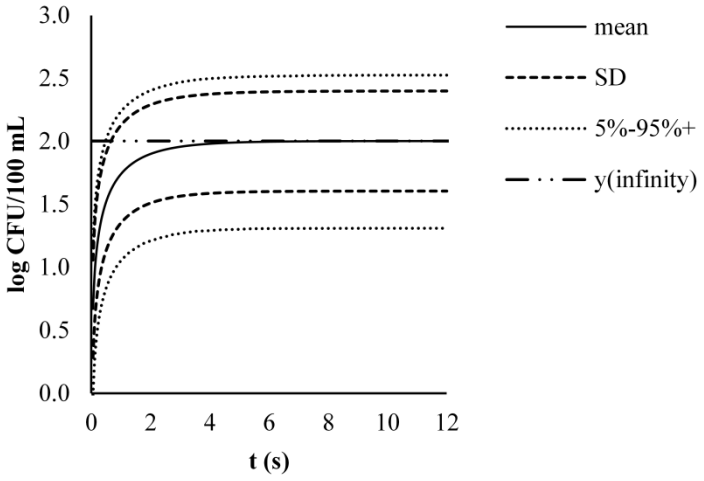


Figure 3.10. Model for *E. coli* O157 contamination during washing of fresh-cut lettuce in tap water with 1.17 ± 0.26 mg/L free chlorine

3.4.1.4. DBPs formation during reconditioning and wash water disinfection

DBPs were measured during the reconditioning trials. In all cases only low amounts of TTHMs were measured. Only chloroform was present in quantifiable amounts: 7.8 ± 1.4, 13.6 ± 2.9 µg/L in water of COD 800 mg O_2/L when adding 100 or 150 mg/L of chlorine respectively and 9.3 ± 3.4,

13.5 ± 7.8 µg/L in water of COD 1500 mg O_2/L when adding 100 or 150 mg/L of chlorine respectively. In the wash water disinfection experiment, the necessary free chlorine dose to maintain a residual of approximately 1 mg/L increased substantially with increasing COD (Table 3.5). As with the reconditioning experiments, the formation of TTHMs was higher in SPW of higher COD, and with addition of higher chlorine concentrations. Besides chloroform, also small amounts (< 6.3 µg/L) of bromodichloromethane were measured in SPW COD 1000 mg O_2/L.

3.4.2. Peracetic acid+ lactic acid

3.4.2.1. Peracetic acid as reconditioning agent

The measurements of selected physicochemical parameters of the SPW/IPW are shown in Table 3.7. When comparing the decay of PAA in SPW with the decay in IPW, it can be observed that UV_{254}(F) is a more universal indicator for PAA decay than COD and turbidity (Figure 3.11). ANOVA analysis showed no difference among initial decay when comparing between varying UV_{254}(F) values or PAA concentration. Nonetheless, for UV_{254}(F) ≥ 0.29 and PAA ≥ 1.4 mg/L there was a significant initial decay, which correlated with UV_{254}(F).

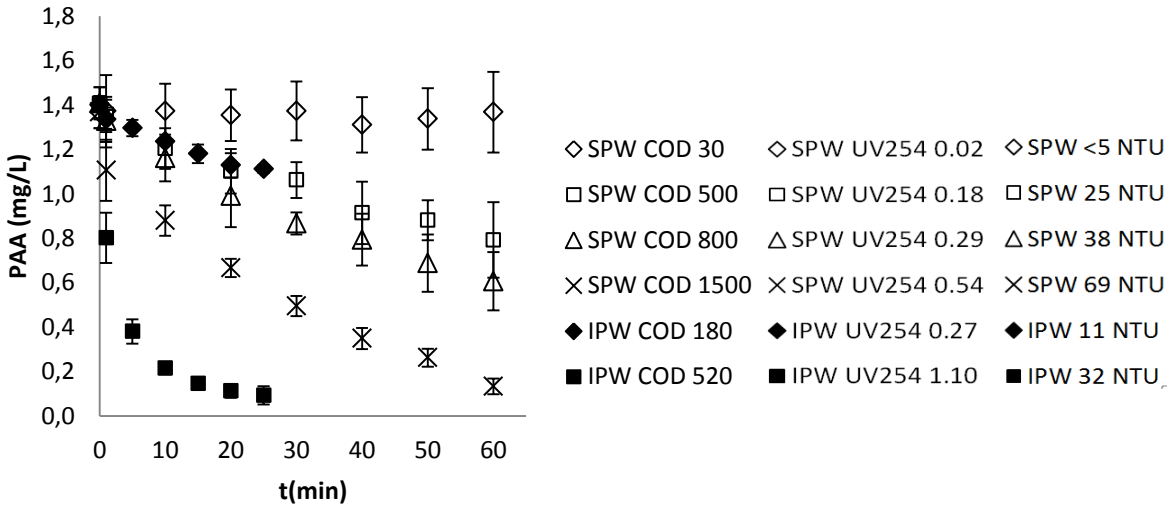


Figure 3.11. PAA decay (1.4 mg/L at t= 0 min; PAA+LA 1:40) in function of time in SPW/IPW of different COD/UV 254 nm content (n=3)

To estimate the reaction order of PAA decay, linear regression of PAA (0^{th}), ln(PAA) (1^{st}), and PAA^{-1} (2^{nd}) in function of time was executed for all PAA concentrations, and physicochemical loads (n=122 time points). Within the studied range of PAA concentration (0.7 – 2.1 mg/L) and physicochemical load (UV_{254}(F) = 0.02 – 0.54), the prediction quality of PAA decay was as followed: zero order (r^2=0.97; RPD = 6.33) > 1^{st} order (r^2=0.95; RPD = 3.15) >> 2^{nd} order (r^2 =

0.78; RPD = 1.41). Note however that these parameter relations are only valid for the considered range of UV_{254}(F) and PAA concentration. At higher UV_{254}(F), i.e. in the IPW with UV_{254}(F) of 1.230, initial decay was dependent on both the UV_{254}(F) and the PAA concentration, and the time dependent decay approximated 1st order kinetics: 1st order (r^2 = 0.97; RPD = 6.29) >> zero order (r^2=0.81; RPD = 2.33) > 2nd order (r^2 = 0.79; RPD = 2.20).

The PAA decay was estimated in function of UV_{254}(F) and PAA concentration with multilinear regression. For the PAA decay in function of time, the three models with the highest RPD value for the calibration also yielded the highest RPD values for the validation, and this for both zero and 1st order approximations (Table 3.8). In addition, the models with lower complexity (lower amount of parameters) showed the highest prediction quality for both zero (Model 1) and 1st order (Model 4) (Table 3.8).

The synergetic properties of PAA+LA were tested and confirmed by comparison of 1.4 mg/L of PAA and 1.4 mg/L PAA + LA (1:40) (Figure 3.12). The inactivation of *E. coli* O157 in function of time was characterized by an increasing microbial inactivation rate in function of time. Both the concentration and physicochemical quality of the SPW influenced the slope of the microbial kill-off (increasing and decreasing influence respectively) (Figure 3.13).

Two models were assessed to predict the microbial inactivation: the shoulder + log-linear model by Geeraerd et al. (2000), and the modified Hom model with disinfectant decay by Haas & Joffe (1994). The modified Hom model can be used to model the observed shoulder effect and incorporate the first order disinfectant decay (eq. 3.14 & 3.15):

$$\ln\left(\frac{N(t)}{N0}\right) = -\left(\frac{m}{n.kPAA}\right)^m (k(PAA(0) - InDecay)^n)(1 - e^{\frac{-n.kPAA.t}{m}})^m \quad (3.14)$$

$$PAA = PAA(0).e^{-kPAA.t} \quad (3.15)$$

With: m, n, and k: the Hom model parameters

kPAA = the first order PAA decay rate constant

InDecay = initial decay

PAA(0) = added PAA dose

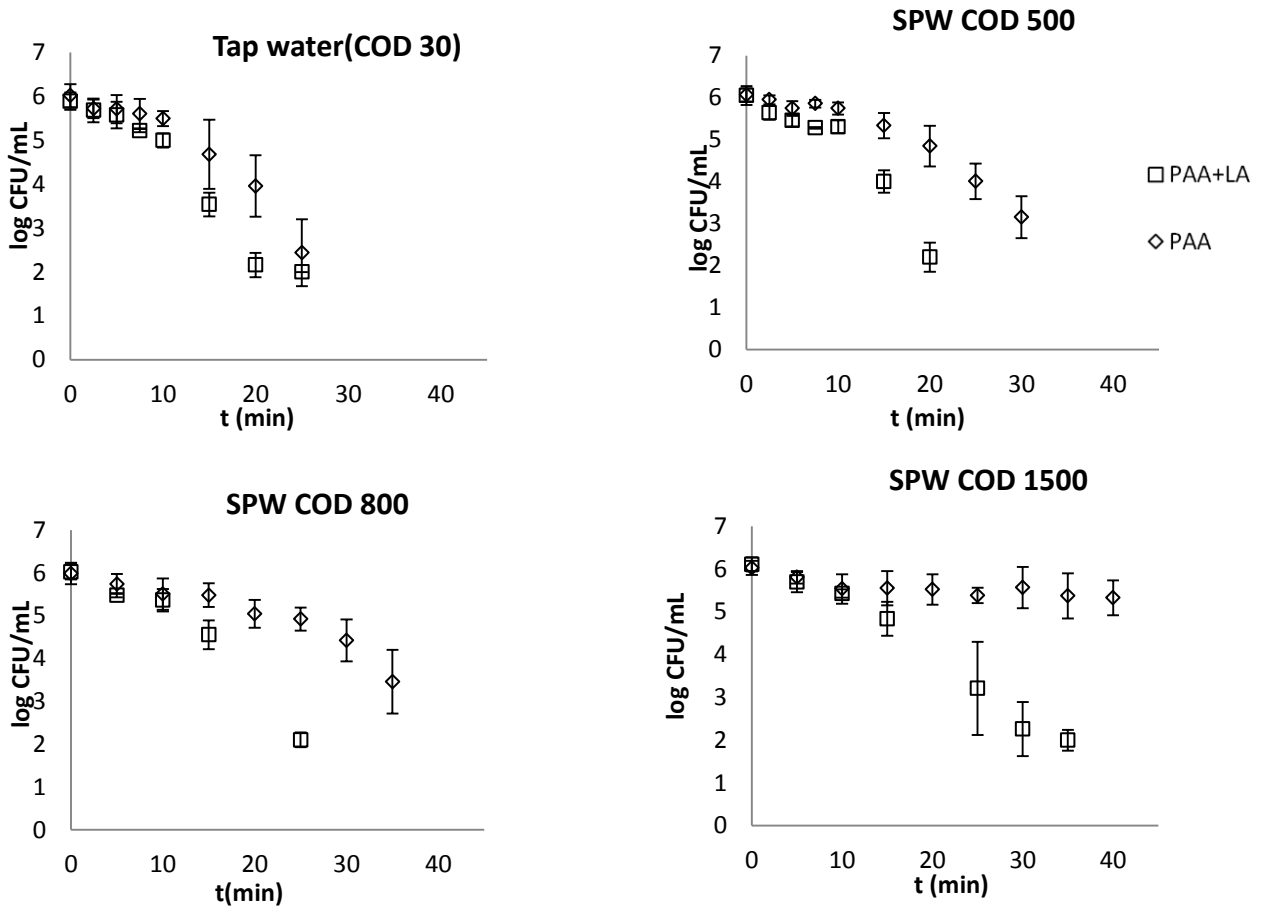
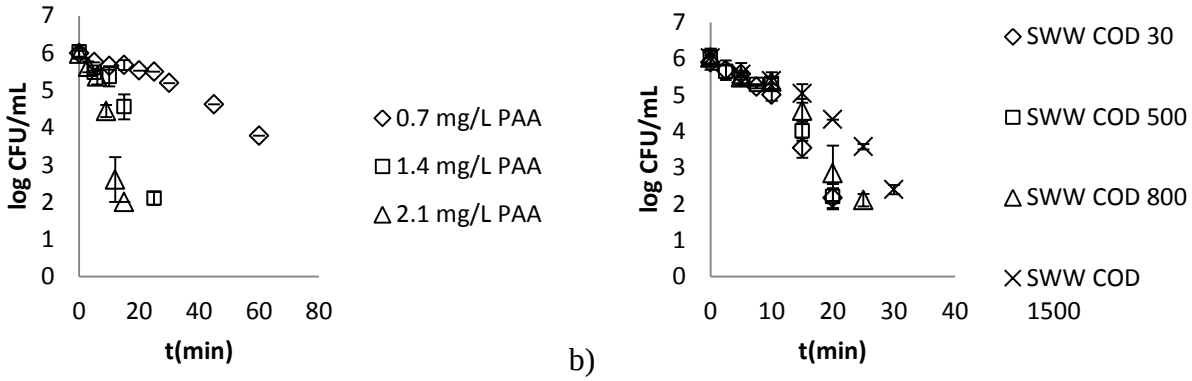


Figure 3.12. Comparison of *E. coli* O157 inactivation with 1.4 mg/L PAA compared to PAA+LA (1:40) (n=3)



a) b)

Figure 3.13. a) *E. coli* O157 inactivation in SPW of COD 835 ± 6 O_2/L with PAA+LA (1:40), b) inactivation in SPW with PAA+LA (1.4 mg/L PAA) (n=3)

The PAA decomposition 1st order model (Initial decay, kPAA) with the highest RPD validation value (Table 3.8), was inserted in the modified Hom model (Table 3.9). With m > 1, a shoulder effect was described. The small k value compared to that in the Hom modeling of chlorine

expresses the considerably slower inactivation kinetics. With an RPD value of 2.76 and 99% of the error due to random prediction errors, the model does not suffer from systematic deviations between the measured and predicted inactivation data.

The Geeraerd equation has the following form:

$$log\frac{N}{N0} = \frac{-kmax.t}{\ln(10)} + \log(\frac{e^{kmax.SL}}{1+(e^{kmax.SL}-1).e^{-kmax.t}}) \quad (3.16)$$

For the Geeraerd model, the data from addition of 0.7 mg/L PAA to SPW with UV_{254}(F) absorption of 0.54 were discarded due to lack of significant microbial inactivation which interfered with the estimation of the maximum inactivation constant (kmax) and the shoulder length (SL). The Geeraerd model was fitted to the calibration set (n = 33; 171 time points) and for each setting (PAA conc, UV_{254}(F) value) and repeat (n = 3) the parameters (kmax and SL) were determined. The relation between the kmax/SL values and the physicochemical parameters was assessed with multilinear regression (eq. 8). The three microbial inactivation models (kmax, SL combinations) that yielded the best RPD values are shown in Table 3.10. Both the Hom and Geeraerd models (Table 3.10 Models 3-5) contained an equal amount of parameters (i.e. 6 parameters). The RPD and r^2 values were slightly higher for the Geeraerd model (Table 3.10 Models 3-5) than for the Hom model. On the other hand, a larger percentage (11% *VS* 1%) of the error was systematic. According to the model, SL is dependent on PAA concentration but not on the physicochemical water quality, whereas kmax is influenced by both parameters. However, these dependencies can only be suggested within the experimental limits (PAA, UV_{254}(F)) of the experiment. Comparing the reduced Geeraerd models 1 or 2 with the more complex model 3 (Table 3.10) resulted in p values $<10^{-5}$ for both comparisons. Therefore, lower model complexity for the Geeraerd models could not be justified

Contrary to the *E. coli* O157 inactivation in SPW, the inactivation in IPW could not be predicted solely based on the PAA exposure (in turn dependent on added PAA concentration and UV_{254}(F) absorbance). Despite similar PAA exposure, the inactivation in IPW was preceded by a longer lag phase (Figure 3.14). As the temperature was kept constant (5°C) in both wash waters, the pH seems the most obvious factor of influence. The acid dissociation constant of PAA is 8.2 (Kitis, 2004). Due to the added LA, the pH dropped from 8.2 to 7.8-7.3 (72-89% PAA in acid form) in the IPW, and from 7.4 to 6.7-6.5 (96-98% PAA in acid form) in the SPW. The influence of dissociation of the PAA was assessed in buffered solutions. Lowering the pH increased the reduction of *E. coli* O157 (Figure 3.15).

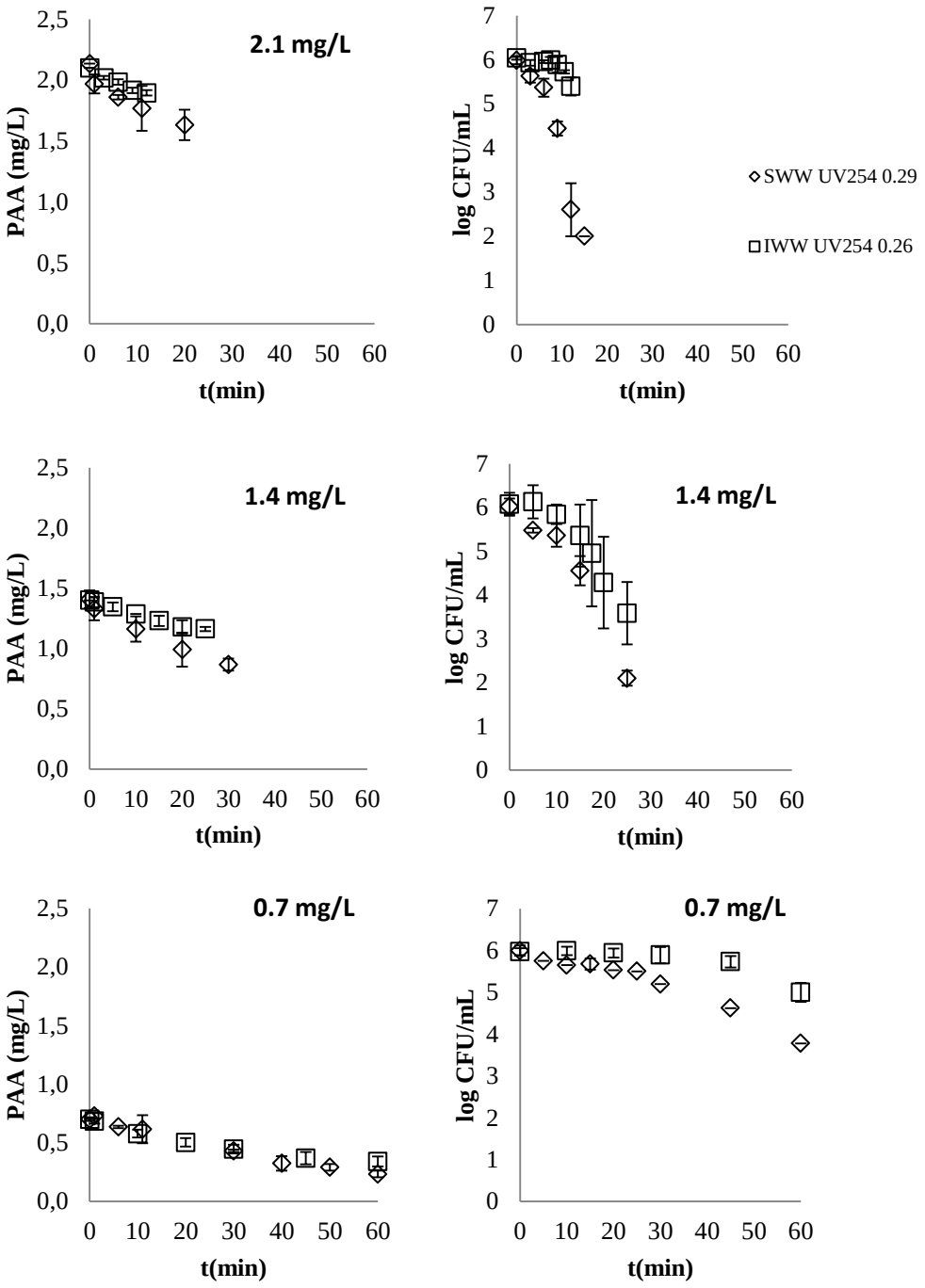


Figure 3.14. Comparison of *E. coli* O157 inactivation in SPW and IPW under similar UV_{254}(F) conditions (n=3)

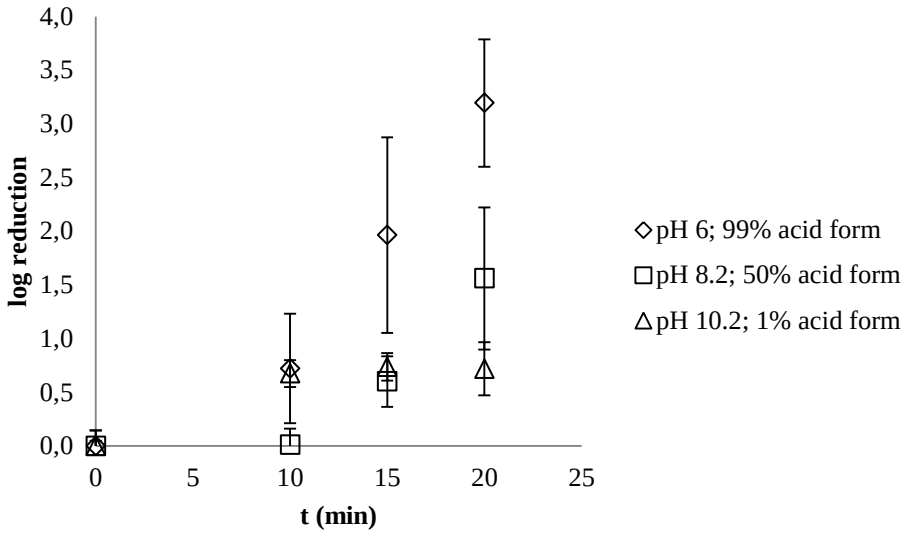


Figure 3.15. Inactivation of *E. coli* O157 in oxidant demand free buffer with PAA+LA (1:40; 1.4 mg/L PAA) (n=3)

Table 3.7. Physicochemical characteristics of the used process waters (n=3)

	Tap water	SPW COD 500 mg O_2/L	SPW COD 800 mg O_2/L	SPW COD 1500 mg O_2/L	Company 1	Company 2
pH	6.9 ± 0.1	7.2 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	8.2 ± 0.1
Turbidity (NTU)	<5	25.3 ± 4.3	37.5 ± 6.1	68.9 ± 10.6	32.1 ± 2.9	11.3 ± 0.1
COD (mg O_2/L)	30	516 ± 4	835 ± 6	1551 ± 33	518 ± 10	177 ± 1
UV_{254} (F)	0.022 ± 0.004	0.18 ± 0.02	0.29 ± 0.03	0.54 ± 0.04	1.23 ± 0.02	0.26 ± 0.03
UV_{254}	0.024 ± 0.003	0.33 ± 0.04	0.53 ± 0.06	0.98 ± 0.10	1.56 ± 0.05	0.43 ± 0.07

Table 3.8. Prediction quality of the PAA concentration during water disinfection in SPW/IPW wash waters with PAA+LA

Model number	InDecay[a]	kPAA	calibration SPW					Validation IPW				
			r^2	U_m	U_r	U_d	RPD	r^2	U_m	U_r	U_d	RPD
	Zero order											
1	$(0.49\pm0.06^{b}).UV_{254}(F)$	$(0.003\pm0.001).PAA+(0.020\pm0.003).UV_{254}(F)$	0.964	0.051	0.016	0.933	5.07	0.997	0.004	0.009	0.987	12.2
2	$(0.49\pm0.06).UV_{254}(F)$	$(-7\pm11).10^{-4}+(0.001\pm0.0007).PAA+(0.034\pm0.006).UV_{254}(F)-(0.054\pm0.008).UV_{254}(F)^2+(0.015\pm0.002).PAA.UV_{254}(F)$	0.966	0.121	10^{-4}	0.879	5.13	0.993	0.028	0.010	0.962	11.5
3	$(0.49\pm0.06).UV_{254}(F)$	$-0.003\pm0.001+(0.0046\pm0.0009).PAA+(0.023\pm0.003).UV_{254}(F)$	0.969	0.119	0.002	0.879	5.30	0.994	0.075	0.166	0.759	11.3
	First order											
4	$(0.49\pm0.06).UV_{254}(F)$	$(0.090\pm0.007).\ UV_{254}(F)-(0.028\pm0.005).\ PAA.UV_{254}(F)$	0.978	0.001	0.082	0.917	6.47	0.990	0.348	0.005	0.647	8.25
5	$(0.49\pm0.06).UV_{254}(F)$	$(6\pm10).10^{-4}.PAA+(0.090\pm0.007).UV_{254}(F)-(0.029\pm0.005).PAA.UV_{254}(F)$	0.981	0.015	0.037	0.948	7.08	0.990	0.362	0.003	0.635	7.98
6	$-0.042\pm0.026+(0.58\pm0.23).\ UV_{254}(F)$	$(6\pm10).10^{-4}.PAA+(0.090\pm0.007).UV_{254}(F)-(0.029\pm0.005).PAA.UV_{254}(F)$	0.981	0.014	0.039	0.947	7.12	0.990	0.362	0.003	0.635	7.98

[a] Presence of an initial decay was only significant for UV254(F) ≥0.29 and PAA ≥ 1.4 mg/L, [b] standard error of mean

Table 3.9. Prediction quality of the modified Hom model with disinfectant decay for *E. coli* O157 inactivation in SPW with PAA+LA

InDecay	kPAA	k	n	m	r^2	U_m	U_r	U_d	RPD
(0.49±0.06[a]).UV_{254}(F)	(0.090±0.007). UV_{254}(F)-(0.028±0.005). PAA.UV_{254}(F)	0.015±0.004	2.12±0.09	1.92±0.08	0.867	0.001	0.001	0.998	2.76

[a] standard error of mean

Table 3.10. Prediction quality of the Geeraerd model for *E. coli* O157 inactivation in SPW with PAA+LA

Model number	SL	kmax	r^2	U_m	U_r	U_d	RPD
1	19.7±1.4[a] (7.27±0.87).PAA	-0.22±0.09+(0.63±0.05).PAA-(0.40±0.12).UV_{254}(F)	0.826	0.001	0.691	0.307	2.00
2	31.0±3.7-(26.1±5.6).PAA+ (6.6±1.9).PAA^2	(0.59±0.05).PAA-(0.78±0.25).UV_{254}(F)	0.878	0.004	0.352	0.640	2.30
3	31.0±3.7-(26.1±5.6).PAA+ (6.6±1.9).PAA^2	-0.22±0.09+(0.63±0.05).PAA-(0.40±0.12).UV_{254}(F)	0.910	0.072	0.040	0.888	3.15
4	31.0±3.7-(26.1±5.6).PAA+ (6.6±1.9).PAA^2	-0.25±0.15+(0.635±0.091).PAA-(0.81±0.33).$UV_{254}(F)^2$	0.918	0.080	0.028	0.892	3.31
5	31.0±3.7-(26.1±5.6).PAA+ (6.6±1.9).PAA^2	(0.26±0.11).PAA+(0.13±0.08).PAA^2-(0.38±0.17).UV_{254}(F)	0.915	0.082	0.022	0.896	3.25

[a] standard error of mean

3.4.2.2. Peracetic acid as fresh-cut lettuce wash water disinfectant

A model to assess the *E. coli* O157 contamination in the washing tank during continuous influx of SPW (COD 504 mg O_2/L;UV_{254}(F) 0.945; 6 log CFU/mL *E. coli* O157) was constructed based on the following assumption: PAA is free to inactivate bacteria, or otherwise stated, knowledge of the residual PAA in the washing bath can be used to estimate the microbial kill-off regardless the physicochemical load.

To estimate the Geeraerd inactivation constants, *E. coli* O157 was inactivated in tap water, i.e. in water with virtually no PAA demand (Table 3.11).

Table 3.11. Prediction quality of the Geeraerd models for *E. coli* O157 inactivation with PAA+LA in tap water.

PAA (mg/L)	kmax	SL	r^2	RPD
14	21.4±2.0[a]	0.23±0.07	0.91	2.78
19	22.6±5.7	0.16±0.08	0.89	2.48
24	21.7±4.1	0.10±0.06	0.95	3.43

[a]standard error of mean

Within the range of PAA 15 to 24 mg/L the kmax and SL did not significantly change. During the dynamic PAA+LA experiment, the PAA concentration was 18.5 ± 1.1 mg/L. Therefore, the parameter estimates of kmax and SL for PAA = 19 mg/L (Table 3.11) were used in the model. The dynamic model was constructed by inserting a water dilution factor in the derivative of the Geeraerd equation, in order to quantify the change in *E. coli* O157 load with respect to time (eq. 3.17):

$$\frac{dNs(t)}{dt} = \left(-kmax.\frac{-kmax}{1+e^{-kmax.t.}\left(e^{kmax.SL}-1\right)} - D\right).Ns(t) \qquad (3.17)$$

Where Ns(t) = *E. coli* O157 wash water contamination (CFU/mL) of a 'single' influx event of microbial contamination

D = dilution factor (s^{-1}), i.e. contamination leaving the washing bath

For the initial condition Ns(t) = N0 with N0 is influx of contamination (CFU/s/mL), solving the differential equation yields (eq. 3.18):

$$Ns(t) = \frac{N0e^{kmax.SL}.(e^{-kmax.t})\frac{D}{kmax}}{e^{kmax.SL}-1+e^{kmax.t}} \quad (3.18)$$

This equation describes a 'single' contamination event in function of time. As for all intents and purposes D <<kmax, the factor D/kmax approaches 0 and the equation is reduced to the Geeraerd equation (eq. 3.19):

$$Ns(t) = \frac{N0e^{kmax.SL}}{e^{kmax.SL}-1+e^{kmax.t}} \quad (3.19)$$

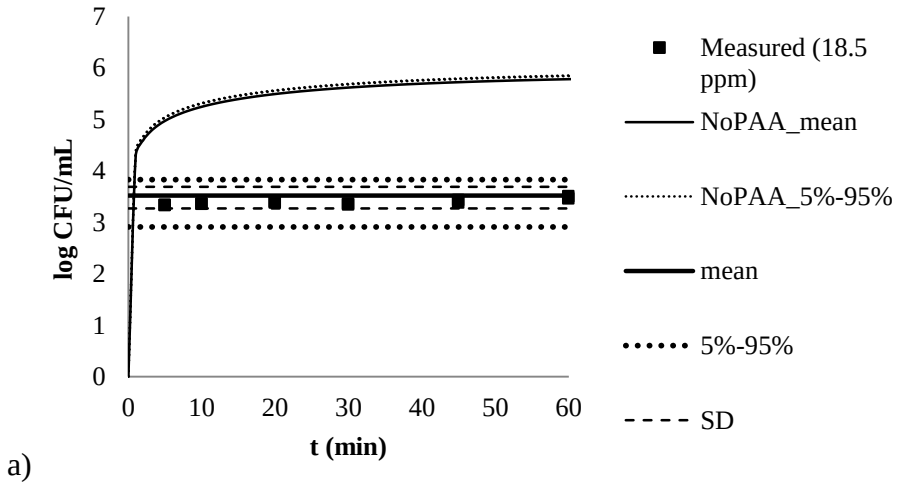
As the inactivation rate is time dependent, the total microbial contamination at a certain time point is described as the sum of all the 'single' contamination events up to that point. Therefore, the total microbial wash water contamination equals the integration of the Geeraerd function (eq. 19) in function of time (eq. 3.20):

$$N(t) = \int_0^t Ns(t)dt = \left[-\frac{N0.e^{kmax.SL}.(ln(e^{kmax.SL}-1+e^{kmax.t})-kmax.t)}{kmax.(e^{kmax.SL}-1)}\right]_0^t \quad (3.20)$$

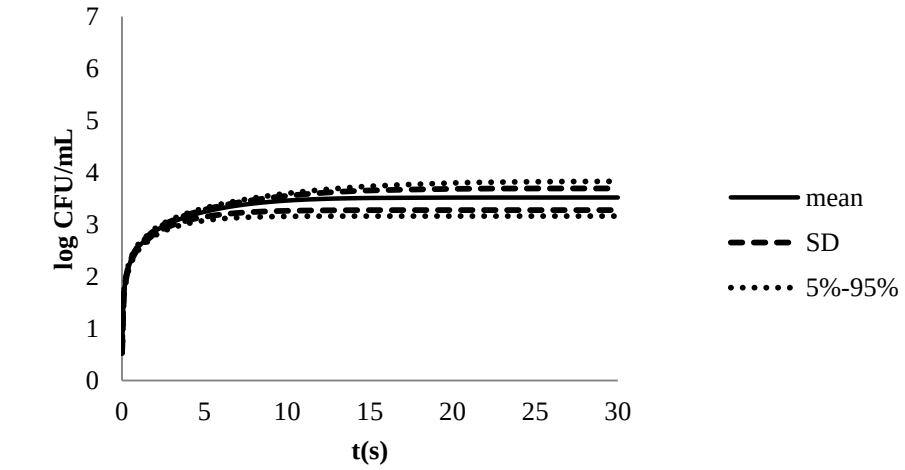
Where N(t) = the *E. coli* O157 wash water concentration at time t (CFU/mL).

As with chlorine, the *E. coli* O157 wash water contamination converged rapidly (Figure 3.16). The model corresponded well to the measured *E. coli* O157 contamination values (TIC = 0.020).

Knowing the added COD and PAA+LA during the washing simulation in SPW from spinach (COD = 504 mg O_2/L; UV_{254}(F) = 0.944), the PAA demand could be calculated. To maintain 18.5 mg/L residual of PAA (to achieve about 2.3 log reduction at 60 min of operation), 0.003 mg PAA per mg COD was consumed. The exact same experiment was executed by Gomez-Lopez et al. (2014) but with free chlorine. To maintain 1 mg/L of free chlorine (to achieve > 3 log reduction at 60 min of operation) in the same conditions 0.124 mg chlorine per mg COD was consumed, i.e. about 41.3 times more free chlorine was needed. On the other hand 18.5 times more PAA is necessary to account for water refreshing. 40 mg/L of PAA (+LA) was needed to achieve > 3 log reduction in an identical dynamic setup (Figure 3.16c). These data were not modelled because of microbial values below the LOQ.



a)



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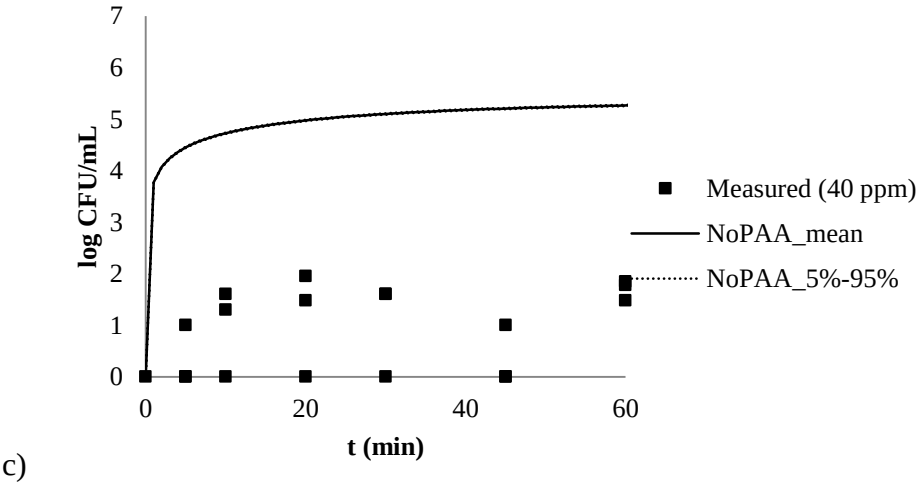

c)

Figure 3.16. Model for *E. coli* O157 contamination during process wash water disinfection in the washing tank with PAA+LA, a) full experimental time interval (18.5 ± 1.1 mg/L PAA), b) model output for first 30 s (18.5 ± 1.1 mg/L PAA), c) full experimental time interval (40.5±4.1 mg/L PAA)

3.5. DISCUSSION

3.5.1. Free chlorine and peracetic acid disinfection in oxidant demand free conditions

That pathogenic and non-pathogenic *E. coli* strains are prone to chlorination in oxidant demand free environments and requires low concentration x contact times values has been known for decades (Hoff & Akin, 1986; Rice et al., 1999; Zhao et al., 2001). Though the Hom model and the presence of some resistant cells suggested the possibility of some tailing, the experimental setup did not allow the confirmation of such an effect. Modeling according to log-linear kinetics provided a decent prediction of the *E. coli* O157 inactivation. A good fit to log-linear kinetics was observed by Lee et al. (2010) for chlorination of *E. coli* (pH 8.5, 4°C, 1 mg/L free chlorine), i.e. when giving equal weights to free chlorine concentration and contact time.

In this chapter, PAA was applied in two conditions of low oxidant demand water, i.e. in tap water and oxidant demand free buffer. In both cases, an initial shoulder effect was observed. Concerning PAA, not much is known about the inactivation rate in oxidant demand free conditions. PAA has mostly been researched in a wastewater context due to its high stability in the presence of organic matter. However, these studies can provide some information regarding the inherent disinfection efficiency of PAA. For example, Dell'Erba et al. (2004) disinfected *E. coli* in wastewater by adding 4 mg/L which decayed to about 3.2 mg/L in 30 min contact time. As such, *E. coli* and total coliforms were exposed to more than 3.2 mg/L of PAA during the experiment. In order to reach 1 log reduction of *E. coli* and total coliforms, about 15 min of contact time were necessary. Although such a reasoning simplifies the process by not considering particle shielding, higher resistance of wild-type strains etc., it is an indication of the relatively slow disinfection rate of PAA. The results in oxidant demand free conditions in the current chapter clearly show the much higher inherent inactivation rate of free chlorine compared to PAA.

3.5.2. Free chlorine and peracetic acid reconditioning in fresh-cut lettuce wash water

COD load had a detrimental effect on disinfection efficiency. As the amounts of iron and manganese were low compared to the COD, these constituents had most likely no considerable influence on chlorine consumption compared to the organic load. Although considerable amounts of organic chloramines were formed, there was only minor potential for inorganic chloramines formation. Organic chloramines possess little or no disinfection

potential against *E. coli* (Donnermair & Blatchley, 2003; Jang, 2009), whereas inorganic chloramines exhibit some disinfection efficiency, yet to a much lesser extent than free chlorine, although they are more stable (Donnermair & Blatchley, 2003). As the inactivation occurred in the first minute of the 30 minute trials it can be concluded that only free chlorine contributed to the inactivation of *E. coli* O157. Regression modeling showed that COD was, next to the added free chlorine dose, the predominate predictor of disinfection efficiency in fresh-cut lettuce wash waters. That the other physicochemical parameters were decent to good estimators in the SPW appeared to be solely due to the correlation between COD and the other parameters. As pH and T were kept constant it can be concluded the microbial inactivation by free chlorine in SPW/IWW depended on COD and free chlorine concentration.

Contrary to free chlorine, the PAA decay correlated most significantly with the UV_{254}(F) absorption. A sharp initial decay of disinfectant concentration often occurs for chemical oxidants in wastewater disinfection, including PAA. This has been attributed to particles, reduced inorganic species such as iron and manganese, microorganisms, volatilization and reaction of the disinfectant with water (Hoff, 1986; john et al., 2005; Falsanisi et al., 2006). However, the results in this chapter strongly point towards organic matter originating from lettuce as the primary determinant of initial and time dependent decay of chlorine and PAA. The PAA decay rate was much slower than that of free chlorine, analogous to the disinfection rate.

The disinfection behavior of PAA was similar in SPW/IPW as in tap water, i.e. a shoulder effect followed by a quasi log-linear inactivation. This behavior is not universal however, as values of m <1 in Hom models, i.e. no shoulder but tailing, has been observed e.g. in wastewater for *E. coli* and total coliforms (Liberti et al., 1999; Dell'Erba et al., 2004; Falsanisi et al., 2006). This occurred in both cases with high initial PAA demand (Falsanisi et al., 2006) and low initial demand followed by low disinfectant decay (Dell'Erba et al., 2004), and as such rapid disinfectant decay cannot fully account for the observed decreased disinfection rate in function of time in those studies. A possible explanation would be the presence of subpopulations with different resistance against PAA within a species (*E. coli*) or varying resistance between species within a microbial group (total coliforms), clumping of microbial cells, or particle association of microbial cells (Gyürek & Finch, 1998). As in the current chapter all *E. coli* O157 cells were derived from two strains that were grown in ideal conditions, such variety among the population might have been absent.

E. coli O157 inactivation during process water recycling with PAA+LA in SPW could be estimated through estimation of the PAA concentration (initial decay and gradient of PAA decay) and the contact time (modified Hom model) or through PAA (+LA) concentration and UV_{254}(F) to predict the kmax and SL values for the Geeraerd model. The *E. coli* O157 inactivation in IPW was lower than expected from the UV_{254}(F) and PAA concentration values and the pH was shown to be influential on the inactivation, presumably because the acid form of the PAA has somewhat greater microbial inactivation (Kitis, 2004, section 1.6.3). This can also serve as an explanation for the synergy between the observed PAA and LA at these low concentrations of LA (i.e. 28 to 82 mg/L) which in itself have no significant disinfectant potential against *E. coli* or *Listeria* spp. (Virto et al., 2006; Grace Ho et al., 2011), but which did lower the pH, i.e. increase the relative abundance of the acid form of PAA.

In conclusion, both microbial inactivation rate and disinfectant decay rate were considerably higher in the case of chlorine compared to PAA+LA. As such, a higher chlorine demand exists in the wash water. Due to the stability in water of PAA, a disinfectant residual can be maintained by adding relatively low doses which interact with the microorganisms during relatively long contact times to achieve disinfection as is being used in wastewater disinfection (Collivignarelli et al., 2000; Dell'Erba et al., 2004). On the other hand a considerably higher PAA+LA residual compared to a free chlorine residual is necessary for equally rapid *E. coli* O157 inactivation.

3.5.3. Free chlorine and peracetic acid dynamic wash water disinfection

For the process wash water disinfection in the washing tank, the model for PAA+LA accurately predicted the microbial wash water contamination, whereas the chlorine model showed some deviations in high COD water. The used models do not incorporate certain phenomena. The used *E. coli* O157 cells were in the stationary phase, yet not exposed to heavy external stress. In real life situations however, cells may have been present on the produce for extended periods of time, and as such the resistance to water disinfectants may be higher from that in the used experimental setup (section 1.5.3). Also, the main assumption of the model (the microbial inactivation depends on the disinfectant residual irrespective of the physicochemical load of the water matrix in which this residual resides) has been shown to be flawed in certain studies, i.e. the "increased demand – increased inactivation" hypothesis (section 1.7) and seems especially relevant for water disinfectants with rapid inactivation/reaction kinetics. In this chapter, it was observed for chlorine that when

maintaining a residual in SPW with a higher COD, the number of microbial samples below the LOD was also higher (Figure 3.6). On that note, with increasing COD load, the variation in free chlorine concentration also increased, due to the difficulty of maintaining low residuals in conditions with a high physicochemical load. In the case of higher organic loads, the greater variations in *E. coli* O157 were not predicted by the model outputs, i.e. the observed increased variation in free chlorine concentration could not account for the increased variation in microbial inactivation. The related increased variation in microbial inactivation in SPW 500 and 1000 mg O_2/L was predominately towards lower and not higher microbial counts compared to the inactivation in tap water (low physicochemical load) (Figure 3.6), corresponding to the "increased demand – increased inactivation" hypothesis. Furthermore, higher chlorine gradients in high COD conditions and the incompletely mixed character of the washing tank were not considered in the model. The PAA+LA model did not suffer from the model simplifications described above. This can be explained by the relatively slow decay of PAA in the presence of organic matter, resulting in lower disinfectant demands of the wash water, and as such lower disinfectant concentration gradients, and overall slower influence of concentration gradients on microbial inactivation.

Concerning the practical use of these models, the inactivation of *E coli* O157 can be predicted in a leafy vegetables washing process when the disinfectant concentration of PAA (+LA) or free chlorine is continuously measured. In addition, the models can predict the influence of water refreshing on the *E. coli* O157 wash water contamination. It can be assumed that by following the same methods (i.e. determining inactivation parameters for the target microorganism and entering these in the dynamic model) these models could be applied for other target microorganisms. These microbial inactivation models should be validated with data obtained from full-scale fresh-cut leafy vegetables washing operations. Also, when considering food quality, inactivating a heterogeneous collection of microbial species is needed. This is very difficult to simulate in oxidant demand free buffer and such experiments should be done with water from fresh-cut leafy vegetables processes that contain these microorganisms, unless model organisms are used to represent a microbial group (e.g. *Candida albicans* for yeasts). In addition, the necessary dosage (disinfectant demand + residual) of free chlorine and PAA (+LA) in function of COD or UV_{254}(F) respectively, was determined in SPW conditions. Therefore, for these disinfectants, the necessary disinfectant dosage could be estimated for a certain process, which is a necessary parameter for operational cost calculation. That is why the disinfectant demand should be studied in more

detail, i.e. determining the demand of different types of fresh-cut leafy vegetables (and other fresh(-cut) produce). In conclusion, the current models can estimate disinfectant efficiency and disinfectant demand. This provides a disinfectant residual value to be maintained through process control. Through estimation of the disinfectant demand, these models can be coupled to dynamic cost models. The resulting model could then be used to estimate the total cost of implementing the disinfectant technology in the fresh produce washing operation.

3.5.4. Influence of target microorganism on chlorine disinfection efficiency

The higher susceptibility to chlorine of *E. coli* O157 than of *L. monocytogenes* in this chapter was also observed in other studies (Korich et al., 1990, Koseki et al., 2001). As the primary action of chlorine is targeted at the interior of the cell (section 1.5.1.1), it can be considered that the difference in resistance between Gram-negative and Gram-positive species might be predominately dependent on differences in resistance to mass transfer of chlorine across Gram-positive and Gram–negative cell surface layers. On that note, a comparison between resistance of Gram-positive and Gram–negative species based solely on the thickness of the peptidoglycan layer is an oversimplification due to the spatial difference of these layers in the cell surface of both bacterial groups, as well as the difference in composition of the Gram-positive and Gram-negative bacterial cell walls. This was noted by Dalrymple et al. (2010) when reviewing the mechanisms of photocatalytic disinfection, but this reasoning seems also sound for chlorination. The higher resistance of *L. monocytogenes* compared to Gram-negative pathogens illustrates the necessity for an accurate indicator organism (Harwood et al., 2005; Sinclair et al., 2012). If two or more pathogens form a significant risk in a given situation, the criteria should be designed to avoid the occurrence of the most resistant one, if practically and economically achievable. This vision has already been adopted in certain water quality criteria, e.g. the "Long Term 2 Enhanced Surface Water Treatment Rule" by the USEPA, which incorporated the necessity for drinking water production systems to fulfill a certain *Cryptosporidium* inactivation, besides the conventional *E. coli* based limits (Dotson et al., 2012; USEPA, 2012).

Although maintaining 1 mg/L of free chlorine during the process wash water disinfection does not keep the wash water free from *E. coli* O157 (and which was the initial goal to allow quantitative modeling), the results show that low chlorine concentrations are highly effective in eliminating *E. coli* O157 in suspended state (e.g. resulting in an average reduction of 3.2 log CFU/100 mL wash water after the 1 hour simulated washing process in tap water). It must

also be noted that although eliminating cross-contamination may reduce the risk for outbreaks by avoiding the spread of contamination during produce washing, ultimately it does not solve fresh produce food borne disease and the best way of eliminating pathogens is to avoid contamination altogether during primary production, although a zero tolerance cannot be expected at the moment (Parish et al., 2003; Lopez-Galvez et al., 2009; Lynch et al., 2009). The fact that chlorination of fresh-cut lettuce wash waters in the assessed model systems is feasible for inactivating vegetative bacterial pathogens, does not make it necessarily effective against other pathogens in these conditions. Concerning viruses, norovirus is considered to be of greatest concern in regard to fresh-cut produce (FAO, 2008; Baert et al., 2011). Although there are large fluctuations in various studies on inactivation of norovirus with free chlorine (dependent on the type of detection, if the viruses are aggregated or dispersed, if free or total chlorine is measured), concentration x contact times from studies with free chlorine in low organics loaded waters show that norovirus and its more practical surrogate murine norovirus are both highly vulnerable to chlorination (Shin & Sobsey, 2008; Cromeans et al., 2010; Kitajima et al., 2010). Protozoan parasites, such as *Cryptosporidium parvum* and *Cyclospora cayetanensis*, have also been associated with outbreaks related to fresh produce consumption, particularly in Latin America, but the issue of protozoan parasites has been increasingly recognized worldwide as pathogens of concern (Chaidez et al., 2005; FAO, 2008; Ortega & Sanchez, 2010; Olaimat & Holley, 2012). If produce wash water should be kept free of these protozoan pathogens, a chlorination system would fail due to the high resistance against chlorine (Gyurek et al, 1998; Duhain et al., 2012) or alternatively excessively high free chlorine concentrations would be required. As such, other disinfection technologies should be applied to inactivate protozoan parasites and some viruses (section 1.5).

3.5.5. The production of DBPs in the wash water and on the lettuce during chlorine reconditioning and wash water disinfection

The TTHMs concentrations, generated during the reconditioning trials, were far below the allowed DBPs concentrations in drinking water in the EU (100 µg/L TTHMs) and in the USA (80 µg/L TTHMs) (EC, 2007; USEPA, 2009). Higher concentrations were formed in the washing bath trials due to continuous exposure of the wash water to free chlorine. In some of the TTHMs measurements high standard deviations were observed. As this occurred both at a low TTHMs concentration and a relatively high concentration (Chapter 5), this is most likely, next to the experimental error, in part due to the fact that only two replicates were executed to be analyzed for TTHMs. Formation of TTHMs increases among others with increasing

chlorine dose and organic load (WHO, 2000, Yang et al., 2005). The results in this chapter, and those by Lopez-Galvez et al. (2010b) and Gomez-Lopez et al. (2014) confirmed this specifically for fresh-cut leafy vegetables derived water matrices. In the case of COD 1000 mg O_2/L, small amounts of bromodichloromethane were detected. Brominated organics are formed through reaction of organic matter with hypobromous acid, which itself is formed because hypochlorous acid oxidizes bromide that is present in the water (Gallard & von Gunten, 2002; Westerhoff et al., 2004). In the case of COD 1000 mg O_2/L, DBPs in the water exceeded the EU and USA TTHMs drinking water limit. Nonetheless, in all cases no measurable amounts of TTHMs were found on the lettuce after the rinsing step, even in the case when the TTHMs in the SPW exceeded the EU legislative limit. The given setup would not pose any chemical residual risk with regard to TTHMS on the end product (pre-washed packaged fresh-cut lettuce) for the consumer. This absence of DBPs on the washed leafy greens was also observed in other studies (COT, 2006; Lopez-Galvez et al., 2010b; Gomez-Lopez et al., 2013). Nevertheless, the results of the washing bath trials, especially in the SPW containing a COD of 1000 mg O_2/L, show that considerable TTHMs can be formed in the water due to prolonged chlorination. As such, formation of DBPs could still be a problem if legislation would pose limits on DBPs in the wash water or the resulting wastewater, as besides the known drinking water limits now also legal limits are arising for treated wastewater (e.g. by the Florida Department of Environmental Protection) to protect the surface water quality and avoid accumulation in the environment (Hua & Yeats, 2010).

3.6. CONCLUDING REMARKS

- Inactivation of *E. coli* O157occurred faster with free chlorine than with PAA+LA
- Free chlorine decomposition (and *E. coli* O157 inactivation in a reconditioning setup) could be predicted based on the COD of the wash water
- PAA decomposition could be predicted based on the UV_{254}(F) of the wash water
- Inactivation of *E. coli* O157 with PAA+LA occurred faster at lower pH (pH range 6 to 10)
- *E. coli* O157 inactivation rate constants of both PAA+LA and free chlorine in oxidant demand free conditions could predict the inactivation in the dynamic wash water disinfection experiments, with prediction based on the disinfectant residual and independent of wash water organic matter

CHAPTER 4

WASH WATER DISINFECTION OF A FULL-SCALE LEAFY VEGETABLES WASHING PROCESS WITH HYDROGEN PEROXIDE AND THE USE OF A COMMERCIAL METAL ION MIXTURE TO IMPROVE DISINFECTION EFFICIENCY

Redrafted from

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4. WASH WATER DISINFECTION OF A FULL-SCALE LEAFY VEGETABLES WASHING PROCESS WITH HYDROGEN PEROXIDE AND THE USE OF A COMMERCIAL METAL ION MIXTURE TO IMPROVE DISINFECTION EFFICIENCY

4.1. ABSTRACT

H_2O_2 was used to maintain the microbial wash water quality of a full-scale leafy vegetables (radicchio, sugar loaf, curled endive, lollo, lollo rosso) wash water process. Despite addition of 300 L/h of 1.8 % H_2O_2 to a 450 L washing bath (333 ± 50 kg/h fresh-cut produce introduction speed), the H_2O_2 quickly decreased and a lower wash water contamination of APC and enterococci than without addition of H_2O_2 could not be maintained. There was no significant difference between the APC on fresh-cut leafy vegetables washed with H_2O_2 and those washed with water.

In a second part, lab-scale experiments were performed to assess the impact of a commercial metal ion formulation (Bacsan®, containing a. o. Cu^{2+}, Zn^{2+}, Ag^{+}) on the stability of H_2O_2 in artificial wash water, made from iceberg lettuce and tap water. Bacsan improved the stability of H_2O_2 in artificial lettuce wash water and fresh-cut leafy vegetables wash water from a processing company and synergistically increased the disinfection efficiency of APC and *E. coli* compared to H_2O_2 or Bacsan. Increasing COD had detrimental effect on the H_2O_2 stability and disinfection efficiency. Addition of Ag^{+} to Bacsan further synergistically enhanced the H_2O_2 stability.

H_2O_2 is not suited as an *in situ* wash water disinfectant to avoid cross-contamination in fresh-cut leafy vegetables washing processes due to the slow water disinfection kinetics and the rapid H_2O_2 consumption.

4.2. INTRODUCTION

H_2O_2 does not produce toxic fumes in the worker space and is an environmentally friendly alternative to chlorine for decontamination of fresh produce, as it breaks down in water and oxygen (Tofant et al., 2006), and does not form carcinogenic disinfection byproducts (section 1.4.3). Considerable research has been conducted on the use of H_2O_2 as produce decontamination agent against bacterial and viral indicator organisms, pathogenic bacteria, or spoilage microflora on fresh (-cut) fruit and vegetables (Parish et al., 2003; Ukuku et al., 2012), among which some experiments have been performed on leafy vegetables (Lin et al., 2002; Allwood et al., 2004; Hadjok et al., 2008; Huang, & Chen, 2011; Li et al., 2011). On

the contrary, its use as a water disinfectant to control the wash water quality of fresh produce washing processes is virtually unexplored. Earlier water disinfection studies that focused on inactivating vegetative bacteria, bacterial spores, viruses, or protozoa have shown that H_2O_2 by itself is a slow acting water disinfectant, requiring high dosages and contact times for microbial inactivation (Raffellini et al., 2008; section 1.5.1). Combined with Ag^+ and $Cu^{1 \text{ or } 2+}$, performance of H_2O_2 can be enhanced (Pedahzur et al., 1997, 2000; Batterman et al., 2000; Orta De Velasquez et al., 2008).

In this chapter, the use of H_2O_2 for process wash water disinfection in a full-scale industrial fresh-cut leafy-vegetables washing process was assessed. Also, lab-scale experiments were performed to assess the use of Bacsan (containing a. o. Cu^{2+}, Ag^+, and Zn^{2+}) to improve the H_2O_2 disinfection efficiency in post-harvest process water recycling practices.

4.3. MATERIALS & METHODS

4.3.1. Water disinfection in a fresh-cut leafy vegetables processing company

4.3.1.1. Experimental setup

Experiments were executed in a Belgian fresh-cut leafy vegetables processing company. First, a run was executed without addition of water disinfectant, i.e. the 'blank' run. A batch of 400 kg mixed salad was processed, containing radicchio (33%), sugar loaf (*Chicorium intybus*) (33%) and curled endive (33%). The leafy vegetables were cut (in pieces of 1 by 5 cm), and transported through two subsequent immersion washing baths (washing bath 1: WB1 and washing bath 2: WB2) with a volume of 450 L each, and a leafy vegetable residence time of 1 min in each washing bath. The washing system consisted of bubble washers, i.e. production of agitation in the washing baths by air bubble injection through underwater air nozzles. Subsequently they were transported by a conveyer belt to a centrifuge for dewatering, followed by a weighing unit (computer controlled weight proportioning scales). Both washing baths were filled with bore hole water, cooled on beforehand to 2 °C. During the washing process, 300 L/h of bore hole water was added to each of the washing baths. Wash water was recirculated within washing baths but not between washing baths. The only water that was transferred from WB1 to WB2 was the water that was attached to the transferred lettuce. Two wash water disinfection experiments were performed. In both experiments, the same types of leafy vegetables were processed during the wash water disinfection experiments of which the first batch (467 ± 55 kg) was the same leafy vegetables mix as in the blank runs. In addition, a second batch (258 ± 31 kg) was processed, consisting of white lollo (*Lactuca sativa* cv. Lollo

Bianco) (50%) and lollo rosso (*Lactuca sativa* cv. Lollo Rosso) (50%). For each type of leafy vegetable and experiment, the crops originated from the same farm, and the crops were processed at the day of harvest. On average leafy vegetables were washed at 333 ± 50 kg/h. In the disinfection experiments, WB1 was operated identically to the blank runs. In the first disinfection experiment, WB2 was filled with 1.8 % H_2O_2 (i.e. 4% EcoClearProx, ABT Belgium, Belgium) and 300 L/h 1.8% H_2O_2 of bore hole water was added. In the second disinfection experiment, WB2 was filled with 1.8% H_2O_2 and 300 L/h of bore hole water was added. During processing, 300 L/h of wash water was tapped from the washing bath and 5.4 L/h H_2O_2 was dosed (again to obtain addition of 1.8 % H_2O_2/L) and sent through a LP UV-C system (Aquada 2, Wedeco, Belgium; 55 W) with fluence of 240 mJ/cm² at a flow of 300 L/h and 98% UV 254 nm transmittance/cm, before recirculation to WB2.

4.3.1.2. Sampling in the fresh-cut leafy vegetable processing company

Samples of the fresh-cut leafy vegetables, water samples from WB1 and WB2, and samples from the food contact surfaces of the conveyer belt and the weighing unit were taken five times throughout the processing: at the start of batch 1, at the middle of batch 1, at the end of batch 1 = start of batch 2, in the middle of batch 2, at the end of batch 2. About 250 g of fresh-cut leafy vegetables was sampled and put directly into a sterile stomacher bag. For sampling the raw material, each lettuce type was sampled separately per batch, and averaged as the microbial count of the raw material. The water samples were collected into a sterile 1 L bottle according to ISO 19458:2006 (ISO, 2006). Excess H_2O_2 was quenched with sterile $Na_2S_2O_3$. The food contact surfaces were sampled with sterile swabs. Aseptic templates covering 50 cm² were used and a sterile swab moistened in 5 mL of buffered peptone water was used to swab a delimited area vertically, horizontally, and diagonally. All the samples were stored and transported in the dark at < 4°C to the lab for further handling and subsequent microbial analysis within 12 h. For each measuring point two independent samples were taken. At each time point and operation unit, water and food contact surfaces were sampled at two consistent points, and each of the two samples for raw materials screening originated from two crops.

4.3.1.3. Microbial analyses

For the fresh-cut leafy vegetables samples and food contact surfaces, APC and *E. coli* were enumerated, whereas in the water also enterococci were enumerated. For the fresh-cut leafy vegetables samples, 10 g of fresh-cut leafy vegetables was weighed in a stomacher bag and homogenized for 1 minute in 90 ml buffered peptone water. The enumeration of APC was done with the reference method ISO 4833:2003 (ISO, 2003), with the exception that the plates

were incubated at 22°C for five days instead of at 30°C for 3 days. *E coli* was enumerated with the pour plate method on RAPID'*E.coli 2* agar (BioRad, France), a selective chromogenic medium, incubated for 24 h at 37°C. For the water and food contact surface samples, APC was measured according to ISO 6222:1999 and incubated for 3 days at 22°C (ISO, 1999). The enumeration of *E. coli* was done according to ISO 9308-1 (i.e. membrane filtration) with the exception that the tergitol 7 medium was replaced by RAPID'*E.coli* 2 agar (Biorad, France) (ISO, 2000a). The detection and enumeration of enterococci was performed using the membrane filtration method ISO 7899-2 (ISO, 2000b).

4.3.1.4. Physicochemical parameters

Alkalinity, turbidity, and COD were determined as described in section 2.3.4. H_2O_2 concentration, pH and T were determined at the fresh-cut leafy vegetables processing company. H_2O_2 was determined with the spectrophotometric I_3^- method by Klassen et al. (1994). H_2O_2 was determined immediately after sampling, to avoid further consumption due to reaction with the water matrix components.

4.3.2. Water disinfection in standardized process water

4.3.2.1. Standardized process water

SPW was made as described in section 3.3.2.2 but with iceberg lettuce (*Lactuca sativa L.*) instead of butterhead lettuce.

4.3.2.2. Industrial process water

IPW was collected at a Norwegian fresh-cut leafy vegetables processing company. The water was collected immediately after a batch of mixed lettuce, i.e. iceberg lettuce, rucola (*Eruca sativa*) and radicchio, had been washed with tap water.

4.3.2.3. Bacterial inoculation

E. coli ATCC 25922 was grown in nutrient broth (Oxoid, France) for 24 hours at 37° C. The *E. coli* cells were washed in phosphate-buffered saline and subsequently added to the SPW to obtain 5-6 log CFU / mL.

4.3.2.4. Physicochemical parameters

The Cu^{2+} ion concentration in SPW was measured with a test kit (MD 200 2IN1 copper, Lovibond, Germany), based on the reduction of Cu^{2+} to Cu^{+}, the reaction of Cu^{+} with

bicinchoninic acid, followed by spectrophotometric measurement of the formed complex. Free chlorine was measured as described in section 2.3.4.

4.3.2.5. Disinfection experiments

H_2O_2 was diluted from a 30% stock-solution (Fluka Analytical, Germany). Bacsan (Labola, Norway) is a patented, commercial formulation from Aqua Chemical Nutrients, marketed as water disinfectant and containing a. o. Cu, Ag, and Zn. The content of the Bacsan solution was analyzed with inductive coupled plasma emission spectrometry to determine the actual metal ion concentrations and were found to be: 84.2 ± 1.1 g/L Cu, 7.3 ± 0.2 mg/L Ag, 23.7 ± 0.4 g/L Zn, 24.0 ± 0.4 mg/L Al, and 56.0 ± 0.2 g/L NO_3^-. 100 mL of continuously mixed, inoculated SPW at 4 ± 2 °C was exposed to 500 mg/L of H_2O_2, with or without the addition of 2 or 10 mg/L Bacsan-Cu (expressed as mg/L Cu^{2+} in Bacsan), or to 500 mg/L of H_2O_2 with the addition of 10 mg/L Cu^{2+} from $CuSO_4.5H_2O$ (Merck, Germany), or to 10 mg/L Bacsan-Cu without H_2O_2. H_2O_2 residual concentration was measured after 5, 30, and 120 min. Microbial samples were taken after 30 and 120 min and immediately quenched with $Na_2S_2O_3$. For each treatment and chosen COD level of SPW, 3 independent experiments were executed. The IPW was similarly treated as the SPW, except for the *E. coli* inoculation which was not executed. Also, exposure of SPW to 500 mg/L of H_2O_2 with addition of 2 mg/L Bacsan-Cu and 0.1 mg/L Ag^+ from $AgNO_3$ (Sigma-Aldrich, Germany) or to 500 mg/L of H_2O_2 with addition of 10 mg/L Bacsan-Cu and 1 mg/L Ag^+ was assessed for H_2O_2 stability in SPW.

4.3.2.6. Microbial analyses

APC was enumerated with the pour plate method on Water plate count agar (Oxoid, England) (incubated for 3 days at 22° C) and *E. coli* with the pour plate method, using RAPID'*E. coli* 2 agar (Biorad, France) (incubated for 24 h at 37°C).

4.3.2.7. Assessment of the interaction of catalase and Bacsan

For investigating the effect of Bacsan and pH on the H_2O_2 consumption caused by the enzyme catalase, SPW was rapidly heated to 80°C and maintained at 80°C for 10 min to inactivate catalase (Hirvi et al., 1996; Anderson, 2002). Thereafter, the SPW was rapidly cooled to 4°C. The heated and unheated SPW were treated with 630 mg/L H_2O_2 (with or without 10 mg/L Bacsan-Cu) and also with 100 mg/L free chlorine for comparison with a disinfectant that is no specific target of an enzyme. Free chlorine was diluted from a chlorine stock solution (28.4 g/L NaOCl, La Croix, Belgium).

Also, experiments using pure catalase from bovine liver (Sigma-Aldrich, Norway) were performed. H_2O_2 was diluted in 0.05 mol/L phosphate buffer with pH 5.5, 6.0 or 7.2 to a final concentration of 590 mg/L H_2O_2. Preliminary tests were done at pH 5.5 and pH 7.2 by adding 0, 2, 10 mg/L Bacsan-Cu, or 10 mg/L Cu^{2+} (as $CuSO_4.5H_2O$) to the buffered H_2O_2 solutions, to which catalase was added to a final concentration of 1.5 mg/L. The consumption of H_2O_2 caused by the added catalase was assessed by measuring the H_2O_2 residual concentration after 5 and 30 min, at 4±2 °C, and under continuous mixing. More detailed experimentation was done with 0 and 10 mg/L Bacsan-Cu in the presence of 590 mg/L H_2O_2 in buffered solutions at pH 5.5, 6.0, and 7.2. The H_2O_2 residual concentration was measured after 5 min and 30 min. The experiments were performed at 4 ± 2 °C and repeated 3 times.

4.3.2.8. Oxidative browning

Lettuce washing experiments were executed to determine the effect of the treatments (i.e. 500 mg/L H_2O_2 with or without Bacsan) on oxidative browning of the lettuce and consisted of washing 30 g of cut lettuce with mechanical agitation for 2 min at 4 ± 2 °C, followed by centrifugation and storage in sterile plastic boxes at 4 ± 2° C. After 3, 4, and 5 days the lettuce samples were observed for visible traces of enzymatic browning.

4.3.3. Statistics

Statistical analysis was performed with SPSS statistics 21 and Microsoft Excel. Comparison of parameter levels was done with one-way ANOVA or Brown-Forsythe when equal variance could not be assumed. Group comparison was done with post-hoc tests (Tukey or Games-Howell). For comparing means of parameters, Wilcoxon-signed rank tests was used for paired samples. For unpaired samples, the student t-test (independent two-sample t-test) was used because the Mann-Whitney U test cannot be used to compare two groups of three values (repeats), and the fact that there is no principal objection to using a student t-test on low sample sizes if caution is taken towards the interpretation and the value of the results (de Winter, 2013). A level of significance of $p \leq 0.05$ was chosen for all statistical analyses. Noted deviations on measurements represent standard deviations.

4.4. RESULTS

4.4.1. H_2O_2 wash water disinfection in a fresh-cut leafy vegetables processing company

Initially, the washing process turbidity and COD increased rapidly and subsequently, in general, the increase diminished as a function of time (Figure 4.1). The turbidity in WB1 was significantly higher than in WB2 during the trials. The COD in WB2 was not measured due to the interference of H_2O_2. The H_2O_2 concentration rapidly decreased during the washing process (Figure 4.1), and a significant negative correlation between turbidity and H_2O_2 concentration ($r^2 = 0.608$; $p < 0.0005$) was observed. The temperature increased to higher values in WB2 compared to WB1. The pH of the bore hole water was 7.4 ± 0.1. The pH value rose to slightly higher values in WB2 compared to WB1 towards the end of the water disinfection experiments, though not significantly (Figure 4.1). The alkalinity did not change significantly as a function of time and was not significantly different between treatments nor washing baths, with 6.36 ± 0.10, 6.38 ± 0.14 and 6.51 ± 0.16 mmole/L bicarbonate in the bore hole water, WB1 and WB2 respectively, indicating it originated predominately from the bore hole water itself. The same was observed in the sugar snaps washing process (section 2.4.1.).

The *E. coli* contamination was below the limit of detection at all times and locations, i.e. < 1 log CFU/g on the fresh-cut leafy vegetables, < 0.3 log CFU/100 mL in the water and < 0.7 log CFU/ 50 cm² on the conveyer belt and the weighing unit. APC and enterococci contamination was significantly higher in WB1 compared to WB2 in all experiments (Figure 4.2). To assess the impact of the water disinfection treatments, the differences between the measured contamination in both washing baths was calculated (WB1 – WB2) in order to be able to compare the wash water disinfection efficiency of the treatments and to incorporate fluctuations in transfer of microorganisms from fresh-cut leafy vegetables to the washing baths between the different water disinfection trials. These differences were significantly higher for both disinfection treatments compared to the blank during the first batch (first 60 to 80 min dependent on the batch size), i.e. the wash water contamination was significantly reduced with the 1.8 % H_2O_2 (with or without UV) treatment. For enterococci, no significant reductions in the wash water were found. The gradually increasing APC concentration in the H_2O_2 treated wash water (Figure 4.2b) reflects the declining H_2O_2 residual during the washing process (Figure 4.1), despite the continuous addition of 300 L/h of 1.8 % H_2O_2 in a 450 L washing bath, due to the build-up of organic matter in the washing bath, indicated in WB2 as increasing turbidity (Figure 4.1).

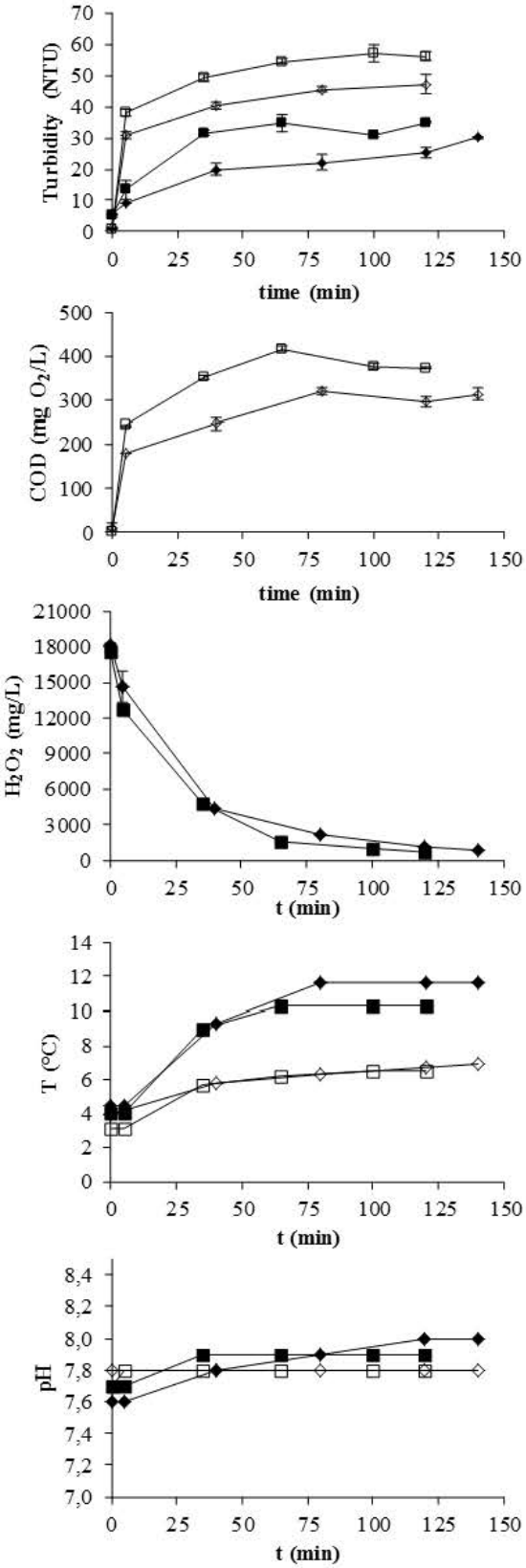

Figure 4.1. Turbidity, COD, H_2O_2, T, and pH during the washing bath trials with 1.8% H_2O_2, measured in WB 1(◊) and WB 2 (♦), washing bath trials with 1.8 % H_2O_2 + UV, measured in WB 1 (□) and WB 2 (■) (n = 3)

The APC and enterococci contamination after the UV/H_2O_2 unit was reduced to below the detection limit (< 2 log CFU/100 mL and < 0.3 log CFU/100 mL respectively) at all times. However, a decrease of the wash water contamination in WB2 compared to the 1.8% H_2O_2 treatment was not observed.

For batch 1, the initial APC load of the fresh-cut leafy vegetables was 7.1 ± 0.4, 6.8 ± 0.3, and 6.8 ± 0.2 log CFU/g for the blank run, 1.8 % H_2O_2, and 1.8 % H_2O_2 + UV respectively, whereas for batch 2, it was 7.4 ± 0.2 and 7.6 ± 0.2 log CFU/g for 1.8 % H_2O_2 and 1.8 % H_2O_2 + UV respectively. The APC load on the fresh-cut leafy vegetables was reduced significantly with 1.8 % H_2O_2 (Figure 4.3), with or without UV, but also with a water wash. The introduction of organic matter lowered the H_2O_2 concentration as the washing process advanced in time. However, processing time had no influence on decontamination efficiency of any of the treatments, and 1.8 % H_2O_2 (with or without UV) did not improve the decontamination efficiency (considering batch 1) compared to a water wash (Figure 4.3). The APC contamination on the conveyer belt increased during processing from 3.1 to 4.8, 2.7 to 4.2 and 2.3 to 4.7 log CFU/ 50 cm² in the blank run, with 1.8 % H_2O_2, and with 1.8 % H_2O_2 + UV respectively, and on the weighing unit from 3.2 to 5, 2.3 to 4.7, and 3.5 to 5.5 log CFU/ 50 cm² respectively.

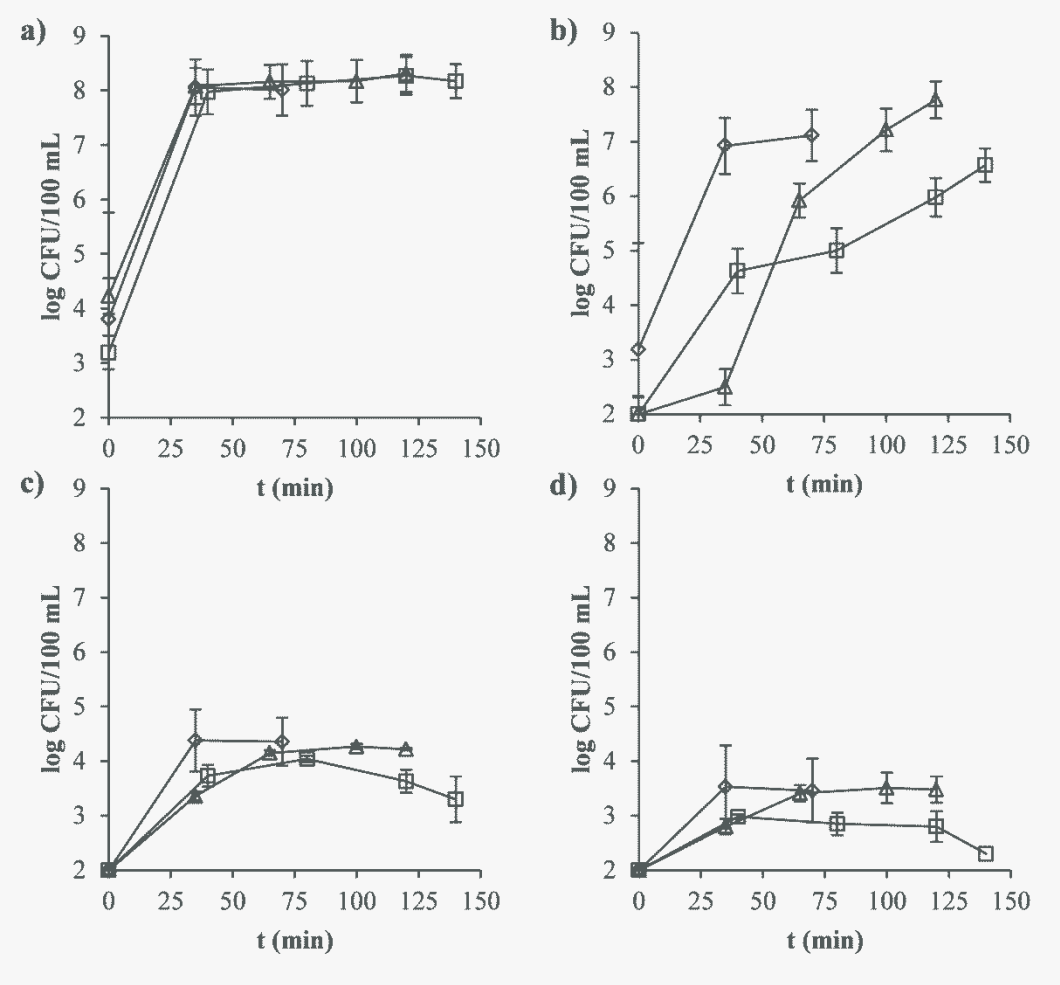

Figure 4.2. Washing bath contamination of APC during the washing trials in the screened company in a) WB1 and b) WB2 and enterococci in c) WB1 and d) WB2; during the blank run (◊), when WB2 was treated with 1.8 % H_2O_2 (□), when WB2 was treated with 1.8 % H_2O_2 + UV (Δ) (n = 2)

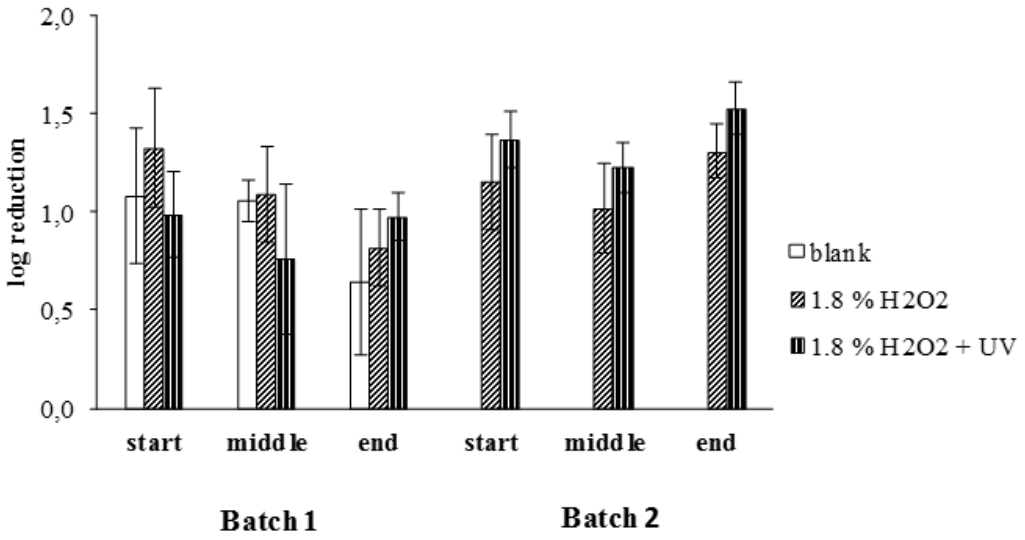

Figure 4.3. APC reduction on the fresh-cut leafy vegetables due to the industrial washing processes in the screened company (n = 2)

4.4.2. H_2O_2 stability in SPW

For all treatments, both COD and contact time had a significant detrimental influence on the H_2O_2 concentration in the SPW (Table 4.1). The rate of H_2O_2 consumption was lowest with H_2O_2 + 10 mg/L Bacsan-Cu. The consumption rate was lower with H_2O_2 + 2 mg/L Bacsan-Cu than with H_2O_2 at COD 497 and 848 mg O_2/L, whereas at COD 1830 mg O_2/L no difference was observed (Table 4.1).

The stability of H_2O_2 in SPW of COD 789 mg O_2/L was higher with H_2O_2 + 10 mg/L Bacsan-Cu compared to H_2O_2 + 10 mg/L Cu^{2+} (from $CuSO_4$), which in turn was significantly higher after 30 min than in the absence of metal ions (Figure 4.4). The addition of 0.1 mg/L Ag^+ to 2 mg/L Bacsan-Cu and 1 mg/L Ag^+ to 10 mg/L Bacsan-Cu in SPW of COD 753 mg O_2/L further enhanced the stability of initially added 500 mg/L H_2O_2 in a synergistic fashion (Figure 4.5).

Table 4.1. Microbial reduction and H_2O_2 concentration during water disinfection trials in SPW of varying COD and IPW with 500 mg/L H_2O_2 and/or Bacsan dosage (n=3)

					SPW						**IPW**	
COD (mg O_2/L)		497 ± 7			848 ± 6 mg			1830 ± 21			509 ± 3	
t (min)	5	30	120	5	30	120	5	30	120	5	30	120
APC (log reduction)												
H_2O_2		0.7±0.1	2.2±0.5		0.5±0.2	1.3±0.3		0.0±0.1	0.0±0.2		0.5±0.2	1.0±0.1
H_2O_2 +2 mg/L Bacsan-Cu		2.0±0.4	2.7±0.1		1.2±0.2	1.3±0.3		0.0±0.2	-0.1±0.3		0.4±0.1	0.8±0.1
H_2O_2+10 mg/L Bacsan-Cu		4.8±0.1	5.0±0.2		4.5±0.2	4.5±0.2		0.1±0.1	0.9±0.1		2.8±0.2	3.3±0.1
10 mg/L Bacsan-Cu		2.6 0.1	>2.7		0.8±0.3	1.1±0.2		0.1±0.2	0.1±0.1		0.3±0.1	0.8±0.1
***E. coli* (log reduction)**												
H_2O_2		0.7±1.1	3.0±0.1		0.6±0.2	0.7±0.2		0.0±0.1	0.1±0.1			
H_2O_2 +2 mg/L Bacsan-Cu		3.8±0.2	4.3±0.5		3.0±0.3	3.0±0.3		0.2±0.1	0.4±0.1			
H_2O_2+10 mg/L Bacsan-Cu		>5	>5		>5	>5		1.2±0.1	1.8±0.2			
10 mg/L Bacsan-Cu		>2.5	>2.5		1.2±0.2	1.9±0.1		0.2±0.1	0.1±0.2			
H_2O_2 (mg/L)												
H_2O_2	314±2	38±1	1.2±0.1	221±2	1.3±0.1	1.4±0.1	94±1	3±1	1.1±0.1	338±3	40±1	1.3±0.1
H_2O_2 +2 mg/L Bacsan-Cu	358±1	57±3	16±2	241±2	27±2	1.2±0.1	92±2	6±1	1.0±0.1	352±6	88±1	35±1
H_2O_2+10 mg/L Bacsan-Cu	374±2	166±9	88±2	319±3	143±8	72±2	157±5	16±1	2±0.1	387±7	202±8	122±1

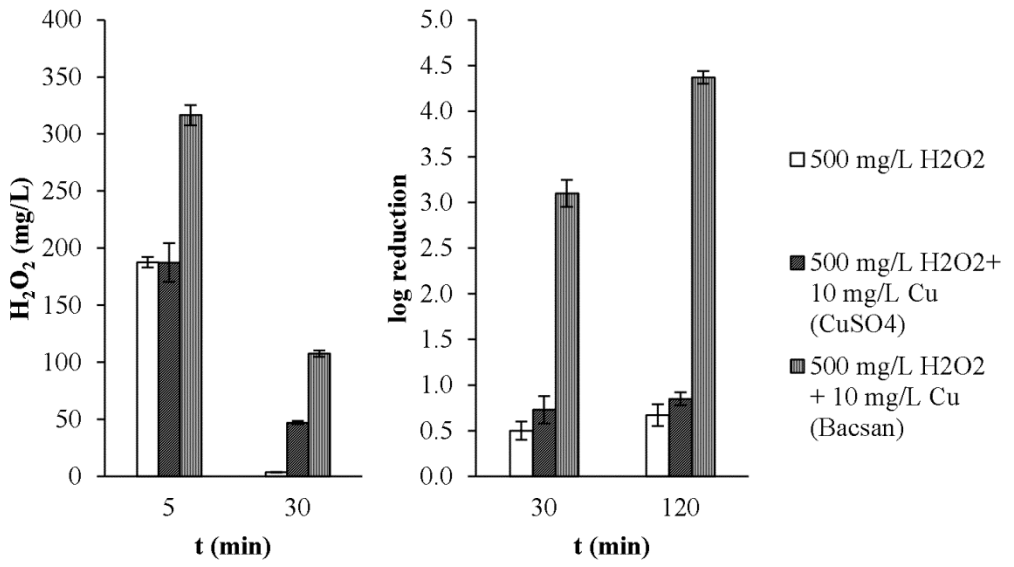

Figure 4.4. Residual H_2O_2 and APC reduction in SPW of COD 789 ± 7 mg O_2/L when comparing treatment with 500 mg/L H_2O_2, H_2O_2 + 10 mg/L Cu^{2+} (as $CuSO_4$) and H_2O_2 + 10 mg/L Bacsan-Cu (n = 3)

The Cu^{2+} concentration in solution decreased only moderately after 120 min treatment time, i.e. 9.5 ± 0.7 %, 3.6 ± 1.3 %, and 5.6 ± 3.1 % for H_2O_2 + 10 mg/L Bacsan-Cu, H_2O_2 + 2 mg/L Bacsan-Cu and 10 mg/L Bacsan-Cu respectively. The pH of the SPW was 7.3 ± 0.2. Addition of 500 mg/L H_2O_2 did not change the pH significantly, whereas addition of 10 mg/L Bacsan-Cu, H_2O_2 + 2 mg/L Bacsan-Cu, and H_2O_2 + 10 mg/L Bacsan-Cu decreased the pH with 0.3 to 0.7, 0.3 to 0.9, and 0.5 to 1.0 respectively, the pH drop increasing with decreasing COD of the SPW, most likely due to an increasing amount of pH buffering molecular species in SPW of higher COD. This pH drop was in part due to the low pH of the Bacsan stock-solution i.e. below the detection limit of the pH meter (pH 0).

When heating SPW of COD 819 mg O_2/L, the COD did not change significantly. Addition of 630 mg/L H_2O_2 to heated SPW led to an initial rapid decrease in the first 5 min, after which no considerable further consumption occurred during the remaining 25 min (Figure 4.6a). Addition of Bacsan had no influence on the H_2O_2 consumption. In the unheated water however, Bacsan decreased the H_2O_2 consumption (as observed before). The H_2O_2 residual in heated SPW was considerably larger than in the unheated SPW, whereas for free chlorine the difference in consumption was much smaller (Figure 4.6b).

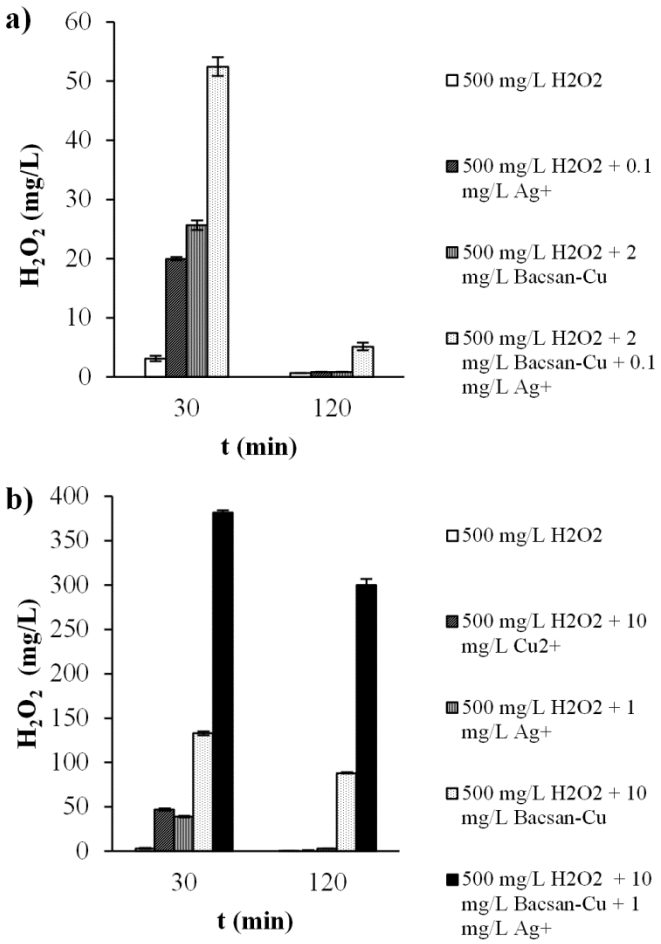

Figure 4.5. H_2O_2 residual in function of time, when adding Ag^+ to Bacsan in SPW of COD 753 ± 5 mg O_2/L, a) combinations with 2 mg/L Bacsan-Cu and 0.1 mg/L Ag^+, b) combinations with 10 mg/L Bacsan-Cu and 1 mg/L Ag^+ (n = 3)

To explain the increased stability of H_2O_2 in the presence of Cu^{2+} and to larger extent in the presence of Bacsan, the impact on the activity of bovine liver catalase activity was assessed. In the absence of catalase the H_2O_2 concentration remained constant in all the phosphate buffered solutions during the experimental period. The pH was measured and found to not be affected by the addition of H_2O_2 and Bacsan. Preliminary experiments (without repeats) showed the following order of H_2O_2 stability: H_2O_2 < H_2O_2 + 2mg/L Bacsan-Cu ~ H_2O_2 + 10 mg Cu^{2+} (from $CuSO_4$) < H_2O_2 + 10 mg/L Bacsan-Cu (data not shown). More detailed experiments showed that at each pH value (5.5, 6, or 7.2), the consumption of H_2O_2 was significantly lower in the presence of 10 mg/L Bacsan-Cu than in absence of Bacsan (Figure 4.7). The pH affected the consumption of H_2O_2 by catalase. The highest residual H_2O_2 concentration after 5 and 30 minutes was measured at pH 5.5 in the presence of 10 mg/L Bacsan-Cu (Figure 4.7).

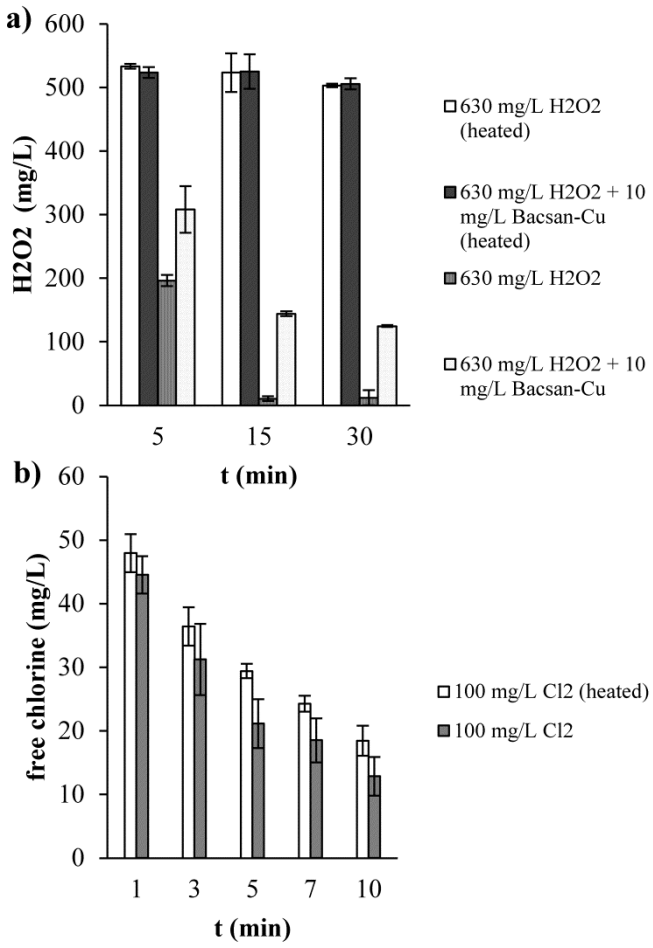

Figure 4.6. a) Residual H_2O_2 concentration of initially added 630 mg/L H_2O_2 in SPW with COD 819 ± 10 mg O_2/L, with or without heating at 80 °C for 10 min, and b) residual free chlorine concentration of initially added 100 mg/L free chlorine in SPW with COD 634 ± 2 mg O_2/L, with or without heating at 80 °C for 10 min (n = 3)

4.4.3. Water disinfection in SPW

At the start of the experiments in SPW, the APC was 6.0 ± 0.2 log CFU/ mL averaged among all experiments, and the inoculated *E. coli* contamination was 5.4 ± 0.4 log CFU/ mL. *E. coli* was more susceptible than APC to H_2O_2 combined with 2 mg/L or 10 mg/L Bacsan-Cu (Table 4.1). The inactivation of APC was significantly higher with H_2O_2 + 10 mg/L Bacsan-Cu compared to the other treatments, whereas for *E. coli*, the reduction was higher with H_2O_2 combined with 2 or 10 mg/L Bacsan-Cu compared to the other treatments. Exposure to increasing concentrations of COD had a detrimental influence on the inactivation of *E. coli* and APC. A significantly higher inactivation of APC and *E. coli* after 120 min compared to 30 min contact time was only observed at COD 1830 mg O_2/L with H_2O_2 + 10 mg/L Bacsan-

Cu and at COD 497 mg O_2/L with H_2O_2, and of *E. coli* at COD 848 mg O_2/L with 10 mg/L Bacsan-Cu. The low improvement on disinfection efficiency in the interval 30 to 120 min contact time is attributed to the low remaining H_2O_2 residual (Table 4.1).

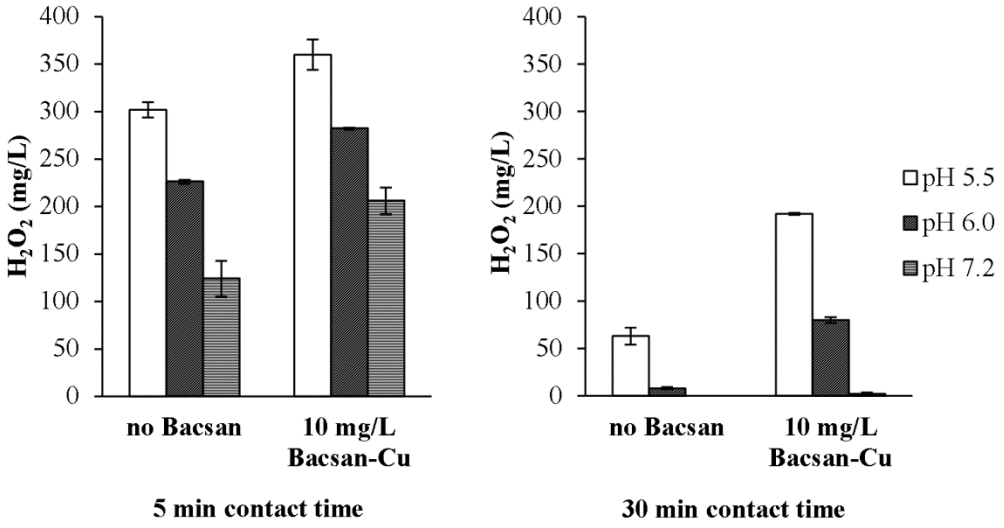

Figure 4.7. Residual H_2O_2 concentration of initially added 590 mg/L H_2O_2, measured after addition of 1.5 mg/L catalase to phosphate-buffered solutions at different pH and in presence and absence of 10 mg/L Bacsan-Cu (n = 3)

Synergy of H_2O_2 + 10 mg/L Bacsan-Cu was observed in SPW of COD 848 and 1830 mg O_2/L (except for APC after 30 min in COD 1830 mg O_2/L) (Table 4.1). In SPW of COD 497 mg O_2/L, observation of possible synergy was hindered by detection limit issues combined with overall higher inactivation due to the lower physicochemical load of the SPW. Only the presence of synergy in the case of APC after 30 min could easily be observed. The reduction of APC in SPW of COD 789 mg O_2/L was higher with H_2O_2 + 10 mg/L Bacsan-Cu compared to H_2O_2 + 10 mg/L Cu^{2+} (as $CuSO_4$) (Figure 4.4). Despite the H_2O_2 concentration being significantly higher after 30 min when adding Cu^{2+} than in the absence of Cu^{2+} (as $CuSO_4$), the APC inactivation with H_2O_2 + 10 mg/L Cu^{2+} was not significantly different from that obtained with only H_2O_2 (Figure 4.4).

4.4.4. H_2O_2 consumption and water disinfection in industrial process water from a processing company

3 CFU/ 100 mL *E. coli* were found in the IPW and none were detected after the disinfection treatments. The inactivation of APC was lower in IPW of COD 509 mg O_2/L compared to in SPW of COD 497 mg O_2/L (Table 4.1) . Nonetheless, the H_2O_2 residual was significantly higher in the IPW than in SPW after 30 and 120 min contact time with H_2O_2 + 2 and 10 mg/L Bacsan-Cu, but not with solely H_2O_2 compared to SPW. As in SPW, both the highest H_2O_2 stability and a synergistic APC inactivation were observed with 500 mg/L H_2O_2 + 10 mg/L Bacsan-Cu.

4.4.5. Impact of washing treatments on browning of stored fresh-cut iceberg lettuce

After washing the lettuce in the water disinfection solutions, some browning appeared after 3 days of storage in the fresh-cut lettuce, and considerable more browning was observed when washing in 10 mg/L Bacsan-Cu and H_2O_2 + 10 mg/L Bacsan-Cu compared with washing in water, 2 mg/L Bacsan-Cu, H_2O_2, and H_2O_2 + 2 mg/L Bacsan-Cu. When the lettuce was rinsed after treatment with 10 mg/L Bacsan-Cu (with or without H_2O_2), the amount of browning was similar to that of tap water treatment (and the other treatments) for the 5 days storage duration.

4.5. DISCUSSION

4.5.1. H_2O_2 as process wash water disinfectant in an industrial leafy vegetables washing process

The results in this chapter show that applying 1.8% H_2O_2 in the washing bath and dosing 300 L/h of 1.8% H_2O_2 in a 450 L washing bath is insufficient for maintaining the microbial wash water quality in the washing process when washing 333 ± 50 kg/h fresh-cut leafy vegetables. The microbial load in the wash water increased due to a rapid consumption of H_2O_2. The H_2O_2 was consumed due to oxidation of the organic matter that accumulated in the washing bath. The exothermic nature of these oxidation reactions (Klais, 1993) explains the higher temperatures in the H_2O_2 treated washing bath compared to the untreated one.

Industrial washing of the leafy vegetables in ≤ 1.8 % H_2O_2 for 1 min did not improve the decontamination efficiency compared to a water wash in this chapter. Other decontamination studies of fresh-cut leafy vegetables have been conducted with H_2O_2 in the range of 1 – 3 %,

with concentrations ≥ 2% showing improved decontamination efficiency compared to washing in water. However, these studies were performed at room or elevated temperature and with artificially inoculated microorganisms (Lin et al., 2002; Allwood et al., 2004; Huang & Cheng, 2011). Possible explanations for higher removals in those experiments than in the present chapter are longer contact time, higher temperature, the use of artificial inocula, the use of specific bacterial pathogens instead of general plate counts, and the full-scale experiments in the present chapter *versus* lab-scale in the other studies. On the other hand, Ramos et al. (2013) noted that at concentrations of 1-2%, H_2O_2 is not effective for produce decontamination. The issue of artificial inocula was illustrated by Hadjok et al. (2008), who used vacuum infiltration in order to achieve infiltration of inoculated *Salmonella* Montevideo in fresh-cut iceberg lettuce, and observed a much lower inactivation with H_2O_2/UV of internalized *Salmonella* than those bound to the surface. Improved decontamination efficiency of fresh-cut leafy vegetables with H_2O_2 in current industrial processes seems unrealistic, due to the requirement of long contact times, relatively high temperatures, high H_2O_2 wash water residual and the high reactivity of H_2O_2 with wash water organics. The observed increase in APC contamination on food contact surfaces illustrates that besides cross-contamination via the wash water, cross-contamination via food contact surfaces is an issue in fresh-cut leafy vegetables processing operations.

The executed case-study with UV/H_2O_2 in the fresh-cut leafy vegetables company confirms the inadequacy of using process water recycling to attempt to control the microbial contamination in a process with rapid and continuous influx of microbial contamination such as a fresh-cut leafy vegetables washing process..

4.5.2. H_2O_2 as process water recycling disinfectant in SPW

The experiments in SPW showed that the stability of H_2O_2 was improved by the addition of Bacsan and to lesser extent Cu^{2+}. Elimination of heat labile molecules (80 °C, 10 min) greatly increased the stability of H_2O_2 in SPW, which was much less the case for free chlorine. The most obvious heat labile compound with a high and selective impact on H_2O_2 stability is catalase, originating from the lettuce tissue and present in the SPW. This chapter showed the inhibiting influence of Bacsan and to lesser extent Cu^{2+} on bovine liver catalase activity. The decrease in catalase activity in the presence of certain metals may be related to direct binding of metal ions (including Cu^{2+}, Zn^{2+}, Ag^{+}) to –SH groups of the catalase enzyme, as such inhibiting the enzyme (Atli et al., 2006; Atli & Canli, 2007; Lemire et al., 2013). Furthermore,

peroxidase (from vegetables) activity is reduced after heating (80°C, 10 min) (Morales-Blancas et al., 2002), and inhibition of peroxidases by metal ions (Zn^{2+}, Ag^{+}) has been reported (Splittgerber & Tappel, 1979). As such it could be that peroxidases were inhibited by Bacsan. The less rapid H_2O_2 consumption by catalase at lower pH is attributed to catalase having to operate at sub-optimal pH. Bovine liver catalase has optimal activity at about pH 7 and isoelectric point (pI) of pH 5.4 (Samejima et al., 1962; Shi et al., 2008), while lettuce catalase has optimal activity in the pH range 7-8, and consists of two izoenzymes (pI 5.8 and pI 6.2) (Bestwick et al., 2001). Also, the H_2O_2 consumption rate is reduced at lower pH, especially below pH 3 (Watts et al., 1999; Ortiz et al., 2000) and the pH drops caused by the addition of Bacsan, and amplified when combined with H_2O_2, potentially slightly contributed to the inhibition of lettuce catalase in SPW.

Cu^{2+} in the presence of H_2O_2 generally does not induce free radical formation (through Fenton-like reactions) in systems that contain biomolecules because of the tendency of Cu^{2+} to tightly bind amino group containing compounds (Gutteridge & Wilkins, 1983; Pham et al., 2013). This is an additional explanation why Cu^{2+} did not accelerate the H_2O_2 consumption. Therefore, the inactivation effect of H_2O_2/Cu^{2+} against bacteria is most likely due to the combined attack of the two disinfectants, rather than the production of radical formation (Macomber et al., 2007; Orta De Velasquez et al., 2008).

In accordance with the increased stability of H_2O_2 when combined with Bacsan, synergistic effects were observed when these disinfectants were combined, both in SPW and in IPW. The lower inactivation of the APC in the IPW, despite the higher H_2O_2 exposure, can be attributed to an overall more resistant microbiota than in the SPW, which is plausible, as APC is a non-discriminative enumeration method. The lower H_2O_2 consumption might be due to the fact that COD is a general parameter that measures the amount of oxygen that is necessary to oxidize the substances in the sample, and is used as an indicator for the organic load of the water. As such, it does not directly inform about the reaction rate of specific molecular species with H_2O_2 (as was observed to be significant in the heated SPW), nor the levels of iron or phosphate, which can also influence the H_2O_2 consumption (Watts et al., 1999). On the contrary, in chapter 3, the COD was found to be a universal parameter that effectively predicted the disinfection efficiency of free chlorine to inactivate inoculated *E. coli* O157 in both SPW and IPW. The chlorination trials in heated SPW in this chapter show the relative independence of chlorine consumption on specific heat labile compounds in the SPW.

The results in SPW confirm that H_2O_2 is a slow acting water disinfectant that quickly decomposes in the presence of high COD of fresh-cut lettuce origin. The inactivation of *E. coli* in oxidant demand free conditions requires a much higher exposure (concentration x contact time) to H_2O_2 than to free chlorine and O_3 (section 1.5.1.1). Bacsan itself also showed antimicrobial activity when dosed at 10 mg/L (as Cu). Ionic Cu and Ag have antimicrobial properties based on interaction with cell structures and components and interfering with cell respiration (section 1.8.1). Cu^+, Ag^+, and Zn^{2+} ions inactivate Fe-S cluster enzymes in *E. coli* and depletion of antioxidant reserves, particularly glutathione, can occur due to metal ions including Ag^+ and Zn^{2+} and exposure of *E. coli* to toxic doses of these metal species can lead to depletion of total cellular thiols (Macomber & Imlay, 2009; Lemire et al., 2013). Also, combining sub-inhibitory concentrations of Ag^+ with certain metal ions, including Cu^{2+} and Zn^{2+}, increased the toxicity of those metal ions on *E. coli* by a factor of 10 (Pedahzur et al., 1997). As such, the combination of measured ions in Bacsan exhibit antimicrobial action.

The synergistic microbial inactivation due to H_2O_2/Bacsan cannot be attributed to the presence of Cu^{2+} alone, but is dependent upon the combination of metal ions added to the SPW, in the presence of H_2O_2. Contrary to this chapter, synergy of H_2O_2 (50 to 250 mg/L) with 1 mg/L Cu^{2+} was observed for inactivation of vegetative bacteria in primary wastewater treatment effluent (Orta De Velasquez et al., 2008). Ag^+/H_2O_2 was more effective than H_2O_2 alone for inactivation of bacteria and fungi (Tote et al., 2009) and synergistically against *E. coli* (Pedahzur et al., 1997; 2000, Batterman et al., 2000). Research has shown that H_2O_2 is less effective against microorganisms with high catalase activity level (Lambert et al., 1999; Armon et al., 2000; Watts et al., 2003; Sacchetti et al., 2009). Monofunctional catalases are not only produced by plants and animals, but also widespread among bacteria (a. o. *E. coli*) and fungi, next to the catalase-peroxidases which are only distributed among bacteria and fungi, and the non-heme catalases that have only been found in certain bacterial species (Nadler et al., 1986; Loewen et al., 2000). The synergy of H_2O_2 and Bacsan can be explained by i) the higher stability of H_2O_2 in SPW in the presence of Bacsan, allowing for higher exposure of the microorganisms to H_2O_2 and combined with the stress from Bacsan itself, ii) the multiple damage mechanism, meaning both disinfectants attack different targets in the microorganism, as such creating different stresses that could make it harder for the microorganism to remain viable compared to separate addition of the damage from both disinfectants (Koivunen & Heinonen-Tanski, 2005a), and iii) the metal ions in Bacsan might inhibit the functioning of microbial catalases, as such rendering them more susceptible to

H_2O_2 attack. Research on inhibition of microbial catalases of target microorganisms to improve the disinfection efficiency, as well as further research on inhibition of water matrix catalase might be interesting towards enhancing the use of H_2O_2 as a disinfectant.

4.5.3. Influence of H_2O_2/Bacsan on fresh-cut lettuce browning

It could be that in the case of washing in 10 mg/L Bacsan-Cu, Cu^{2+} was transferred to the lettuce in amounts that induced the activity of polyphenoloxidase, whereas a subsequent rinsing step leached the Cu^{2+} back from the lettuce and as such avoided additional browning (Vandekinderen, 2009). 500 mg/L of H_2O_2 does not influence the rate of fresh-cut lettuce browning and is much lower than the concentrations normally applied to decontaminate fruits and vegetables (mostly 1 to 5 %), and the concentrations that potentially cause sensorial quality issues towards the lettuce (McWatters et al., 2002a, 2002b; Ukuku et al., 2012; Lopez-Galvez et al., 2013). In case H_2O_2/Bacsan would be implemented as process water recycling technique in fresh-cut leafy vegetables washing processes, most of the Cu^{2+} (from the Bacsan) would remain in the SPW after process water recycling, and could come into contact with the fresh-cut leafy vegetables when the water is reused. This is advantageous from a cost perspective, because it would enable the reuse of the metal ions for water disinfection. Nonetheless, implementation of a rinsing step with tap water would be required to avoid presence of Cu^{2+} on the packaged lettuce. Furthermore, regulations (for Cu) or guidelines (for Ag and Cu) exist to govern their presence in drinking water (Council Directive 98/83/EC; WHO, 2003, 2004; USEPA, 2009, 2013) to avoid ingestion of aesthetically altering (discoloration of the skin and white part of eye in the case of Ag intake) or toxic dosages of these metals. From the perspective of the fresh-cut produce processor and the consumer safety, the primary concern is the possible transfer of these metals to the fresh-cut leafy vegetables and the influence of a rinsing step on the removal of these metals from the fresh-cut leafy vegetables; more so than directly assessing the quality of the wash water itself as this is not consumed.

4.6. CONCLUDING REMARKS

- Dynamic wash water disinfection with H_2O_2 was executed in a full-scale fresh-cut leafy vegetables washing process

- A rapid build-up of COD and rapid decrease of H_2O_2 residual occurred, the latter despite addition of 300 L/h of 1.8 % H_2O_2 to a 450 L washing bath (333 ± 50 kg/h fresh-cut produce introduction speed)
- The applied disinfection setup was unable to avoid the build-up of APC and enterococci
- Bacsan (containing a. o. Cu^{2+}, Zn^{2+}, Ag^{+}) improved the stability of H_2O_2 in SPW and fresh-cut leafy vegetables wash water from a processing company and synergistically increased the disinfection efficiency of APC and E. coli compared to H_2O_2 or Bacsan
- Bacsan decreased the activity of catalase

CHAPTER 5

COAGULATION OF TURBIDITY AND ORGANIC MATTER FROM LEAFY VEGETABLES WASH WATER USING GALLOTANNINS AND CHITOSAN TO IMPROVE WATER DISINFECTANT STABILITY

Redrafted from

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5. COAGULATION OF TURBIDITY AND ORGANIC MATTER FROM LEAFY VEGETABLES WASH WATER USING GALLOTANNINS AND CHITOSAN TO IMPROVE WATER DISINFECTANT STABILITY

5.1. ABSTRACT

The use of gallotannins and chitosan for coagulation of artificial fresh-cut lettuce wash water, made from butterhead lettuce and tap water, was assessed for reducing particulate and dissolved matter in order to improve the physicochemical quality, as such lowering the disinfectant demand and allowing longer reuse of postharvest wash water. Chitosan was better at removing turbidity and organic matter than gallotannins. Chitosan was used to coagulate industrial leafy vegetables wash water from a processing company. Lowering the pH improved the reduction efficiency. Although the turbidity reduction was very high (> 90%), virtually no dissolved organic matter was reduced. Coagulation lowered the disinfectant demand (of free chlorine and PAA) to some degree but had no positive impact on the THMs formation. As such, coagulation does not seem to be a viable option to lower the DBPs, the disinfectant dose or the water use.

5.2. INTRODUCTION

During the industrial washing of fresh produce, (in)organic particulate and dissolved matter is transferred to the wash water. In the case of fresh-cut produce, exudates, leaking from cut surfaces, increase the organic matter content of the wash water. As the water is recirculated during these processes in order to conserve water, a build-up of (in)organic matter in the water occurs (Selma et al., 2008a,2008b; chapters 3 & 4). As such, a higher consumption of water disinfectant, as well as (if relevant) a higher production of DBPs take place (chapters 1 & 3).

Besides the use of disinfectants that do not produce harmful DBPs, strategies to lower the organic matter content could be applied to allow a longer reuse of water in this industry, as well as to lower the disinfectant consumption. Coagulation-flocculation is widely applied in water and wastewater treatment to destabilize colloids and particles, resulting in particle aggregation, allowing removal by gravity (sedimentation, flotation) and/or filtration. The most frequently used coagulants are mineral additives such as alum and polyaluminum chloride, and synthetic polymers based on polyacrylamide. However, aluminum coagulants are often overdosed to ensure coagulation efficiency, which results in water or sludge with high

aluminum content that contributes to the accumulation of aluminum in the environment. Ingestion of elevated doses of aluminum may contribute to some neurological diseases, i.e. Alzheimer's disease and premature senility. Also, the spread of the neurotoxic and probable human carcinogen acrylamide mono- and oligomers might be hazardous (Ahmad et al., 2006; Renault et al., 2009; Chen & Chung, 2011; Vinci et al., 2012). There is increased interest in biopolymers with coagulating properties because they are biodegradable, usually nontoxic, and eliminate the problems related to dealing with potentially toxic sludge, even allowing recycling of sludge from food processing waste water (Mishra & Bajpai, 2005; Chi & Cheng, 2006; Bina et al., 2009). In this chapter, two biocoagulants were tested: chitosan and gallotannins. Tannins have been applied for dewatering sludge, reducing turbidity from wastewater, and clarification of beer. Chitosan has been applied for coagulation-flocculation treatment of various effluents, including wastewaters from several food processing operations (Shahidi et al., 1999; Renault et al., 2009). One of the main uses in food processing operations is based on the ability of chitosan to recuperate lipids and proteins from food wastewaters by flocculation (Fernandez & Fox, 1997; Ahmad et al., 2006; Chi & Cheng, 2006; Boeris et al., 2011).

Implementation of coagulation-flocculation in produce washing processes could be a method to prolong the water use during operation. The efficiency of the method depends on the capability of the coagulation-flocculation process to reduce sufficient amounts of organic matter from the wash water, translating into a lower disinfectant demand of, and lower DBPs production in the wash water, which were assessed in this chapter.

5.3. MATERIALS & METHODS

5.3.1. Experimental setup

Experiments were performed in both SPW and IPW from a leafy vegetables processing plant (Figure 5.1). Coagulation-flocculation-sedimentation experiments were performed with a jar test apparatus. Efficiency was assessed based on turbidity and COD reduction. The reduction of APC, total coliforms, and inoculated *E. coli* O157 strains after coagulation-flocculation-sedimentation-rapid sand filtration was assessed in SPW.

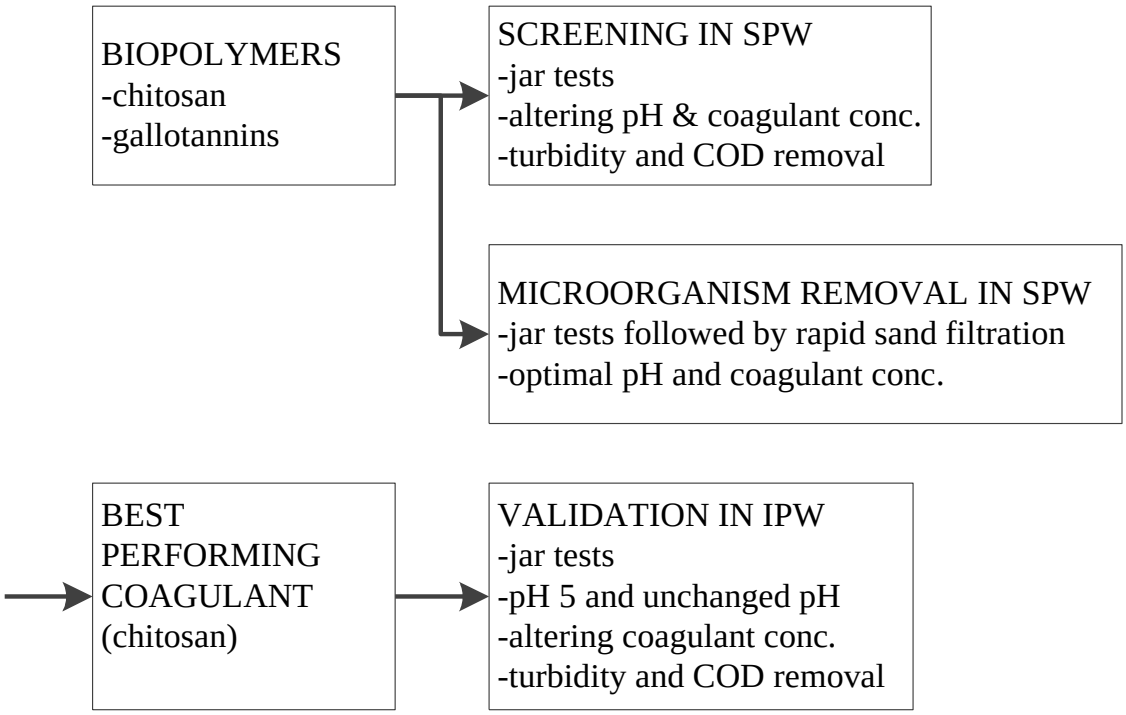


Figure 5.1. Experimental setup

5.3.2. Standardized process water

Butterhead lettuce (*Lactuca sativa)* was purchased from a local market in Belgium and transported at < 4°C to the lab for further handling. After discarding the outer leaves, 67 g of lettuce was put in a stomacher bag (0.5 mm pore size), 200 mL of tap water was added, and the mixture was homogenized for 2 min. The COD of this suspension was determined, and subsequently the suspension was diluted with tap water to obtain SPW containing about 800 mg O_2/L COD.

5.3.3. Wash water from a fresh-cut leafy vegetables processing company

IPW was collected during the leafy vegetables washing operation. Water was collected in sterile recipients and transported to the lab < 4°C, where it was stored at 4°C till execution of the experiments. In the company, ground water was used as water source during washing of butterhead lettuce, iceberg lettuce, endive and radicchio (ca. 250 kg leafy vegetables/h; 450 L washing bath volume). Water from the same processing line, processing the same leafy vegetables, was sampled on two different days and process duration: after 1 h of vegetable processing (IPW Low), after 4 h of vegetable processing (IPW High).

5.3.4. Coagulants

Chitosan of lower molecular weight (LMW; ~200 cps), and higher molecular weight (HMW; ~ 800 cps) were purchased from Sigma-Aldrich. Chitosan solutions were made by dissolving 0.5 g in 100 mL 0.1 M hydrochloric acid. Gallotannins HMW, extracted from Chinese gallnuts were obtained from Ajinomoto Omnichem. This extract consisted of gallotannins in the range penta- to dodecagalloylglucose (information provided by the supplier). Also, gallotannins LMW, extracted from Alepo nuts were obtained from Ajinomoto Omnichem and consisted of gallotannins in the range tetra- to octagalloylglucose (information provided by the supplier). Solutions were made by dissolving 1 g of gallotannins in 100 mL distilled water.

5.3.5. Determination of degree of deacetylation and molecular weight of chitosan

The degree of deacetylation (DD) was determined with the two abrupt change potential titration method as described by Wang et al. (2006b). The apparent pKa values were estimated from the titration curves by locating the half-equivalence point. The molecular weight was determined with an Ubbelohde viscosimeter (PM Tamson Instruments, Germany) as described by Wang et al. (2006b).

5.3.6. Coagulation-flocculation-sedimentation experiments

Different concentrations of gallotannins HMW and chitosan were added to the SPW at pH 5 and unchanged pH (approximately 7.5). To change the pH of the water, hydrochloric acid (1 M) was added. Chitosan was also tested at pH 9, to assess the reduction efficiency in the absence of any protonated amine-groups in the chitosan molecule. As such, in the case of chitosan, the pH was controlled at pH 9 by adding NaOH (1 M) to compensate for the pH drop caused by the addition of acidified chitosan. In the case of pH 5, the pH drop was insignificant (up till 35 mg/L chitosan) and in the case of unaltered pH (7.5) the alkalinity of the tap water functioned as a buffer which resulted in non-significant pH drops (up till 50 mg/L).

Jars were filled with 1 L SPW and the water temperature controlled at 7-8°C to simulate the water temperature at the processing plant. Coagulants were added during stirring at 200 rpm with a conventional jar test apparatus (RER, IKA, Germany). The SPW was stirred for 2 min at 200 rpm and subsequently for 28 min at 30 rpm, after which the formed flocs were allowed

to settle for 30 min. The upper 400 mL were withdrawn from the jar using a pipette and analyzed for turbidity and/or COD. The same set of experiments that were executed in SPW, were repeated in IPW with the coagulant that showed the best reduction efficiency, i.e. chitosan.

The optimal dose of each coagulant, i.e. the lowest dose that provides the greatest turbidity reduction after sedimentation and obtained in the screening process, was used to repeat the experiment in order to assess the microbial reduction but this time with subsequent filtration over 70 cm of filter sand (effective size = 0.52 mm; uniformity coefficient = 1.5) in a borosilicate glass column (2.5 cm internal diameter) and at a filtration speed of 10 m/h, using a peristaltic pump (Sci-Q 323, Watson-Marlow, Belgium). For this purpose, phosphate buffer (containing *E. coli* O157 cells) was added to the SPW before execution of the jar test experiment under mixing of the SPW to obtain ca. 6 log CFU/mL. Experiments on *E. coli* O157 were executed separately from those on the naturally present microorganisms (total coliforms and APC), as to make sure *E. coli* O157 did not contribute to the APC counts. *E. coli* O157, total coliforms and APC in the SPW were enumerated at the start, after sedimentation and after filtration.

5.3.7. Assessing the impact of coagulation on disinfectant decay and DBPs production

IPW High (both coagulated and untreated) was exposed to 100 mg/L free chlorine (28.4 g/L NaOCl; La Croix) or 5 mg/L PAA (Chriox 5; Christeyns Chemicals) at 4°C and, while continuously being mixed, periodically sampled to measure the disinfectant concentration. IPW (both coagulated and untreated) was exposed to a dose of 500 mg/L free chlorine and stored, while continuously being mixed, at 4°C. This high free chlorine dose was added to simulate the ultimately high dose that results from continuous addition of free chlorine to maintain an opted chlorine residual. After 7 hours, samples for TTHMs (i.e. chloroform, bromoform, dichlorobromomethane, and dibromochloromethane) quantification were taken. The TTHMs were analyzed as described previously by Lopez-Galvez et al., 2010b.

5.3.8. Physicochemical measurements

Turbidity was determined with a turbidimeter (HI98703; HANNA Instruments; Belgium). COD was measured according to the small-scale sealed-tube method (LCI 400; Hach Lange; Belgium). Soluble COD (CODs) is the COD measured after filtration through a 0.45 µm polytetrafluoroethylene filter (Macherey-Nagel, Belgium). The particulate COD (CODp) was

calculated by subtracting the CODs from the COD. UV at 254 nm (UV_{254}) was measured using a UV-VIS spectrophotometer (UV1601; Shimadzu; Belgium) and quartz cuvets of 1 cm pathlength (Hellma, Belgium). Free chlorine was measured as described in section 2.3.4. PAA was measured as described in section 3.3.3.1.

5.3.9. Microbial inoculation and enumeration

Two attenuated nalidixic acid-resistant *E. coli* O157 strains (Laboratory of Food Microbiology and Food Preservation (Ghent University) strains 662 and 679) were used. The strains were grown at 37°C for 24 h in Brain Heart Infusion broth (Oxoid, United Kingdom) containing 50 µg/ml nalidixic acid (Sigma-Aldrich, Belgium). The cocktail of strains was centrifuged (4°C at 1800 g for 10 min). The pellets were washed twice in phosphate buffer (pH 7, 0.01 M), with intermittent centrifugation, and subsequently resuspended in phosphate buffer. *E. coli* O157 was enumerated by the pour-plating method on Chromocult Coliform-agar (Merck, Belgium) containing 50 µg/ml nalidixic acid (incubation at 37°C, 24 h). Total coliforms were enumerated on RAPID'*E. coli* 2 chromogenic medium (Biorad, France) (incubation at 37 °C, 24 h), and APC on Plate Count Agar (Oxoid, Belgium) (incubation at 22 °C, 3 days) using the pour-plating method.

5.3.10. Statistical analysis

When relevant, the student t-test (independent two-sample t-test) was used because the Mann-Whitney U test cannot be used to compare two groups of three values (repeats), and the fact that there is no principal objection to using a student t-test on low sample sizes if caution is taken towards the interpretation and the value of the results (de Winter, 2013).

Disinfectant decay rate orders were assessed through linear regression of disinfectant conc. (0 order), ln(conc.) (1^{th} order), and $(conc.)^{-1}$ (2^{nd} order) in function of time and the prediction quality of this decay was quantified by the r^2 and the ratio of prediction to deviation (RPD) (section 3.3.4.1). The effect of the coagulation treatment on the disinfectant decay was assessed with Analysis of Covariance (ANCOVA) in SPSS statistics 22 (IBM). Presence of a significant interaction term (disinfectant concentration x contact time) indicates a difference in disinfectant decay rate (i.e. difference in slope). A significance of $p < 0.05$ was applied for all analyses. Noted deviations on measurements represent standard deviations.

5.4. RESULTS

5.4.1. Sedimentation of SPW and IPW without addition of coagulant

The physicochemical parameter values of the SPW and IPW are shown in Table 5.1. At alkaline pH, the colloids and particles in SPW remained stable during 4 h of settling without addition of coagulant. In the range of pH 3 to 5 destabilization, floc formation and subsequent sedimentation of the SPW occurred (Figure 5.2). In the IPW, the particle sedimentation was independent of pH after 4 h of settling (turbidity reduction of 28.3 ± 3.6 %).

Table 5.1. Physicochemical characteristics of the used process waters (n=3)

	SPW	**IPW Low**	**IPW High**
pH	7.5±0.1	7.7±0.1	7.8±0.09
Turbidity (NTU)	116.7±18.4	59.1±8.0	88.6±2.1
Alkalinity(mmole/L)	5.52±0.02	6.43±0.09	ND[a]
UV_{254}	2.43±0.12	1.42±0.11	2.23±0.06
UV_{254} (F)	1.39±0.14	0.90±0.02	1.58±0.04
COD (mg O_2/L)	778±26	486±17	936±13
COD_s	505±12	371±13	ND
COD_p	273±4	115±10	ND

[a] not determined

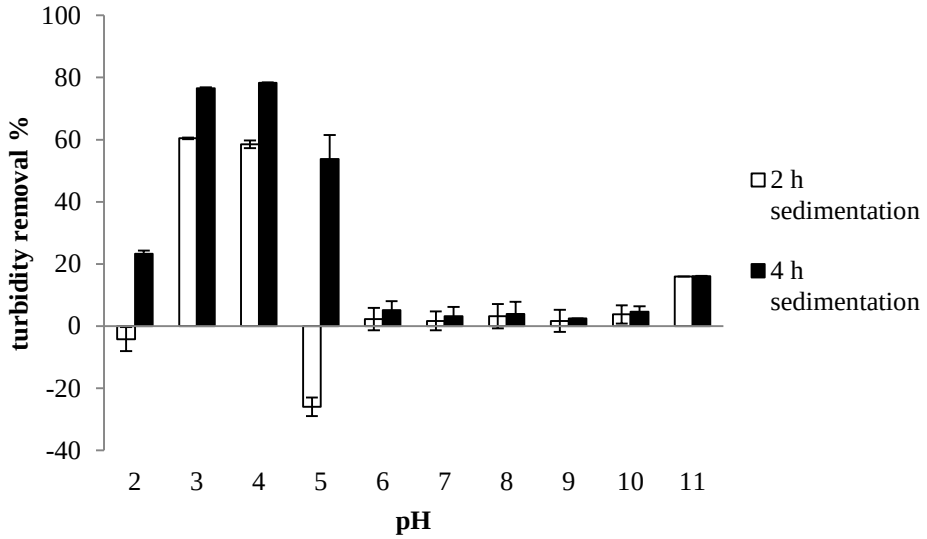


Figure 5.2. Turbidity reduction in SPW (turbidity 141 ± 2.0 NTU; COD 921 ± 13 mg O_2/L) without addition of coagulant (n=3)

5.4.2. Screening of coagulant efficiency in SPW

5.4.2.1. Influence of pH and coagulant concentration on coagulant efficiency

In none of the experiments, be it for reduction of turbidity, COD, or microorganisms, a significant difference was observed between reduction efficiency with chitosan HMW or chitosan LMW. In addition, the DD of chitosan HMW and LMW were not significantly different (Table 5.2). As such, the results from the experiments were pooled independent of chitosan molecular weight. When comparing the turbidity reduction efficiency of gallotannins HMW with LMW, the gallotannins HMW, i.e. with a higher amount of galloyl-groups, were better at reducing particulate matter (turbidity reduction of 88.5 ± 3.9 % *versus* 75.8 ± 1.4 % at pH 5 and 61.6 ± 2.1 % *versus* -73.4 ± 4.0 % at pH 7.5 for HMW and LMW respectively). Therefore, gallotannins HMW were used in further experiments.

Table 5.2. Characteristics of the used chitosan species (n=3)

	intrinsic viscosity (η)	**molecular weight (kDa)**	**DD(%)**	**pKa**
HMW	675 ± 108	(1.51 ± 0.22) x 10^3	68.4 ± 2.4	6.53 ± 0.11
LMW	229 ± 10	579 ± 22	67.9 ± 2.4	6.39 ± 0.12

Assessment of the influence of pH on gallotannins HMW and chitosan performance was executed by adding 25 mg/L gallotannins HMW or 5 mg/L chitosan to SPW with varying pH (Figure 5.3). The reduction of turbidity and COD was optimal in the pH range 2 to 6 for gallotannins HMW, and in the pH range 3 to 5 for chitosan. At pH 5, lower coagulant concentrations were necessary to reach maximum turbidity reduction than at pH 7.5, and less chitosan was required than gallotannins HMW to reach the maximum reduction (Figure 5.4). Whereas suspension restabilization occurred at pH 5 and 7.5 when dosing chitosan beyond the optimal coagulant dose, this was not the case for the gallotannins HMW. When dosing chitosan at pH 9, a lower maximum turbidity reduction was achieved than at the lower pH values, and considerably higher dosage was needed for turbidity reduction.

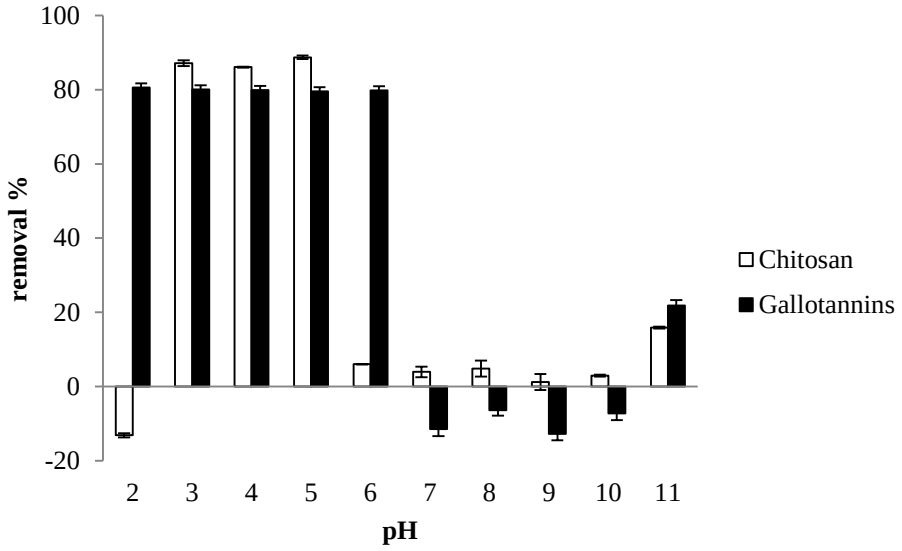


Figure 5.3. Reduction of turbidity from SPW (turbidity 141 ± 2.0 NTU; COD 921 ± 13 mg O_2/L) treated with 25 mg/L gallotannins or 5 mg/L of chitosan at different pH values (n=3)

At the optimal dosage, COD reductions at pH 5 (15 mg/L chitosan) and pH 7.5 (35 mg/L) were 49.2 ± 0.9 % and 50.6 ± 0.9 % respectively. For gallotannins HMW, the COD reductions were considerably lower, at pH 5 (50 mg/L) and pH 7.5 (150 mg/L), the COD reductions were 39.9 ± 0.7 % and 29.9 ± 0.9 % respectively.

5.4.2.2. Influence of turbidity variation on coagulant efficiency

In SPW, at pH 5, with addition of 15 mg/L chitosan LMW in the initial turbidity range 58 – 154 NTU, and at pH 7.5, with addition of 35 mg/L chitosan LMW, in the range 75 – 130

NTU, the residual turbidity after coagulation and filtration through 10 cm filter sand was virtually independent of the initial turbidity (Figure 5.5).

At lower and higher initial turbidities, the coagulation process was sub-optimal. In those cases an additional filtration step had a greater added effect on turbidity reduction.

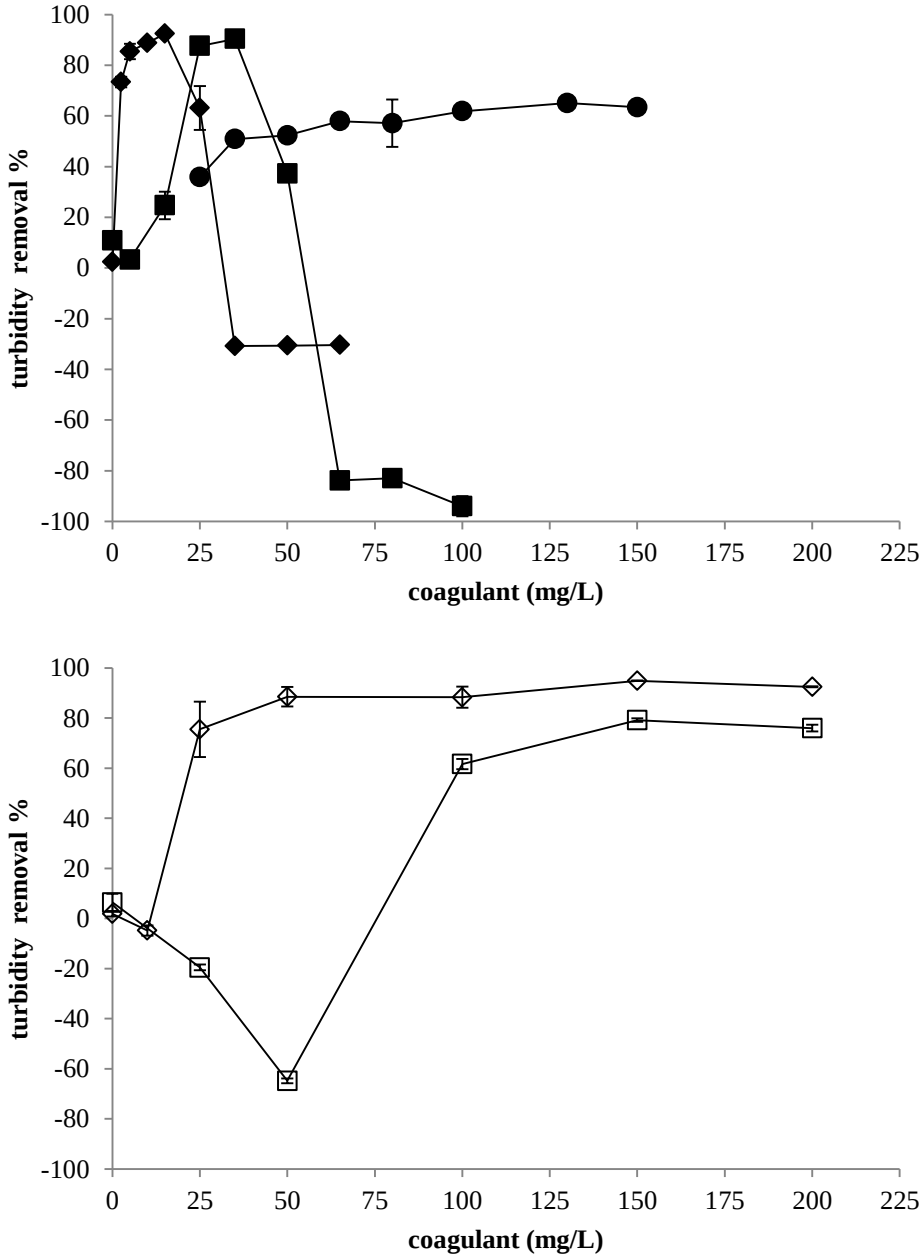
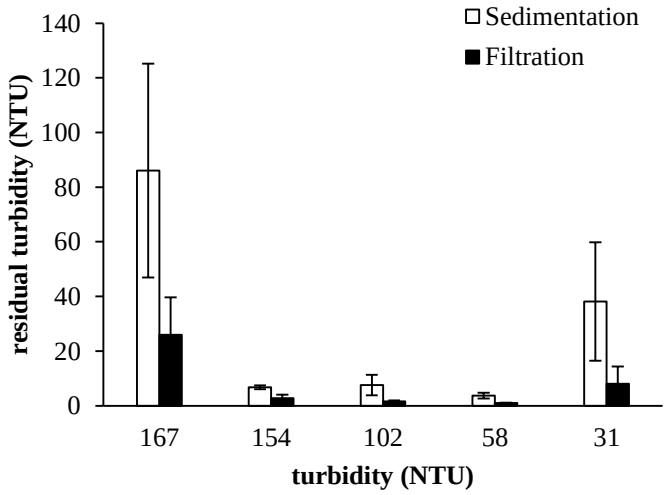
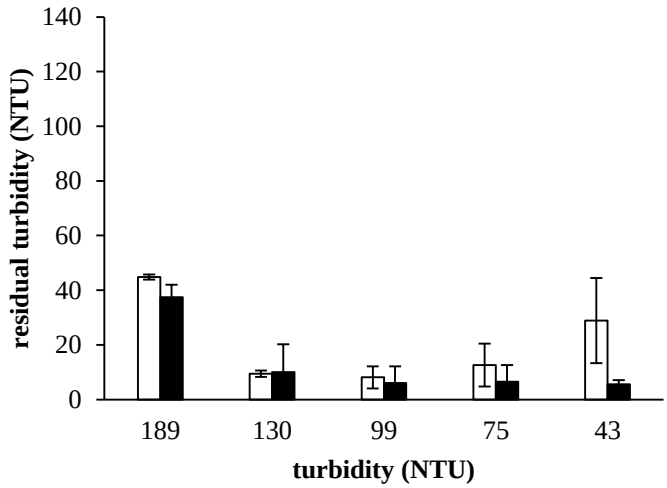


Figure 5.4. Reduction of turbidity in SPW (133 ± 1.4 NTU; COD 792 ± 8 mg O_2/L) treated with chitosan at (♦) pH 5, (■) pH 7.5, (●) pH 9, or treated with gallotannins HMW at (◊) pH 5 and (□) pH 7.5 (n=3)



a)



b)

Figure 5.5. Residual turbidity after treatment of SPW of variable initial turbidity with LMW chitosan at a) pH 5 (15 mg/L) and b) pH 7.5 (35 mg/L), both after sedimentation and filtration (n=3)

5.4.3. Microorganism reduction efficiency in SPW due to coagulation-flocculation-sedimentation-filtration treatment

The initial microbial contamination in the SPW was 5.3 ± 0.4 and 4.2 ± 0.4 log CFU/mL APC and total coliforms respectively. The initial inoculated *E. coli* O157 contamination was 5.9 ± 0.3 log CFU/mL. Chitosan treatment reduced total coliforms and APC to a higher extent than did gallotannins HMW (Table 5.3).

Table 5.3. Microbial reduction (log reduction) after treatment of SPW by coagulation-sedimentation-filtration (n=3)

	Total coliforms		**APC**		***E. coli* O157**	
	Sed.	Fil.	Sed.	Fil.	Sed.	Fil.
Blanc	0.1±0.3[a]	0.4±0.2[a]	0.0±0.1[a]	0.5±0.2[a]	0.1±0.2[a]	0.2±0.1[a]
Chitosan (LMW; pH 7.5; 35 mg/L)	1.0±0.1[b]	2.2±0.2[b]	1.9±0.1[c]	2.3±0.1[c]	0.5±0.2[a]	0.7±0.2[b]
Chitosan (LMW; pH 5; 15 mg/L)	1.2±0.2[b]	2.3±0.4[b]	1.9±0.3[c]	2.6±0.5[c]	0.5±0.2[a]	0.5±0.2[ab]
Gallotannins (HMW; pH 5, 50 mg/L)	0.4±0.2[a]	0.4±0.1[a]	1.3±0.2[b]	1.4±0.3[b]	0.2±0.2[a]	0.2±0.1[a]

[a,b,c] a different letter denotes a significant difference in microbial reduction from the other treatments (i.e. compared to other values in the same column)

Gallotannins HMW only reduced APC significantly compared to the blank treatment (sedimentation and filtration without coagulant). For chitosan treatments, there was no difference in microbial reduction with the optimal dose at pH 5 and pH 7.5. *E. coli* O157 was not effectively reduced by any of the treatments.

5.4.4. Coagulant efficiency of chitosan in IPW

The industrial wash water was treated at pH 5 and unchanged pH (7.7-7.8). As with the SPW, lower doses were necessary at pH 5 to achieve maximum turbidity reduction in IPW Low, i.e. 5 and 10 mg/L at pH 5 and 7.7 respectively (Figure 5.6); doses that were overall lower than necessary in the SPW. The reduction of COD in IPW Low (with optimal coagulant dose) was 28.0 % and 14.6 % at pH 5 and 7.7 respectively (Figure 5.7).

Reduction of organic matter was predominately the reduction of CODp, as observed from the turbidity reduction, and the reduction of CODs was negligible. Reduction of UV_{254} was 37.2 ± 3.5 % and 30.1 ± 0.3 % at pH 5 and 7.7 respectively.

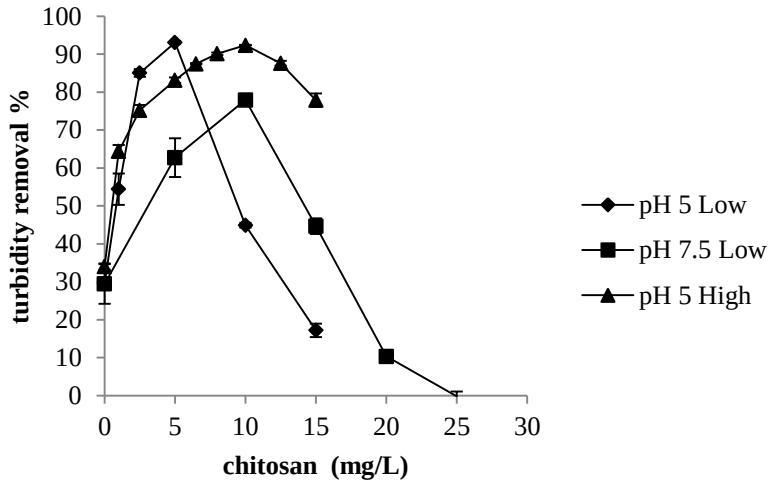


Figure 5.6. Reduction of turbidity from IPW chitosan (n=3)

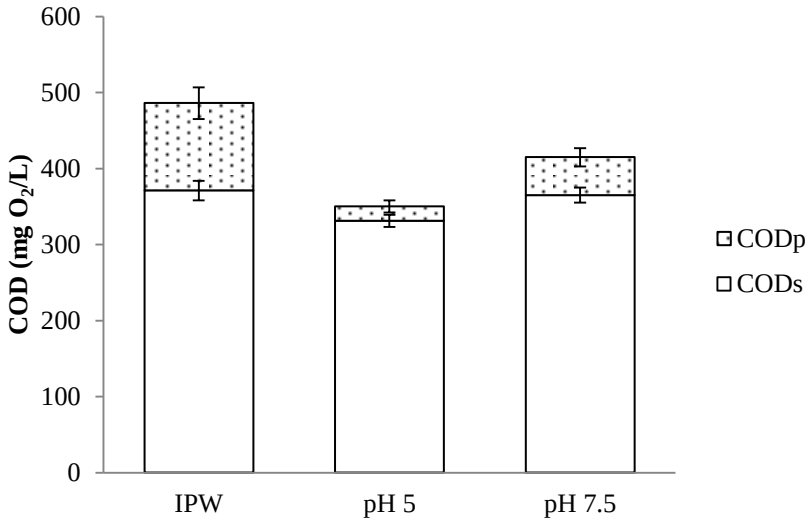


Figure 5.7. Reduction of COD from IPW under ideal coagulant conditions, i.e. using 5 mg/L chitosan at pH 5, or 10 mg/L chitosan at pH 7.5 (n=3)

5.4.5. The influence of the chitosan treatment on disinfectant stability and DBPs production in IPW

The influence of the chitosan treatment on disinfectant stability and DBPs production was assessed in IPW High. The optimal reduction (92.3±0.1 % turbidity, 15.5 ± 0.9 % COD, 29.1 ± 0.5 % UV_{254}) at pH 5 required twice the dose necessary in IPW Low (Figure 5.6). The disinfectant decay showed an initial rapid decay within the first 30 s of contact time. This initial decay was substantially lower in coagulated water for free chlorine (initial decay of

87.0 ± 0.2 *versus* 74.2 ± 1.8 mg/L in SPW and coagulated SPW respectively), but not for PAA (Figure 5.8).

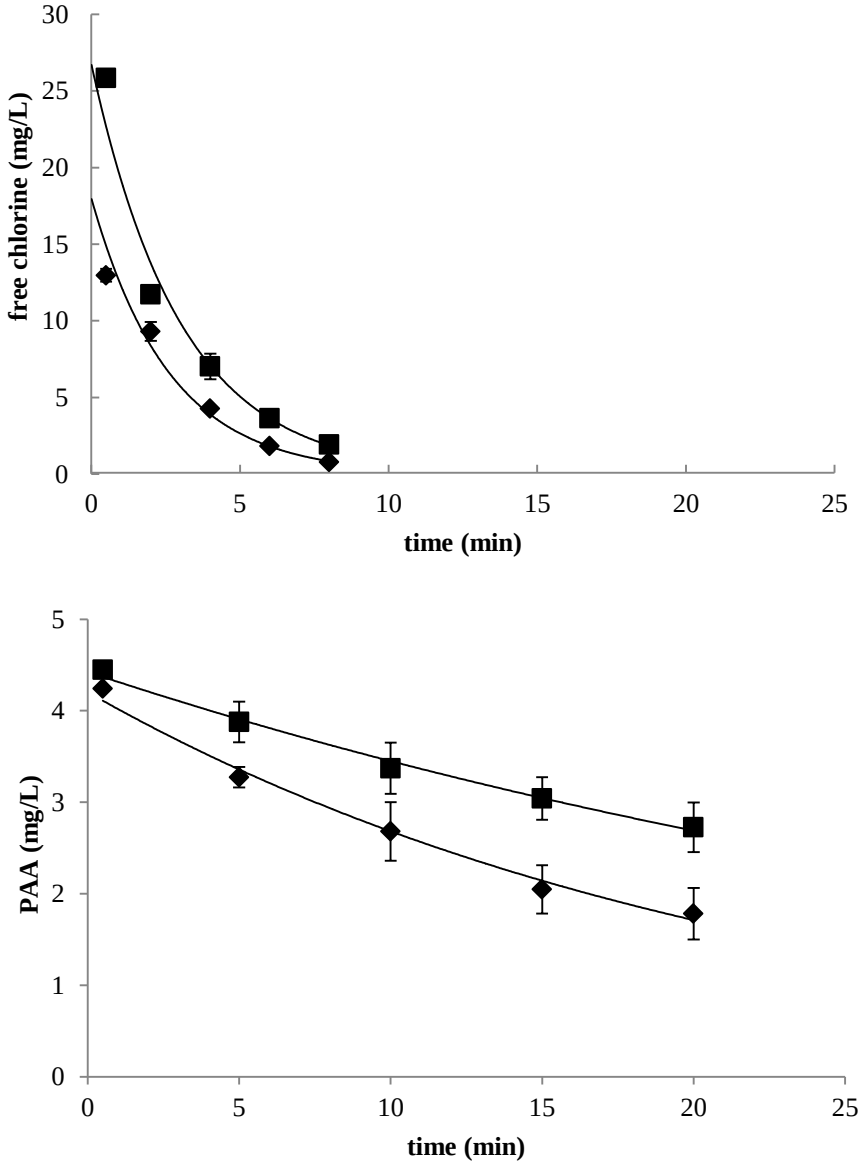


Figure 5.8. Comparison of disinfectant decay in untreated and chitosan treated IPW High; (♦) untreated IPW; chitosan treated IPW (■) with a)100 mg/L free chlorine and b) 5 mg/L PAA (fitted lines: 1st order decay) (n=3)

Further decay in function of time (and within the time period of the experiment) approximated first order for chlorine (R^2 = 0.98; RPD = 7.17) and about equal approximation of zero order (R^2 = 0.91; RPD = 3.39) and first order (R^2 = 0.91; RPD = 3.44) for PAA. The disinfectant decay rate was significantly lower in coagulated IPW for both chlorine and PAA (Figure 5.8), indicating the chitosan treatment lowered the disinfectant demand of the IPW to some extent.

To put it in terms of gains in disinfectant, after 0.5 min of contact time, 12.9 ± 1.8 % and 4.1± 4.4 % of the initially added free chlorine and PAA respectively were "saved" in the coagulated water compared to the untreated water, whereas at the end of the experimental run, for free chlorine, 1.2 ± 0.5 % (8 min) and for PAA, 18.9 ± 3.6 % (20 min) of the initially added disinfectants were less consumed in the coagulated water. An exposure of IPW to 500 mg/L free chlorine for 7 hours resulted in formation of chloroform that was not significantly different between coagulated (438.3 ± 8.5 µg/L) and untreated (371 ± 60.5 µg/L) (n=2) IPW High. Only traces (< 6.3 µg/L) were detected of the other THMs.

5.5. DISCUSSION

5.5.1. Coagulation efficiency in SPW

Chitosan was more dose-effective than gallotannins for COD reduction from SPW. Chitosan coagulation functions primarily through two mechanisms: charge neutralization and polymer bridging (Bolto & Gregory, 2007; Renault et al., 2009). In natural systems, the surface potential of particles is invariably negative, regardless the nature of the primary particles (Beckett & Le, 1990). Addition of positive charges to the water neutralizes the particle charge, and destabilizes the particle. Chitosan attains a high cationic charge at acidic pH due to protonation of its amino groups, and possesses a long linear polymeric structure (Renault et al., 2009). At pH 9, considering the estimated pKa values (Table 5.2), virtually no dissociation of the amine groups occurred. At pH 7.5, about 7 to 10 % of the amine-groups were protonated, while at pH 5, 96 to 97 % of the chitosan amine-groups were protonated. Due to charge neutralization, as the cationic character of chitosan increased with lower pH, the necessary dose for coagulation decreased with decreasing pH. At pH 9, the turbidity reduction was substantially lower than in the range pH 5 to 7.5. However, as turbidity reduction did occur up till 65%, polymer bridging, i.e. interlinkage of particles by polymers, was likely involved (Strand et al., 2003; Bolto & Gregory, 2007).

5.5.2. Reduction of microorganisms with coagulation-filtration

For coagulation/flocculation processes, most bacteria and protozoa can be viewed as particles, while most viruses can be considered as colloidal organic particles. Optimized coagulation-flocculation processes can result in 1 to 2 log reduction of bacteria, viruses, and protozoa. Also, without proper coagulation-flocculation, a rapid sand filter is no effective barrier for microorganisms (Bell et al., 2000; LeChevallier & Au, 2004). In water treatment processes,

the efficiency of flocculating microorganisms also depends on the particulate and dissolved matter present in solution. Microorganisms can get trapped in flocs, be associated with particles or bridging can occur between microorganisms and other particles (LeChevallier & Au, 2004).

5.5.3. Coagulation efficiency in IPW

Schmidt et al. (2005) determined the composition of leafy vegetables, among which iceberg and romaine lettuce. They found considerate fractions of the dry matter consisted of protein, polysaccharides and some fatty acids; e.g. for iceberg lettuce: 16.9% hemicelluloses, 13.1% cellulose, 12.2% starch, 1.4% fructan, 0.7% pectin, 21.9% crude protein, 6.3% fat. Also, relatively large amounts of free sugars were found: 16.8% fructose, 9.7% glucose, 7.3% sucrose, and to lesser extent organic acids (3.9%), predominately consisting of malate (Haila et al., 1992; Ke et al., 1993; Schmidt et al., 2005). Chitosan, as a cationic flocculant, can in principle interact with a.o. negatively charged pectin, soluble starch, protein, oil and grease, in addition to other interaction mechanisms that do not rely on the cationic state of chitosan, such as hydrophobic interactions and hydrogen bonding (Marudova et al., 2004; Renault et al., 2009; Li et al., 2010; Boeris et al., 2011; Domingues et al., 2012; Ghorbel-Bellaaj et al., 2012; Wang et al., 2012). However, the results in this chapter show that the coagulation-flocculation-sedimentation treatments have negligible ability to reduce dissolved substances from fresh-cut leafy vegetables wash water. This concords with studies on the clarification of fruit juices with chitosan which showed that, although chitosan effectively reduced the turbidity of fruit juices, total soluble solids and titratable acidity (indicating concentrations of soluble sugars and organic acids) were only slightly or not significantly reduced (Chatterjee et al., 2004; Domingues et al., 2012, 2014; Ghorbel-Bellaaj et al., 2012). Overall, the COD reduction was considerably lower in the industrial wash water than in the SPW. This can in part be attributed to the relatively higher fraction of CODp in SPW (35 % *versus* 24 % of total COD), most likely because the matter in SPW was artificially created through destruction of lettuce tissue, whereas in the IPW through cutting of the lettuce tissue before washing, resulting in leakage of predominantly cellular exudates containing more dissolved substances. This illustrates that although artificial systems are useful for control of parameters in experimentation, eventually a validation in IPW is necessary.

The inefficient reduction by coagulation of dissolved organic compounds and especially small organic compounds is not restricted to matrices from fruit and vegetable origin. Coagulation

efficiency of raw water originating from surface or ground water to be used for drinking water production has been shown to be proportional to molecular weight (Chang et al., 2001). Of the small soluble molecules in fresh produce, free amino acids, some carboxylic acids, and some phenolic acids react very rapidly with free chlorine, whereas reaction with carbohydrates is much slower (Toivonen & Lu, 2013). Despite the high reduction of turbidity, the effect on COD reduction was limited, because of the small contribution of CODp to the total COD. Although the disinfectant decay is lower in coagulated IPW, the DBPs production was not significantly different in coagulated water. Small molecules (especially small hydrophobic acids) seem to have the highest DBPs formation potential. As these molecules are inefficiently reduced by coagulation, the reduction of DBPs formation is limited (Chang et al., 2001), and as such lowering the organic load in the water matrix does not necessarily imply a reduction of the DBPs formation potential (Bekbolet et al., 2005).

5.6. CONCLUDING REMARKS

- Gallotannins and chitosan were used as coagulants in leafy vegetables wash water
- The greater part of COD in leafy vegetables wash water was soluble
- Both coagulants effectively reduced particulate matter but not dissolved matter, chitosan being the more effective coagulant
- Coagulating SPW did not reduce the THMs formation in subsequent chlorination of SPW
- Due to the high particulate organic matter reduction, the chitosan coagulation process shows promise as a pre-treatment for a biological treatment (to reduce soluble organic matter), but as a stand-alone treatment it is not feasible

CHAPTER 6

SELECTION CRITERIA FOR WATER DISINFECTION TECHNIQUES IN AGRICULTURAL PRACTICES

Redrafted from

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6. SELECTION CRITERIA FOR WATER DISINFECTION TECHNIQUES IN AGRICULTURAL PRACTICES

6.1. ABSTRACT

This chapter comprises a selection tool for water disinfection methods for fresh produce pre- and postharvest practices. A variety of water disinfection technologies is available on the market and no single technology is the best choice for all applications. It can be difficult for end users to choose the technology that is best fit for a specific application. Therefore, the different technologies were characterized in order to identify criteria that influence the suitability of a technology for pre- or postharvest applications. Introduced criteria were divided into three principal components: i) criteria related to the technology and which relate to the disinfection efficiency, ii) attention points for the management and proper operation, and iii) necessities in order to sustain the operation with respect to the environment. The selection criteria may help the end user of the water disinfection technology to obtain a systematic insight into all relevant aspects to be considered for preliminary decision making on which technologies should be put to feasibility testing for water disinfection in pre- and postharvest practices of the fresh produce chain.

6.2. INTRODUCTION

Outbreaks, traced to fresh fruits and vegetables, show that the quality of water used for pre- and postharvest practices is of importance to assure food safety. Furthermore, wash water may function as a vector for transfer of microorganisms from contaminated to uncontaminated produce within a batch and between different batches over time (section 1.1). Therefore, efficient water treatment of water intended for irrigation and postharvest practices and maintaining water quality during produce processing to avoid spread of produce associated pathogens to uncontaminated produce is necessary (Steele & Odumeru, 2004; Gil et al., 2009).

A variety of disinfection technologies is available on the market and the objective of this chapter was to characterize water disinfection methods and to develop selection criteria to support the “fit for purpose” judgment of their use for water disinfection in irrigation practices, hydroponics, and produce processing washing and cooling operations.

6.3. SELECTION TOOL APPROACH

An overview of relevant criteria for selecting a water disinfection technology in pre- and postharvest produce practices was composed based on available scientific literature, regulatory prescriptions and guidance documents. A comprehensive approach for elaboration of the selection tool was taken, grouping the selection criteria into three main aspects, namely technological, managerial, and selection criteria related to sustainability (Figure 6.1).

The technological selection criteria encompass those factors that directly influence the required disinfection efficiency. Technological criteria relate to the water source and the opted water quality, the physicochemical and microbial parameters associated with the water source which will determine in turn the requirements of a disinfection method to achieve the opted water quality that is needed for a defined application. The technological criteria also include the process parameters of a water treatment that can be manipulated to improve the disinfection efficiency.

The managerial selection criteria include the capital and operational costs, factors related to the operation (e.g. complexity of the technology, required monitoring, safety issues) and the legal considerations.

Last but not least, factors that determine the sustainability of the disinfection technology were also taken into consideration in the selection tool. Selection criteria related to sustainability comprise criteria for maintaining the disinfection technology (maintenance, choice of corrosion resistant construction materials, supporting a reliable supply chain), equipment retrofitting and environmental considerations.

Selected disinfection technologies were evaluated on the defined criteria (Table 6.1). The disinfection technologies on which data is most prevalent in scientific and grey literature and thus taken up in this chapter were chlorine, ClO_2, O_3, PAA, H_2O_2, membrane filtration and UV.

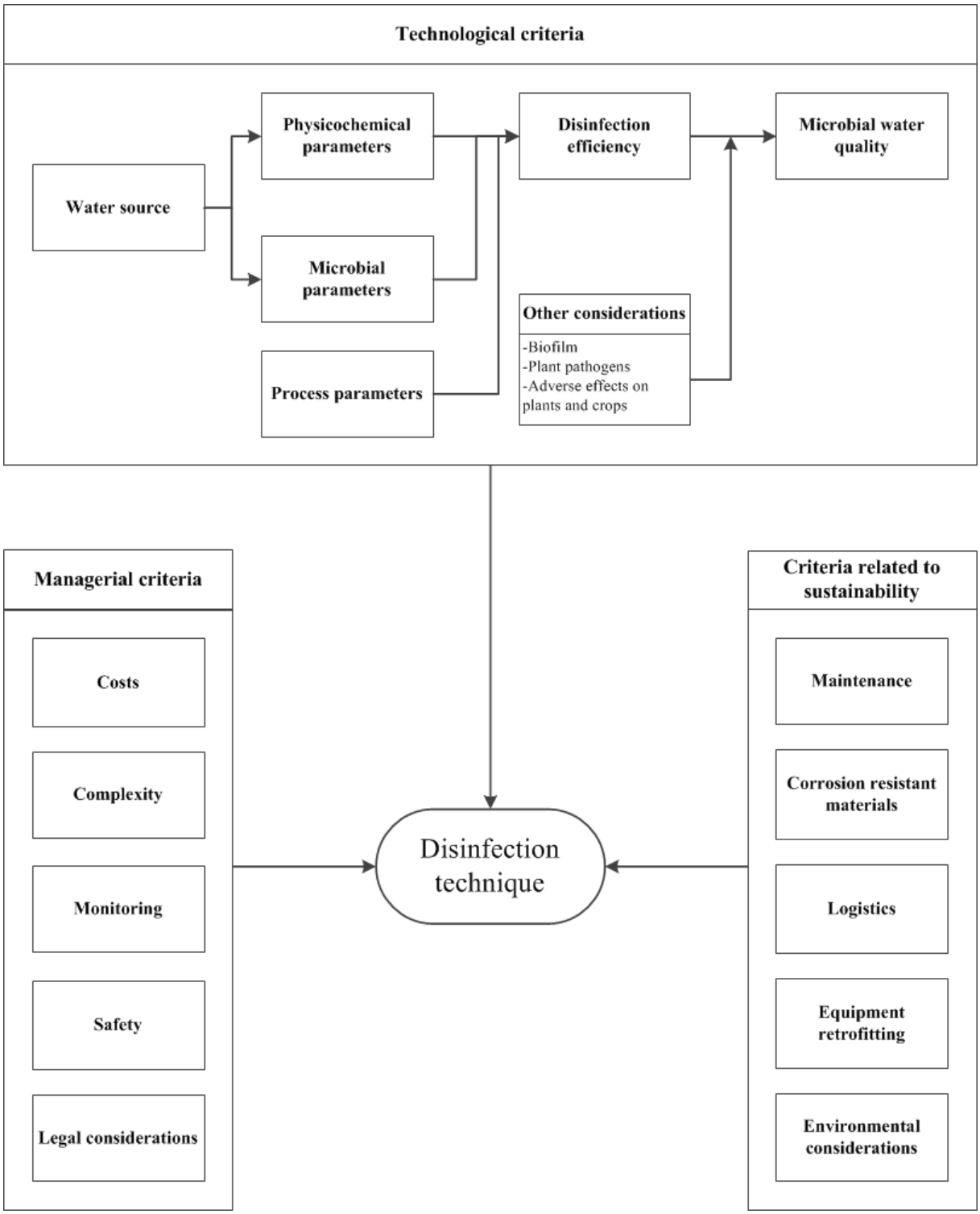

Figure 6.1. Overview of the selection criteria for choosing a water disinfection technique for pre- and postharvest practices

6.4. TECHNOLOGICAL CRITERIA

6.4.1. Water sources and microbial water quality used in fresh produce practices

The potential water sources that can be used in pre- and postharvest water practices and their respective microbial water quality are described in section 1.2.

6.4.2. Microbial, physicochemical and process parameters

6.4.2.1. Microbial parameters

Ideally an implemented disinfection technique provides adequate performance against a broad range of microorganisms. As microorganism types (vegetative bacteria, bacterial spores, viruses, protozoa) differ in morphology and physiology, the resistance towards different disinfection mechanisms depends on the microorganism type. A detailed description of the impact of different disinfection mechanisms on different microorganism types is given in section 1.5. Besides the established water disinfection techniques, there are other technologies that have been and are being studied at present and which potentially could become mainstream technologies for water disinfection purposes (described in detail in section 1.8).

The above description of microbial inactivation efficiency by different disinfection technologies is not complete, as in practice disinfection efficiency is highly influenced by physicochemical parameters.

6.4.2.2. Physicochemical parameters

In practice, water disinfection is dependent on the physicochemical quality of the water, i.e. the particulate and dissolved (in)organic matter, the temperature and the pH. These factors influence the disinfectant stability, the disinfection kinetics, and the molecular forms of chemical disinfectants that are present in the water matrix. A detailed description of the influence of the physicochemical water quality is given in section 1.6, and the basics of disinfection kinetics in section 1.7.

6.4.2.3. Process parameters

Certain parameters can be manipulated to create a better functioning environment for disinfection i.e. process parameters. As mentioned, T and pH influence the disinfection process and control of these parameters can be beneficial (Table 6.1). Control of the wash

water matrix constituents by reduction of turbidity and organic matter can be attempted by a series of available water treatment techniques (section 1.9).

6.4.3. Other technological considerations

6.4.3.1. Biofilms

Presence of biofilms is a persistent source of contamination that can lead to food spoilage or compromise food safety (Van Houdt & Michiels, 2010). Biofilm formation on the produce itself is a main factor in the failure of decontamination practices to remove or inactivate human pathogens on produce surfaces (Annous et al., 2006). In addition, biofilm formation can also negatively influence the process (e.g. down time of the process, pressure drop in pipelines leading to decreased energy efficiency) and the equipment (corrosion).

Biofilms in fresh produce facilities can be controlled by regular disinfection. Sufficient pre-rinsing and cleaning to remove material deposits reduces the loss of applied chemicals during processing (Simões et al., 2010; Van Houdt & Michiels, 2010). The problem of biofilms is not limited to piping and surfaces in direct contact with the produce. Environmental pathogens that have been introduced in a processing facility, can potentially spread throughout the facility if water is allowed to pool on the floor near equipment and employee walkways. This mechanism supposedly contributed in the spread of *L. monocytogenes* in a cantaloupe packing facility in southeast Colorado, which was implicated in a multi-state listeriosis outbreak in the USA in 2011. Applying good agricultural and management practices in processing facilities, such as avoiding water pooling and regular cleaning and disinfection should be used to control the introduction and spread of *L. monocytogenes* in processing facilities (FDA, 2011).

Table 6.1. Evaluated criteria of selected water disinfection technologies for pre-and postharvest applications

	Chlorine	**O_3**	**ClO_2**	**PAA**	**H_2O_2**	**Membrane Filtration**	**UV**
Technological criteria							
Vegetative bacteria	Good	Very good	Good	Good (1) [a]	Weak	Very good	Good
Bacterial spores	Weak	Moderate	Weak	[b](3)	Very weak	Very good	Weak
Viruses	Moderate [c]	Good [c]	Moderate [c]	Moderate (2) [c]	Very weak [c]	Very good [d]	Moderate [c]
Giardia	Weak	Good	Moderate	[b] (4)	Very weak	Very good	Good
Cryptosporidium	Very weak	Moderate	Weak	[b] (4)	Very weak	Very good	Good
Scale	All sizes	Medium to large	Medium to large	Small to medium	All sizes	Generally small to medium	All sizes
T	Better at higher T Not above 40°C	Better at low T Increased decomposition rate and decreased solubility at higher T	Better at higher T	Better at higher T	Better at higher T	< 80°C Lower viscosity at higher T, thus higher flux Higher T may promote biofouling	Better at lower T (of both water and lamp) to reduce fouling
pH	pH < 7 to assure presence of HOCl	6 (optimum) Higher decomposition rate at higher pH	Relatively constant in the range 3 - 9, Slightly better at alkaline pH	Relatively constant in the range pH 5,5-8,2 Reduced efficiency > pH 8,5	Better at lower pH Higher decomposition rate at alkaline pH	Working range 1-13 pH may influence fouling and electrostatic interactions of membrane with MO	No significant influence
Influence of suspended solids, organic matter and reducing compounds	High	High	Medium No reaction with ammonia Overall less influenced than chlorine	Lower than other chemical disinfectants	High	Fouling Fouling and particle association may enhance retention capacity	High Particle shielding Reduction of UV transmittance

[a] In order of decreasing disinfection efficiency 1 > 2 etc. (Kitis, 2004). [b] Relatively little is known. Liberti et al. (1999) observed low efficiency against protozoa in municipal treated wastewater. [c] viral disinfection is quite dependent on virus type. [d] MF generally has a too great pore size to remove viruses, although certain properties of membrane, water matrix and microbial load can result in some retention capacity. UF can achieve very high retention of viruses.

Table 6.1 (continuation). Evaluated criteria of selected water disinfection technologies for pre-and postharvest applications

	Chlorine	**O_3**	**ClO_2**	**PAA**	**H_2O_2**	**Membrane Filtration**	**UV**
Managerial criteria							
Complexity of technology	Low to moderate (in case of gaseous chlorine)	High	Moderate to high	Low	Low	High	Low to moderate
Operational monitoring [e]	Chlorine pH T [f] Ambient gaseous chlorine	O_3 (at generation, transfer to water, off-gass unit) [g] pH T [f]	ClO_2 [g] T [f]	Peracetic acid T [f]	Hydrogen peroxide pH T [f]	Transmembrane pressure or permeate flux Turbidity	UV intensity UV transmittance
Safety issues	Handling, storage, transport Ambient gaseous chlorine	Exposure to high electrical voltages Ambient ozone, ozone leaks	Explosion due to ClO_2 build up in air Ambient ClO_2 Issues related to chlorine when used	Handling, storage, transport	Handling, storage, transport	Handling and disposal of chemicals for cleaning. Contaminated backwash (low risk)	Exposure to UV radiation and mercury during lamp disposal Electric shock (overall low risk)
Capital cost	Low	High	Moderate	Low	Low	High	Low to moderate
Operational cost	Low	High	Moderate	Moderate	Low	High	Low to moderate
Legal status in EU	Processing aid	Processing aid	Processing aid	Processing aid	Processing aid	Not applicable	Not applicable

[e] flow rate measurements are advisable in many disinfection practices, and indicate contact times for chemical disinfectants and UV. [f] monitoring T might not be necessary when the process is T controlled or not subject to high fluctuations. [g] Additional monitoring of the in situ generation process. [h] RO treatment could lead to water with corrosive properties, [i] In-situ generation by indirect electrosynthesis is possible (Chaenko et al., 2011).

Table 6.1 (continuation). Evaluated criteria of selected water disinfection technologies for pre-and postharvest applications

	Chlorine	**O_3**	**ClO_2**	**PAA**	**H_2O_2**	**Membrane Filtration**	**UV**
Criteria related to sustainability							
Corrosive	Yes (all forms)	Yes	Yes but less than chlorine and ozone	Yes	Yes	Not applicable [h]	No
Maintenance	Low to moderate Issues: corrosion	High and careful Issues: corrosion and ozone electrode	High and careful Issues: corrosion	Low Issues: corrosion	Low Issues: corrosion	High and careful Issues: membrane	Low to moderate but careful Issues: lamp and sleaves
Storage	Yes	No, in situ	No, in situ Precursor storage	Yes [i]	Yes	Chemicals for cleaning	Chemicals for cleaning
Availability for developing countries/rural areas in general	NaOCl (high), gas(moderate,equipment only available in large cities), $Ca(OCl)_2$ (moderate)	Very low, equipment needs to be purchased in developed countries	Very low, equipment needs to be purchased in developed countries	Relatively low, PAA is relatively expensive, in part due to limited production capacity		Very low, equipment needs to be purchased in developed countries	Distant or rural areas dependent on external suppliers
Hazardous DBPs	Trihalomethanes (THMs) Haloacetic acids (HAAs) Aldehydes	Bromate and bromo-organic DBPs (dependent on bromide) Aldehydes	Less halogenated DBPs than chlorine (no THMs/THAAs) More iodinated DBPS (if iodine is present) Chlorite and chlorate Aldehydes	Negligible or low levels of aldehydes	Negligible or low levels of aldehydes	Not applicable	Normally none Nitrite with MP lamps if high nitrate concentration and/or very high UV MP doses are applied

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6.4.3.2. Control of plant pathogens

Regardless the increased attention for foodborne pathogens related to fresh produce, not in the least because of possible economic losses due to recall or trade restrictions, the economic problem of reduced crop yield and postharvest produce spoilage that plant pathogens cause has been recognized since early in the last century. Control of plant pathogens is an important practice and the importance of this issue will increase with the growing implementation of recycling water in agriculture. These plant pathogens include *Phytopthora*, *Pythium*, fungi, bacteria, viruses and parasitic nematodes (Hong & Moorman, 2005). Protection from plant pathogens has been especially relevant in closed hydroponic systems due to recycling of nutrient solutions, leading to a higher risk for accumulation of (plant) pathogens. The conventional disinfection methods applied in hydroponics include heating, O_3, UV, iodine, H_2O_2 and slow sand filtration (Ruina, 1995,1996; Ohtani et al., 2000). Other studied disinfection technologies for hydroponics include MF (Ohtani et al., 2000), solar radiation (Osorio et al., 2005), peroxone (Langlais et al., 2001) and H_2O_2/UV (Runia & Boonstra, 2004).

Disadvantages associated with certain disinfection techniques in hydroponics include: the energy consuming process of heating and cooling, the oxidation of iron and manganese by O_3 resulting in precipitation and therefore the need of replenishing them in the nutrient solution, and the precipitation of oxidized ions on UV lamps (Ohtani et al., 2000; Déniel et al., 2006). As a proportion of the microbiota may be antagonistic towards plant pathogens, a technique capable of selectively eliminating pathogens while maintaining these beneficial microorganisms would contribute to suppressing plant diseases. Most disinfection methods do not possess this discriminating ability. Slow sand filtration, however, has shown promise as selective technique for maintaining beneficial microbiota while eliminating pathogens. Non-pathogenic species of *Bacillus* and *Pseudomonas* that inhabit the filter act antagonistically against plant pathogens by competition for nutrients (*Pseudomonas spp.*) or production of antimicrobial compounds (*Bacillus* spp.). In addition, it requires less energy and is less costly than established disinfection methods like O_3 treatment (van Os, 1999; Déniel et al., 2004, 2006; Osorio et al., 2005; Martinez et al., 2010).

6.4.3.3. Adverse effects of disinfectants on plants and crops

It has been noted that the presence of too high concentrations of chemical disinfectants in hydroponic solutions can lead to stunted growth, root and leaf damage, and effects on nutritional quality (Vines et al., 2003; Premuzic et al., 2007). Similarly, crop damage can occur when irrigating fields with reconditioned wastewater that contains disinfectant residuals, which is for example generally the case with wastewater containing more than 5 mg/L chlorine (Pedrero et al., 2010). In postharvest practices, the use of sanitizers in washing operations may also have negative effects on crops. If no proper process conditions are met i.e. excessive concentration, contact time, and possibly temperature, all dependent on the disinfectant applied and the targeted crop, washing of produce with sanitizers may influence discoloration, off flavours, changes in nutrient content, softening of plant tissues etc. (Delaquis et al., 2004; Allende et al., 2008; Tefera et al., 2008) and it may affect physiological processes such as respiration, enzymatic browning and electrolyte leakage in fresh-cut produce (Vanderkinderen, 2009).

6.5. MANAGERIAL CRITERIA

6.5.1. Costs

When the needed degree of disinfection effectiveness is defined, the associated costs become the dominant factor in decision making. Capital investments to acquire the necessary equipment are supplemented by amortization costs, site specific constraints, and hiring consultants in order to implement the technology. Operational costs should be calculated in cost per cubic meter of treated water (USEPA, 1986; Kerwick et al., 2005b) and include cleaning chemicals, supplies and other maintenance costs, equipment repairs, the storage of chemicals and spare parts, operator and management personnel costs, training of personnel, and power consumption (USEPA, 1986; Tzimas et al., 2006). Furthermore, shifts in the market situation influence total costs (Liberti et al., 2000b).

Exploitation scale influences the type of techniques that can be used. Some disinfection technologies have a too high investment or require excessive safety measures that are not feasible for small scale operations (Salsona & Méndez, 2003). However, feasibility can increase due to benefits of scale e.g. O_3 is generally unfeasible at low scale (Leverenz et al., 2006), yet better cost-effectiveness can be achieved at higher scale (Collivignarelli et al., 2000; Tzimas et al., 2006). Likewise, chlorine gas is the cheapest form of chlorine, but the

equipment to operate gaseous chlorine is more expensive than sodium hypochlorite dosing systems. Because of this, chlorine gas tends to be used in greater facilities, while sodium hypochlorite is the chlorine disinfectant of choice in smaller ones (USEPA, 1999a). On the other hand, techniques which require relatively low, simple investments combined with relatively high chemical costs, like PAA, are more cost-effective at lower scale than at larger scale (Collivignarelli et al., 2000; Kitis, 2004). Cost-effectiveness also depends upon the water quality, the refreshing rate, the needed pre-treatment, the imposed/aspired level of safety, the degree of automation and local labor costs. During the evaluation of the scale-up from concept to a large operational process, care must be taken when extrapolating costs (Collivignarelli et al., 2000; Kerwick et al., 2005b).

A cost-analysis for comparison of wash water disinfectants would provide valuable insight on the economical feasibility of different technologies. Garrett et al. (2003) performed a cost analysis for selected disinfectants. As chemical disinfectants can have significantly different reaction rates with water matrix constituents, the actual needed disinfectant dose can differ substantially among disinfectants because during operation the physicochemical wash water quality deteriorates very quickly. Due to lack of published information on comparing wash water disinfectants in actual washing operations, assumptions had to be made by Garrett et al. (2003). It was assumed that the water was of potable quality. Therefore, only the inherent disinfection capacity of the technologies was considered. In addition, the scale of operation was fixed to one size, and therefore the possible increase of economical feasibility at higher scale of potent technologies which require high capital investments was not considered. This illustrates the necessity for additional research that compares the different technologies in actual postharvest washing conditions and which makes a cost analysis possible that considers influencing factors, i.e. the pathogens of concern, the amount of dissolved and particulate matter introduced in the wash water and the scale of operation. For example, Collivignarelli et al. (2000) performed a cost-analysis of disinfection systems for wastewater disinfection by applying different technologies on the same secondary effluent of a wastewater treatment plant and total costs were calculated for different plant sizes, which showed significant influence on costs. In the given case inactivation of total coliforms demanded higher concentration x contact time (CT) values for O_3 than for PAA and ClO_2, again illustrating that physicochemical quality is significant when determining the needed disinfectant dose. A similar approach for investigation of costs of wash water disinfectants could be very valuable to the fresh produce industry.

6.5.2. Complexity

Complexity of the used technology and the aspired level of safety positively correlate with the required amount of operator skill and training (USEPA, 1999a). Disinfection techniques like O_3 and ClO_2 are relatively high tech. This complexity translates in high and careful maintenance requirements and control systems (Salsona & Méndez, 2003), while simple systems for dosing e.g. hypochlorite solutions or PAA require much less maintenance and operating skills (USEPA, 1999a; Kitis, 2004). Complexity is not completely inherent to a defined disinfection technique. Considerable variations can exist between various designs of a disinfection technology e.g. basic UV equipment is relatively simple, but more complex operating systems can be implemented to achieve energy savings or a higher safety level (Solsona & Méndez, 2003).

Higher degrees of automation will decrease the needed operator attention during normal operation, although during initial start-up, additional operator attention is needed to assist with functional and performance testing and to establish site-specific operation and maintenance procedures (USEPA, 2006). This reduced need for operating personnel can be beneficial in developed countries, whereas in regions with low labour costs, the application of labor-intensive, low automated techniques is more attractive.

6.5.3. Monitoring

Accurate monitoring and recording of disinfection procedures is an important component of a reliable postharvest food quality and food safety program (Suslow, 2004b). Monitoring of pathogens or indicator organisms should be done during pilot tests and initial start-up to validate the effectiveness of the process in general i.e. to assess if the applied operational parameters guarantee sufficient inactivation or retention. Incorporation of water disinfection in the HACCP of the fresh produce operator confines the microbial monitoring to key points in the disinfection process, thus limiting needed laboratory resources through proper allocation (LeChevallier & Au, 2004). The relations between the operational, physicochemical parameters and the microbial inactivation, obtained during validation, can be used to establish a (continuous) monitoring system based on these rapidly measurable physicochemical parameters (USEPA, 1980, 1999c, 2006; Reith & Birkenhead, 1998; Madaeni, 1999; Cabassud et al., 2001) (Table 6.1).

In postharvest washing operations the concentration of the wash water disinfectant should be controlled through real-time monitoring (i.e. in an automated fashion continuously measuring the disinfectant and adapting the disinfectant dosing rate) as it decomposes by reacting with organic matter, and the pH if relevant. Based on these measurements, disinfectant and pH altering substances can be added. For example, free chlorine is often monitored with an (on-line) ORP control system. As the ORP is also dependent on the pH, it reflects not only the concentration of free chlorine, but also its state, i.e. the more lethal hypochlorous acid has a higher ORP than hypochlorite ion at the same concentration. ORP values of 650-700 mV, which correspond to about 3 to 5 ppm free chlorine, are considered to be sufficient for controlling suspended vegetative bacteria in the wash water, although in practice sometimes somewhat higher values are aimed for e.g. 850 mV (Suslow, 2004b; Barrera et al., 2012). Another example is the monitoring of membrane filtration, a technology which does not apply a measurable chemical concentration or a UV light intensity. When applying membrane filtration, e.g. for reconditioning purposes of wash water, monitoring is needed to assess both the status of the membrane and the microbial retention. A decreasing membrane flux, or alternatively, an increasing transmembrane pressure at a constant flow, indicates membrane fouling (Reith & Birkenhead, 1998; Madaeni, 1999; Cabassud et al., 2001). Microbial retention of membranes may be compromised by integrity breaches i.e. leaks, which can be monitored indirectly by measuring the turbidity in the effluent (USEPA, 2005).

6.5.4. Safety

Worker safety issues due to the presence of hazardous compounds, danger of irradiation, possibility of electric shocks and proximity of water to electrical equipment etc. need attention in the operation of disinfection systems (Solsona & Méndez, 2003; USEPA, 2006). When applying gaseous chemicals, like chlorine gas, ClO_2 or O_3, ambient concentration levels in the workplace are regulated by law and should be monitored (USEPA, 1999a; Pascual et al., 2007; Tarrass et al., 2010; White, 2010). Transport of strong oxidants, often in diluted solutions meaning only part of the transported weight is active compound, handling, storage and loss due to decomposition during storage, all generate costs and require safety management. This can be partially circumvented by using physical disinfection techniques or in-situ generation of chemical disinfectants. However, the latter requires additional capital investments, knowhow, and transport of precursor chemicals if applicable. In addition, storage of precursor chemicals is still necessary for ClO_2 and chlorine generation, and precursors can

be hazardous compounds like hydrochloric acid or chlorine (USEPA, 1999a, 1999b, Solsona & Méndez 2003; Leverenz et al., 2006).

Safety measures, whether or not enforced by authorities, can directly influence decision making. For example, in the USA, Risk Management Program regulations, enforced by the USEPA, state that facilities, including agricultural establishments, that store, handle, and use chlorine in quantities greater than 2500 pounds (1134 kg), must develop and implement a program to prevent accidental release (40CFR68, 2010). These imposed measures contributed in part to the increased retro-fitting of wastewater treatment facilities from chlorination to UV disinfection (Johns et al., 2002). Another example is the increased use of sodium hypochlorite compared to chlorine gas. Despite chlorine gas being economically favorable for large scaled operations, several large facilities switched to hypochlorite to circumvent the transport of chlorine gas through populated areas (USEPA, 1999b).

6.5.5. Legal considerations of water sanitation

In the EU, chemical disinfectants to control the microbial quality of wash water or produce are classified as processing aids. This implies that disinfectants can be introduced in the process water but also that washing of produce with sanitized water should be followed by a rinsing step of the produce with sanitizer free water. This is to make sure that levels of residual or byproducts that remain in the vegetable tissue pose no health risk and the disinfectant may not perform any function in the final product so only the unintentional presence of the sanitizer substance in the final product is allowed (Council Directive 89/107/EC; Council Directive 178/2002; Ölmez & Kretschmar, 2009). In the EU, the use of sanitizers is not uniformly allowed, because up till now legislation on processing aids is not harmonized at European Community level (Gil et al., 2009). Some member states have explicitly stated some boundary conditions for chlorine use in fresh produce washing processes, e.g. the United kingdom poses limits concerning free and total chlorine in the wash water, the pH, the produce residence time in the washing tank and the produce to water ratio (CFA, 2010), whereas France mentions free chlorine residual limits in the wash water and limits the halogenated organic compounds on the produce (DGCCRF, 2009). Compounds for use as wash water disinfectant cannot be chosen solely based on microbial inactivation potential. Only those biocides that can be considered as processing aids are possible candidates for use as disinfectant. A list, though not a positive list, of antimicrobial substances that can be considered processing aids is provided in the ‘Inventory of substances used as

processing aids', composed by the Codex Alimentarius commission on food additives (Codex Alimentarius, 2012). This document is not a Codex standard, but is meant as a reference tool.

In the USA, for raw agricultural commodities that are washed in e.g. a fresh-cut facility, both the Food and Drug Agency (FDA) and the USEPA have judicial power. Sanitizers used for fresh produce are regulated as secondary direct food additives by the FDA, meaning they exhibit a technical effect during processing yet not in the finished product, although in some cases they are considered Generally Recognized As Safe (GRAS). Disinfectants are registered as pesticides with the USEPA (Gil et al., 2009; FDA, 2011).

6.6. CRITERIA RELATED TO SUSTAINABILITY

6.6.1. Maintenance

Maintenance of disinfection equipment is necessary to avoid damage to the equipment, in order to maintain disinfection efficiency and normal operation. Maintenance consumes a certain amount of time (with possible downtime of the system), requires personnel, and has a certain cost. The various components of the system e.g. meters, valves, piping need to be cleaned periodically for removal of organics, and iron, manganese and calcium deposits if applicable, and parts should be replaced when necessary (USEPA, 1999a, 1999b; LeChevallier & Au, 2004). Dependent upon the technology, specific parts require careful attention by skilled technicians. Furthermore, the frequency of maintenance is related to site-specific conditions like the physicochemical water quality (Leverenz et al., 2006) and the amount of maintenance may also in part be dependent on the specific design of the disinfection technique. For example, due to the higher operating temperatures of UV MP lamps compared to LP lamps, fouling is accelerated with certain water qualities, requiring a specific cleaning mechanism i.e. on-line mechanical or on-line mechanical-chemical cleaning systems in order to be able to clean while maintaining operation (USEPA, 2006; Anonymous, 2008).

6.6.2. Corrosion resistant materials

The use of proper materials, resistant to the applied chemical oxidant, are critical to avoid rapid corrosion of the equipment and associated loss of structural integrity. Sediment formation of accumulating insoluble corrosion by-products can serve as habitat for microbes, as well as protect them from disinfectants (Tarrass et al., 2010; White, 2010). Use of

corrosion preventing materials is especially important in parts of the system coming into contact with undiluted oxidant solutions, gasses, or precursor chemicals like strong acids i.e. those parts preceding the actual dosing systems (USEPA, 1999a). Water treated by RO attains aggressive properties as the ionic concentrations are very low and also pH decreases, so piping and other equipment subsequent to the RO unit should be of adequate materials (USEPA,2005; Pressdee et al., 2007).

6.6.3. Logistics

Crucial to sustaining the disinfection process is maintaining an appropriate supply chain of chemicals and spare parts, a reliable electrical power source if necessary, and the possibility of technical support. Besides money issues, these factors are significant causes in the possible failure of water treatment facilities in developing countries, especially in rural areas (Burch & Thomas, 1998; Gadgil & Derby, 2003; Solsona & Méndez, 2003; Okpara et al., 2011).

6.6.4. Equipment retrofitting

Water disinfection systems in general need to take the present design into consideration when implementing a new disinfection technique. As scientific knowledge and engineering experience evolves, new or improved technologies arise and efficient retro-fitting can help accomplish the effective implementation of a new technology into an existing system design (Lazarova et al., 1999). Is the new disinfection technique compatible with target water flow rates, with the system in general (e.g. is the available capacity of the electrical power distribution system sufficient for operating the new technique), what structural modifications might be needed and will there be enough space available for installing the new system (Johns et al., 2002; Pressdee et al., 2007)?

6.6.5. Environmental considerations

In postharvest practices, a sustainable water disinfection system can be interpreted as one that negatively affects the environment as little as possible, minimizing DBPs formation and persistence of toxic residuals and generating less volumes of wastewater (i.e. allowing recycling) (Ölmez & Kretschmar, 2009).

Disinfectant residuals in wastewater effluents can have detrimental effects on the environment (Moore & Calabrese, 1980; Vedachalam, 2009). The formation and adverse effects of DBPs in drinking and wastewater from chlorine and other disinfectants has been well studied

(described in section 1.4). The formation of DBPs due to chlorination in fresh-cut produce washing water and the transfer to the produce was studied for carrots (Klaiber et al., 2005) and fresh-cut leafy vegetables (COT, 2006; Lopez-Galvez et al., 2010b; Gomez-Lopez et al., 2014), but up till now no significant transfer to the fresh-cut produce (after rinsing) has been observed despite significant formation in the washing bath (chapter 3).

Use of disinfected wastewater in irrigation can augment the DBPs in ground water aquifers through infiltration. This may lead to DBPs accumulation and might have long term effects on the quality of ground water (Bouwer, 2000). Due to the accumulation of DBPs in ground water, now also legal limits of DBPs in treated wastewater are arising (e.g. by the Florida Department of Environmental Protection, i.e. 470.8, 22, 34, and 360 µg/L for chloroform, bromodichloromethane, dibromochloromethane, bromoform respectively) in order to protect surface water quality and avoid accumulation of these compounds in the environment (Hua & Yeats, 2010). Akande et al. (2010) noted that up till now, little attention has been paid as to how the presence of DBPs in disinfected wastewater used for irrigation could affect crop performance, cause physiological changes and the potential bioaccumulation of these compounds in edible plant tissues.

Sustainable development is increasingly employed in decision making. Future environmental and social implications are of importance when evaluating possible disinfection technologies. The impact of factors such as the generation and use of energy, transportation and storage of hazardous chemicals, and disposal of caustic chemicals and contaminated backwash from membrane processes should be considered (EPA Victoria, 2002; Kerwick et al., 2005a).

6.7. DISCUSSION

The selection criteria may help the end user of the treatment to obtain a systematic insight into all relevant factors to be taken into account in preliminary decision making on which techniques should be put to feasibility testing for water disinfection in irrigation practices, hydroponics, and produce washing and cooling operations.

In primary production, water disinfection can be applied to control the microbial load in irrigation water in ponds, wells and irrigation networks, in hydroponic and fertigation solutions in general, and for disinfecting wastewater destined for reuse in agriculture (Dell'Erba et al., 2004; De la Hoz et al., 2009; Martinez et al., 2010).

Due to recycling operations, microorganisms may accumulate in postharvest wash water and be present in higher concentrations on the end product (Luo et al., 2007; Doyle & Erikson, 2008; Holvoet et al., 2012). In postharvest practices, the application of disinfection consists mostly of decontaminating produce during processing and maintaining the microbial quality of washing and cooling water during processing, and in dump tanks and water flumes etc. in packing houses.

A set of water disinfection technologies on which considerable amounts of research has been published were chosen as cases for evaluation with the selection tool (Table 6.1). By evaluating available water disinfection technologies in the context of the specific application of the end user, a screening of the technologies can be executed. In order to make a selection, some hierarchy should be made to assess which criteria are most critical. Loo et al. (2012) constructed a methodology for emergency water treatment selection. They made use of a ranking system based on assigning weighing factors to criteria to adjust their relative importance, as well as defining scores for each criterion expressing the degree to which a certain criterion is met. The most favorable water treatment was selected based on total weighted scores. Concerning the selection criteria for agricultural practices which were defined in this chapter, the amount of accumulated knowledge was considered insufficient to actually quantify the compliance with the criteria. The comparison of the costs of the disinfection technologies and the scale on which they should be used are based on literature review only. This provided only relative comparisons between technologies, i.e. no absolute values of costs and scale were found. Some of the defined criteria are simply essential and if not completely fulfilled the disinfection treatment is not fit for purpose. Those criteria were denoted as knockout criteria (Figure 6.2). Based on these criteria a qualitative analysis can be done to assess fit for purpose status of water disinfection technologies.

Scientific literature relating to water disinfection in pre- and postharvest practices is relatively scarce. In preharvest practices, more research is needed towards disinfection of alternative water sources for irrigation use, because of the issue of groundwater shortages. Also, maintaining the water quality of hydroponic solutions is a relevant topic, and foremost towards techniques which selectively eliminate foodborne and plant pathogens. In postharvest practices both the prevention of cross-contamination during the washing process by applying sanitizers, and the reconditioning of used water for subsequent reuse are topics which should receive additional attention. In order to be able to gain insight in the cost-effectiveness of

postharvest washing processes, performance of relevant technologies should be assessed and compared, especially with consideration of the consumption rates of different disinfectants by organics present in the wash water. This to gain insight into the actual dosage that is needed to maintain residuals that prevent cross-contamination, in order to estimate the cost-effectiveness of different techniques.

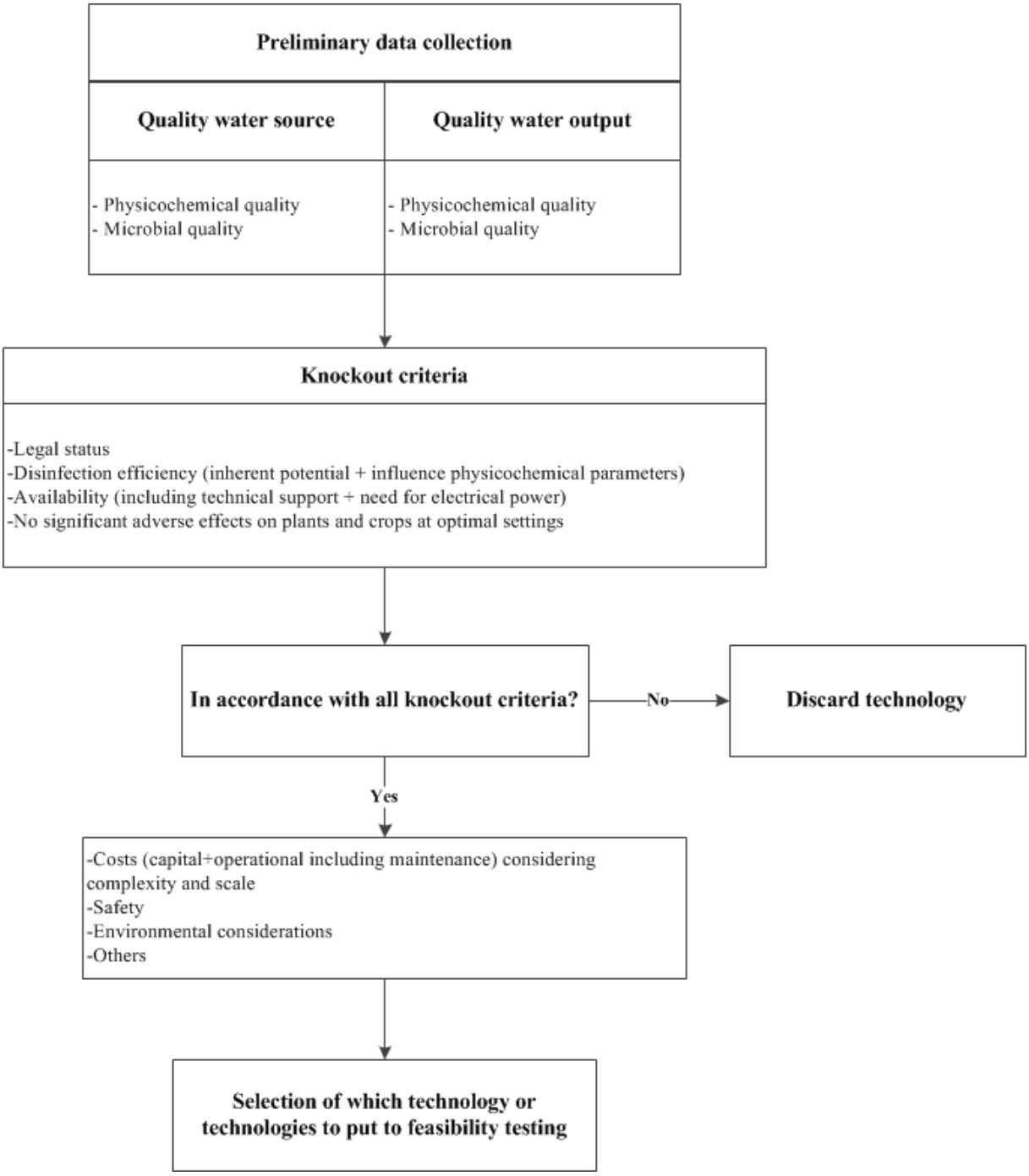


Figure 6.2. Methodology for applying selection criteria to decision making for water disinfection treatments in pre- and postharvest practices

6.8. CONCLUDING REMARKS

- Through literature study selection criteria were defined that could be considered when deciding on the implementation of water disinfection in pre- and postharvest practices
- Criteria concerning the cost-effectiveness, the operation (both towards the process and the people) and sustainability were defined as being of importance
- A quantitative analysis of the decision making process is not possible at the moment, because the cost-effectiveness (and other criteria) are not quantitatively understood for the different disinfection techniques
- Therefore the selection criteria is mostly informative at the moment

CHAPTER 7

GENERAL DISCUSSION

7. GENERAL DISCUSSION

7.1. INTRODUCTION

Among fresh produce, leafy vegetables are one of the commodities most frequently implicated with food disease outbreaks, the culprit most often being *E. coli* O157: H7 or *Salmonella* spp. (Olaimat, & Holley, 2012; Tomas-Callejas et al., 2012a). Therefore, the focus of the PhD was on leafy vegetables. Washing of fresh (-cut) produce is often the only processing step able to reduce the microbial load on the fresh produce (Artés et al., 2009). Current washing treatments with the purpose of decontaminating fresh-cut produce for microbial safety or quality reasons, have evolved from processes that were originally developed to remove soil from fresh-cut produce, to a water disinfection process for removal of microbial targets from fresh-cut produce (Sapers, 2001). However, the efficiency of decontamination has been shown to be limited. Therefore it is preferable to avoid contamination wherever possible by respecting good agricultural and manufacturing practices during the production and processing of fresh produce (Sapers et al., 2001; Keskinen et al., 2009; Lopez-Galvez et al., 2010a; Holvoet et al., 2012; 2013). The post-harvest washing water is a vehicle for microbial cross-contamination and to counter this, a process wash water disinfection (i.e. disinfection in the washing bath) can be performed. Water disinfection can also be used to treat the wash water before reusing it (i.e. process water recycling) for a similar or different purpose. Contrary to decontamination, inactivation of microorganisms in the wash water is quite effective, and therefore disinfection (in the forms of process wash water disinfection and water recycling) and continuous refreshing of fresh produce (and especially fresh-cut leafy vegetables) wash water was studied in this PhD research. Fresh water is a renewable yet finite resource. Concern over continued availability of high quality fresh water and the growing consensus that pollution of the environment should be minimized, have resulted in increased costs for tap water and for waste water discharges (Beekman, 1998; Casani & Knochel, 2002). The use of water disinfection (and treatment in general) offers solutions to lower the impact of the food industry on the (high quality) fresh water scarcity such as the reuse of water in food processing operations, the use of a wash water disinfectant to reduce the water usage, and the use of treated alternative water sources (of initial lesser quality) for use in pre- and postharvest practices (Casani et al., 2005). As such, there are two main drivers for the use of water disinfection in fresh produce production and processing: i) to improve the fresh produce safety and quality, and ii) to lower the impact of the fresh produce industry on the fresh water resources.

Research in this PhD thesis was done both in the lab as well as through study of a full-scale washing process in a processing company. Lab scale experiments were performed to assess the influence of the physicochemical water quality, the disinfection concentration, the contact time and the pH on the microbial inactivation of the bacterial pathogen *E. coli* (O157). Experiments were executed in oxidant demand free conditions, in SPW and in IPW. Based on the acquired data, models were made to predict the efficiency of fresh-cut leafy vegetables wash water disinfection, and more fundamentally in order to understand which variables govern the changes in water disinfectant stability and *E. coli* O157 inactivation.

7.2. THE USE OF PROCESS WATER RECYCLING OR WATER REFRESHING TO COPE WITH PREVENTING CROSS-CONTAMINATION IN THE WASHING BATH

Based on the models constructed in chapter 4, the use of process water recycling and water refreshing can be compared with process wash water disinfection for prevention of *E. coli* O157 cross-contamination in the washing bath.

Suppose a lettuce washing process occurs with the following parameter values:

- volume washing bath = 500 L,
- lettuce washed at 6 kg/min,
- every crop is contaminated with 5 log CFU/g *E. coli* O157,
- 80% transfer of *E. coli* O157 from lettuce to water,
- the water that is transferred with the lettuce is always refilled (even in the case of no refreshing) in order to keep a constant volume,
- all the water that is reconditioned is made devoid of *E. coli* O157 (i.e. complete inactivation).

In essence, process water recycling and refreshing strategies are the same, because both exhibit an antimicrobial action (be it disinfection or removal through water dilution) on only part of the total microbial wash water load. This explains why both strategies are unable to effectively control the wash water contamination (Figure 7.1a). A complete disinfection of *E. coli* O157 in the 500 L washing bath each minute (or a complete refreshing of the wash water each minute) is less efficient than maintaining a free chlorine residual of 1 mg/L.

In chapter 3, all process wash water disinfection in the washing tank was treated as a worst-case scenario in which all crops were contaminated with *E. coli* O157. Therefore, Figure 7.1a

more accurately represents a wash water disinfection process of the total microbial content in the context of food quality, i.e. to control cross-contamination of spoilage microorganisms. In reality, pathogenic contamination is mostly not widely distributed among a batch of fresh-cut leafy vegetables, although there are extreme cases, for example 12.1 % (4/33 positive samples) prevalence of *Salmonella* spp. on cabbage sampled in a field in India irrigated with partially treated municipal wastewater (Rai & Tripathi, 2007), or 76.9 % (10/13) and 61.5 % (8/13) prevalence of respectively *Cryptosporidium* spp. and *Giardia* spp. on lettuce sampled in a field in Spain irrigated with contaminated water from an irrigation canal. Nonetheless, the prevalence of bacterial pathogens (*Salmonella* spp., pathogenic *E. coli*, *L. monocytogenes*, *Campylobacter* spp.) is, as far as microbial screening studies show, much lower (<1% of the crops) (Johannessen et al., 2002; University of Georgia, 2011). In those cases only a very minor part of the crops are contaminated (i.e. a point contamination scenario).

Suppose a lettuce washing process occurs with the following parameters:

- volume washing bath = 500 L,
- lettuce washed at 6 kg/min,
- 1 in 1000 crops is contaminated with 3 log CFU/g *E. coli* O157,
- 80% transfer of *E. coli* O157 from lettuce to water,
- the water that is transferred with the lettuce is always refilled (even in the case of no refreshing) in order to keep a constant volume,
- all the water that is reconditioned is devoid of *E. coli* O157.

The model shows that the reconditioning/refreshing strategies are much slower at microbial inactivation than in the process wash water disinfection in the washing tank strategy (Figure 7.1b & c). In order to prevent cross-contamination between contaminated and uncontaminated crops, the incoming microbial contamination needs to be inactivated rapidly. As such, process water recycling/refreshing strategies are unfit to protect from cross-contamination. Process water recycling/refreshing could reduce the risk of cross-contamination to some degree. However, to assess this risk reduction, the process of cross-contamination from water to produce will have to be studied in a quantitative manner, both concerning microbial concentration in the wash water (as Holvoet et al. (2014) did for *E. coli* and lettuce), as well as the time dependent cross-contamination. Growth of bacterial pathogens on fresh-cut leafy vegetables during postharvest storage is mostly not significant at temperatures below 7°C, but they do survive. Even *Listeria monocytogenes*, which is psychrotrophic, does not readily grow on fresh-cut leafy vegetables at temperatures below 7°C. Therefore, if no temperature abuse

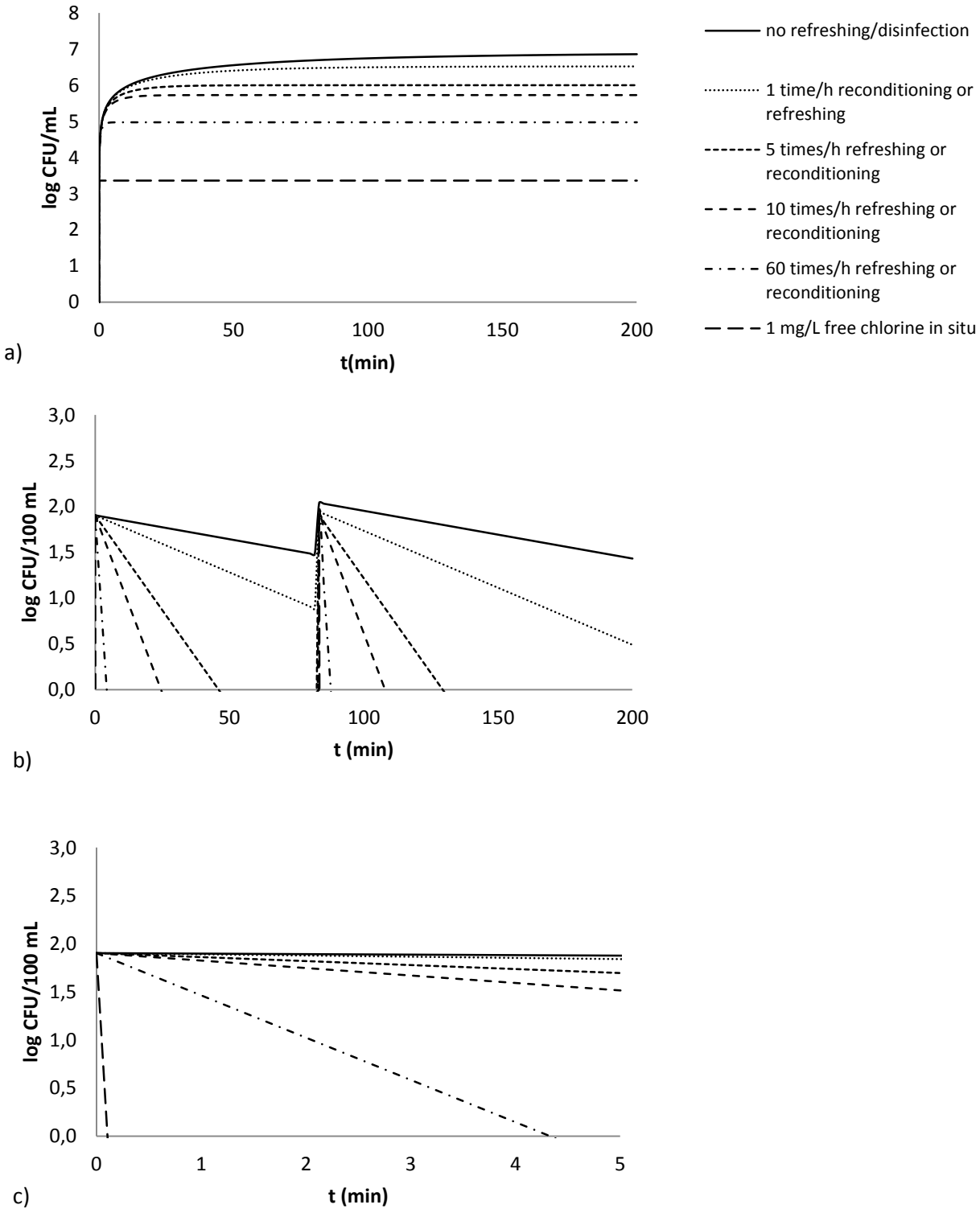


Figure 7.1. Scenario analyses of lettuce washing operations, showing the *E. coli* O157 wash water contamination for a) continuous influx of contamination, b) point contamination c) point contamination (0-5 min interval)

occurs, cross-contaminated pathogens, via wash water, will normally not grow significantly on the newly contaminated fresh-cut leafy vegetables, but the contamination can persist (Sant'Ana et al., 2012; Tian et al., 2012; Possado-Izquierdo et al., 2013; Zeng et al., 2014).

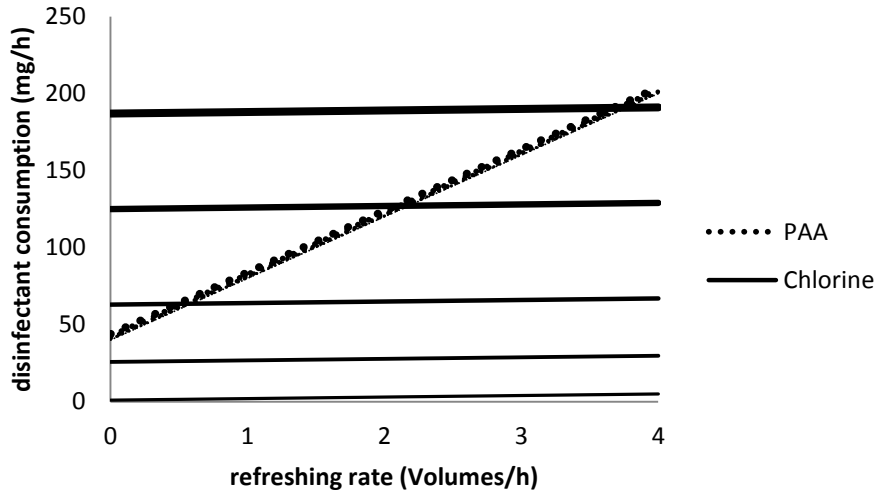
Present-day postharvest washing operations refresh the water to some degree i) to maintain a positive transfer of matter from the lettuce to the water, ii) because of the misconception that it can effectively be used to control the microbial load, iii) to control the accumulation of pesticides, mycotoxins etc., iv) to control the concentration of DBPs. Refreshing has also impact on the necessary disinfectant dose to maintain an opted residual. As free chlorine and PAA show vastly different behaviors in both inactivation kinetics and disinfectant stability, comparing these disinfectants is an interesting setup to assess **how the COD load and refreshing rate influence the disinfectant consumption.**

Suppose a fresh-cut spinach washing process with the following parameters:

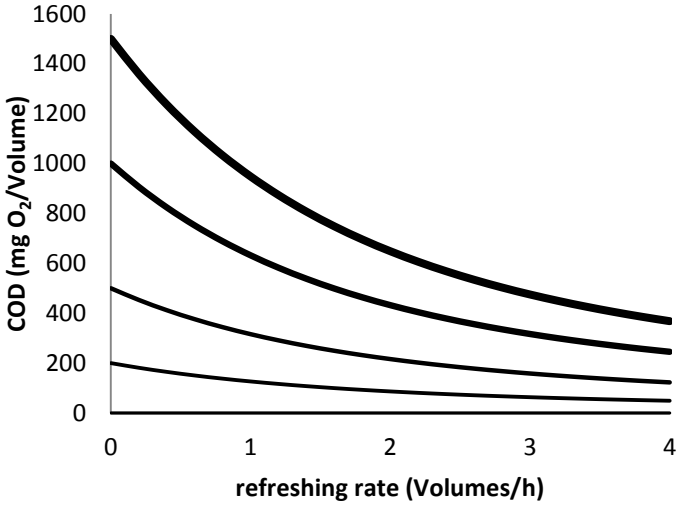
- influx of organic matter (COD) from spinach washing (range 0 to 1500 mg O_2/h),
- assume a disinfectant demand of 0.003 mg PAA/mg COD and 0.124 mg free chlorine/mg COD (the data for the latter not shown in the thesis but obtained from the study by Gomez-Lopez et al., 2014),
- a reduction of > 3 log *E. coli* O157 is aimed for, i.e. a residual of 1 mg/L of free chlorine or 40 mg/L of PAA+LA (1:40) is used.

From figure 7.2a it can be seen that the required PAA dosage to satisfy the COD demand is negligible compared to the dosage required to cope with the refreshing, whereas the vice versa is observed for free chlorine. Although the COD per water volume decreases (Figure 7.2b), the total COD load remains the same and is merely "spread out" over larger water volumes.

As such, PAA seems only interesting when high COD loads are present and low refreshing rates applied. As these are also the conditions in which the highest concentrations of DBPs would be generated with free chlorine, the two disinfectants seem complementary in this regard. However, in this research, PAA+LA was used. The high required PAA residuals (and as such forty times higher LA dosage) and the expected application of water refreshing to some degree make it at least questionable whether there is any cost-effective (not to mention sensorial) advantage of using the combined disinfectant instead of solely PAA.



a)



b)

Figure 7.2. a) consumption of disinfectant in function of influx of COD and refreshing rate, b) COD present in the washing tank after 1 hour operation in function of influx of COD and refreshing rate; influx of COD in the range 0 – 1500 mg O_2/h; increasing line thickness denotes increasing influx of COD

7.3. WHAT IS KNOWN CONCERNING MICROBIAL CROSS-CONTAMINATION VIA WATER, ITS PREVENTION AND HOW MUCH PREVENTION IS SUFFICIENT?

Throughout the thesis prevention of cross-contamination has been equated with maintaining the microbial wash water quality. Holvoet et al. (2014) illustrated for *E. coli* that the quantity of cross-contamination from contaminated wash water to lettuce was directly proportional to the *E. coli* wash water contamination. A cross-contamination event via water basically consists of the following steps:

i) dislodging of microbial cells from fresh produce and transfer to the water,
ii) certain residence time of the microorganisms in the wash water,
iii) adherence of microorganisms to other pieces of fresh produce.

Attempting to prevent cross-contamination is synonymous with decreasing the residence time in the water of the microorganisms as much as possible. As this is water disinfection, the disinfection efficiency is function of the exposure (concentration x contact time), and as such the process parameter that can be manipulated is the disinfectant residual. For example, Luo et al. (2011) observed that although 5 mg/L (1.7 mg/L after chlorine dissipation due to organic matter) inactivated *E. coli* O157 to below the detection limit in the wash water, some cross-contamination from inoculated to uninoculated lettuce was observed. When applying 10 mg/L or more, no cross-contamination was observed.

From equation 4.8, i.e. the microbial wash water contamination in the presence (or absence) of chlorine residual respectively, the steady state can be derived (dN/dt = 0) for the processes without and with free chlorine residual (eq. 7.1 and 7.2 respectively):

$$Ns = \frac{LR_1V}{V_L} \tag{7.1}$$

$$Nsd = \frac{LR_1R_2}{kCl_2{}^n + \frac{R_2V_L}{V}} \tag{7.2}$$

Where Ns = *E. coli* O157 load in the washing bath (CFU/100mL) at steady state and without disinfectant

Nsd = *E. coli* O157 load in the washing bath (CFU/100mL) at steady state and with chlorine residual

It can be seen that the quotient of these 2 equations does not contain L (eq. 7.3), i.e. is independent of the initial microbial contamination of the lettuce.

$$\frac{Nsd}{Ns} = \frac{R_2 V_L}{kCl_2{}^n + R_2 V_L} \quad (7.3)$$

This implies that the microbial logarithmic reduction does not depend on the initial microbial produce contamination. To illustrate this, suppose a lettuce washing process occurs with the following parameters:

- volume washing bath = 500 L,
- lettuce washed 5 kg/min,
- all crops are contaminated varying *E. coli* O157 contamination, from 2 to 5 log CFU/g,
- 80% transfer of *E. coli* O157 from lettuce to water,
- the water that is transferred with the lettuce is always refilled in order to keep a constant volume, no additional refreshing is applied,
- 1 mg/L free chlorine residual is maintained during washing.

Figure 7.3 shows that **the wash water contamination depends on the initial produce contamination, but the microbial logarithmic reduction is independent of the initial produce contamination.**

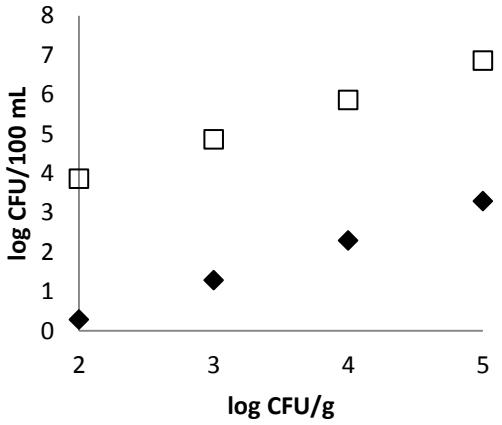


Figure 7.3. the *E. coli O157* wash water contamination after 3 h of processing at a rate of 5 kg produce/min and with varying produce *E. coli* O157 contamination,♦) 1 mg/L free chlorine residual, □) without disinfectant

Considering again the study by Luo et al. (2011) as example. They observed that dosing 10 mg/L of free chlorine resulted in absence of *E. coli* O157 cross-contamination. However, in that study, the lettuce was inoculated with 4 log CFU/g and washed in a process with a H_2O/produce ratio of 20. If the contamination was lower, a lower residual might have sufficed to prevent cross-contamination.

It can be concluded that choosing a disinfectant residual to enable sufficient probability of preventing cross-contamination should, among other factors, be based on identification of the worst case contamination event against which prevention is desired. **As such, cross-contamination studies should be executed, based on target produce contamination values that were declared through preceding risk analysis.**

7.4. IN A FRESH PRODUCE WASHING PROCESS THE ORGANIC LOAD, THE DISINFECTANT DEMAND AND RESIDUAL, AND THE WATER REFRESHING RATE ARE INTERCONNECTED

The problem of washing fresh-produce through immersion in water containing wash water disinfectants has often been studied through a single event, i.e. a single event of washing fresh produce in a batch of tap water or deionized water and mostly to assess the decontamination efficiency. Gil et al. (2009) give a good overview of this type of studies in the period 2002 to 2009. The problem with such a setup is that microbial build-up in function of time, disinfectant demand due to influence of organic matter, and the influence of water refreshing are not fully considered. The process of fresh produce washing with water disinfectants is determined by the physicochemistry of the wash water, the disinfectant demand and residual, and the water refreshing rate.

In chapter 2, sugar snaps were washed in a lab-experiment in tap water with chlorine or organic acids. To lower the APC on the sugar snaps the use of LA in the range 0.8 – 1.6 % was equally successful as 200 mg/L free chlorine. However, free chlorine was more effective to lower the APC in the water. When solely considering produce decontamination, one could argue there is no (only considering effectiveness and not disinfectant dose) difference in effectiveness of the washing processes with LA and free chlorine. However, with increasing wash water contamination, the chances of cross-contamination increase as well (Holvoet et al., 2014). As such, free chlorine tends to be a better water disinfectant for the sugar snaps washing process, as cross-contamination is inhibited to a higher extent.

As with the microbial load (considering continuous introduction of microorganisms), the continuous washing of produce **increases the physicochemical load of the wash water** as observed in chapters 2 to 4. In chapter 3, analysis of the wash water showed that the relative abundance of organic matter (measured as COD or UV_{254}) is much higher than the inorganic load (present as e.g. reduced iron or manganese). Therefore, the prime determinant of disinfectant decay, posed by the water matrix constituents, is the organic load. In chapter 3 it

was shown that free chlorine demand of the wash water is considerably higher than the PAA demand. This illustrates that **the influence of the wash water matrix needs to be considered when studying these processes**. On that note, the physicochemical build-up when washing a **whole produce** type, such as sugar snaps (chapter 2), is much lower than a **fresh-cut produce** process, such as fresh-cut leafy vegetables (chapter 3 and 4), as in the case of fresh-cut produce exudates from cut surfaces are introduced in the wash water. Also, the type of fresh-cut produce has an impact on the build-up of organic load (Selma et al., 2008a). The influence of pH on chlorine disinfection was already fully established before the start of this PhD research and as such controlled at pH 6.5 during the disinfection experiments. Concerning PAA, research has also established that somewhat better inactivation occurs at lower pH (Kitis, 2004). This was confirmed in this research, and the possible mechanism is the greater antimicrobial activity of the acid form of PAA, analogous to free chlorine (Kitis, 2004). In conclusion, the necessary disinfectant dose is not solely determined by the microbial resistance, but also by the disinfectant demand posed by the organic matter of the wash water matrix, and as such the physicochemical water quality is a parameter that needs to be considered in this research topic.

In fresh produce washing processes, some **water refreshing is applied**. The necessary disinfectant residual is a function of the antimicrobial activity of the disinfectant. However, the disinfectant dose is determined by not only the disinfectant demand of the water (i.e. the organic load), but also by the water refreshing rate. This can be illustrated with Figure 7.2. **The higher the necessary disinfectant residual, the higher the needed disinfectant dose per unit of water that is refreshed in order to cope with the water dilution effect.** From scenario analysis in section 7.2, it also became clear that water refreshing and process water recycling/reconditioning are not viable tools to maintain the microbial wash water quality.

The influence of pH, organic load, and water refreshing rate on the disinfectant demand, disinfectant residual and as such the disinfection effectiveness indicate that the value of research in this domain will increase when a holistic approach is applied, i.e. identification and consideration of all the most influencing factors.

7.5. ARTIFICIAL WATER SYSTEMS NEED TO BE VALIDATED WITH INDUSTRIAL WASH WATER

Artificial water systems were used to manipulate selected parameters at fixed values, as such allowing repetitions of the experiment and quantitatively evaluate variation in those selected parameters. However, the SPW in this research was made by diluting a stock solution. Therefore, the physicochemical parameters were interlinked and the identification of which parameter actually created the effect on the disinfection efficiency remained hidden. Validation in IPW of the reconditioning experiments with free chlorine and PAA (chapter 3) showed the pH effect on PAA disinfection and that COD dictated the free chlorine inactivation of *E. coli* O157 whereas UV_{254}(F) influenced the PAA decay (and as such the *E. coli* O157 inactivation). **Basically, the validation in IPW is necessary for disinfection experiments to identify the influential parameters of which the influence was quantified in artificial water systems (SPW or oxidant demand free buffer).**

The coagulation with chitosan (chapter 5) showed considerable difference in both the optimal chitosan dose (which was lower in IPW) and the COD removal efficiency (again lower in IPW). This exposed some other possible weaknesses of the artificial water, namely the fact that the SPW was created by destruction of butterhead lettuce whereas the organic load in IPW was predominately the consequence of exudates from cut surfaces of leafy vegetables, as such possibly containing less structural leafy vegetable compounds and more cytoplasm.

7.6. SOME WATER DISINFECTANTS ARE INADEQUATE FOR WATER DISINFECTION IN FRESH PRODUCE WASHING OPERATIONS

H_2O_2 should not be considered as a process wash water disinfectant/process water recycling disinfectant for fresh produce. It requires a too high residual coupled to a high disinfectant demand (chapter 4). Also, it is less effective than PAA in every regard, and just as H_2O_2, PAA does not produce significant amounts of harmful DBPs (chapter 1).

Organic acids are less effective than free chlorine for inactivation of suspended microorganisms and this with organic acid concentrations orders of magnitude higher than those of free chlorine. Also, at these organic acid concentrations decontamination occurs, which is in itself beneficial, but adverse effects on the sensorial quality of the sugar snaps did occur in some instances (Chapter 2).

UV disinfection requires close proximity to the lamps of the target microorganisms because of the intrinsic functioning of this technology. If the water layer passing the lamp is too thick, some of the water will pass through regions of lower UV intensity, resulting in exposure of the microorganisms to a lower UV dose (USEPA, 2006). The traditional washing tank designs do not facilitate such a close proximity of all the water to the lamps at all times, which is a prerequisite to prevent cross-contamination. As such, unless new washing tank designs arise that cope with this issue, UV disinfection can only be functional for process water recycling/reconditioning practices. In addition, because the UV_{254} absorbance in fresh-cut leafy vegetable processes is quite high (chapters 3 to 5), the delivered UV dose decreases severely, i.e. **fresh produce washing processes do not consist of a sequential treatment system that gradually removes particulate and dissolved matter to allow disinfection of the treated water as a final step such as in drinking or wastewater treatment. Disinfection in fresh produce washing processes occurs in suboptimal conditions.**

7.7. DESPITE KNOWLEDGE GAPS, A SELECTION TOOL AS GUIDE FOR END-USERS IS AT PRESENT A VIABLE TOOL FOR PRELIMINARY DECISION MAKING

Analysis of the impact of microbial water quality on food safety and food quality in irrigation practices, hydroponics, at harvest rinsing, and postharvest transporting, cooling, washing, and rehydration practices shows that water disinfection has its use in all these water related fresh produce production and processing practices (chapters 1 and 6). **Although the knowledge related to water disinfection practices is incomplete, important considerations have been identified and can be applied as a framework for preliminary decision making** (chapter 6). A variety of water disinfection technologies are currently available on the market but no single technology can be put forward as the perfect solution for all needs in water disinfection. It can be difficult for end users to choose the appropriate water treatment technology that is best fit for their purpose. Therefore, several water treatment technologies were characterized and selection criteria developed to support the fit for purpose judgement of their use for water disinfection in irrigation practices, hydroponics, and produce washing and cooling operations. All disinfection techniques have pros and cons, the appropriateness of the water treatment being dependent upon its cost-effectiveness. The inactivation effectiveness and the cost of disinfectant (disinfectant dose) is determined by the inherent microbial inactivation ability of the disinfection technique, the target microorganisms, the applied water refreshing rate, and the physicochemical parameters of the water source to be treated (section

7.4). Further associated costs are influenced by the capital investment of the technology, operational costs and the scale of operation. However, additional aspects such as maintaining a long-term successful disinfection operation, providing worker safety, possible adverse effects on the fresh produce and legal constraints need to be considered as selection criteria, as well as the impact of potential DBPs on human health, and the environment. The defined selection criteria can be applied by the end user as preliminary screening instrument in order to decide which technologies can be tested.

Consider the case of a farmer in a developed country. The farmer can use water from a creek to supplement rainwater use. Suppose the amount of surface water needed is limited. Microbial analysis of the surface water indicates presence of *Cryptosporidium* and *Giardia* spp. Physicochemical analysis showed turbidity of 10-20 NTU and a COD of 30-60 mg O_2/L. All considered technologies can be applied and as such there are no legal constraints. When considering the inactivation potential, the presence of *Cryptosporidium* reduces the possible technologies to O_3, UV, and membrane filtration (MF or UF should suffice). All technologies are negatively influenced by the physicochemical load, O_3 in inactivation efficiency, membrane filtration through fouling and UV through both, fouling resulting from both organics and inorganics such as iron or calcium. Nonetheless, all three technologies are expected to be able to provide the necessary disinfection efficiency, not considering the amount of chemicals or energy consumption it will require. The physicochemical load in this case is not particularly high, but a coagulation process coupled to a rapid sand filtration pretreatment would be beneficial for all three technologies, as e.g. a turbidity < 10 NTU is recommended for UV disinfection (USEPA, 2006). In addition, a rapid sand filter may remove protozoan organisms to some degree, although an optimized coagulation process would be necessary to achieve a 2 log *Cryptosporidium* reduction (Gitis, 2008). Regardless the possibility for additional microbial removal, particle removal would also reduce clogging of irrigation equipment. As it is a simple and low cost pretreatment, implementation would be advisable in all cases. The farmer is situated in a developed country and it is presumed that the remaining technologies can be delivered and installed by the manufacturer, that technical support and spare parts can be provided at all times and that electrical power is accessible. No chemical residual is generated with any of these technologies, so there is no adverse effect on the crops. However the lack of a residual implies that the irrigation piping should be regularly cleaned, as well as any recipients that serve as storage of disinfected surface water. As there are no further knock-outs (chapter 6), the three remaining technologies are evaluated further.

Regarding costs, O_3 and membrane filtration have a higher cost and require more expert knowledge than a simple UV unit, which is well suited for on-site, small scale treatment (Massé et al., 2011). As the farmer does not need to treat great amounts of surface water, such an investment might not be justifiable. Also, both membrane filtration and O_3 generators have higher and more complex maintenance requirements, which might require technical assistance. In addition membrane filtration creates a contaminated waste stream that must be treated with respect to the environment and ozonation might lead to production of bromate and bromoorganic DBPs in bromide-containing waters. There are some inherent safety issues, but proper design combined with periodic maintenance and creating awareness with the farmer could limit those. After evaluation of these technologies UV disinfection pretreated with a rapid sand filter seems best fit to be tested for this application, as it provides inactivation potential, is relatively low cost and has no environmental issues.

However, evaluating the selection criteria does not always reduce the number of technologies to one technology which is suspected to be best fit, e.g. a company wants to implement a disinfection barrier in order to eliminate the cross-contamination risk of bacterial pathogens when washing fresh-cut onions. The water is characterized by a quick accumulation of organic matter and turbidity and reaches high values (COD of 900 mg O_2/L and turbidity 70 NTU). The company is situated in a developed country. All technologies are allowed by legislation. Regarding inherent disinfection capacity, all considered technologies are able to inactivate vegetative bacteria efficiently, except for H_2O_2 which requires high concentration x contact time values. To guarantee the absence of cross-contamination, presence of a residual in the washing water is obligatory, not considering the actual needed concentrations. A process water recycling/reconditioning strategy does not operate *in situ* and as such cannot fully guarantee the absence of cross-contamination. This implies that the technique has to be a chemical disinfectant and therefore membrane filtration and UV disinfection are discarded. The negative impact of organic matter on the remaining technologies can be ranked as following on a general basis: O_3 > chlorine > ClO_2 > PAA. However, O_3 has the greatest inherent disinfection capacity of these technologies and through reaction it can significantly reduce the organic load of fresh produce washing water (Selma et al., 2008b). Based solely on maintaining residual, PAA can be expected to require the least amount of disinfectant. In theory, all four technologies will be able to avoid cross-contamination, it is only a matter of residual. Availability is not an issue for any of the technologies as infrastructure, technical assistance and electrical power are present. The potential for disinfectant concentrations,

necessary to maintain water quality, to exhibit adverse effects on the produce is limited, except in the case of PAA that requires a considerably higher residual. Studies concerning influence on respiration, enzymatic browning, microbial degradation etc. mostly utilized much higher residuals than necessary here because the goal was to decontaminate (Martinez-Sanchez, 2006; Allende et al., 2009; Vandekinderen, 2009; Tomas-Callejas et al., 2012b). Nonetheless, when testing a technology for process wash water disinfection, the potential impact on sensorial quality and shelf life should be assessed. Regarding costs, O_3 will require a higher capital investment than the other technologies. PAA has the highest operational cost per mass unit of disinfectant. O_3 and ClO_2 generators require more careful maintenance than the chlorine and PAA dosing units. All these factors contribute to the cost-effectiveness. **The relation between the selection criteria 'disinfection efficiency' and 'costs' is not sufficiently characterized due to the lack of data on comparison of wash water disinfectants.** Furthermore, there are some specific considerations for these technologies related to worker safety, the environment and their impact on the operation. Use of O_3, ClO_2 and gaseous chlorine will require ambient monitoring of concentrations in the working place to uphold worker safety. In addition the generation of O_3 may require some restructuring in the workplace or an enclosed space where the O_3 is generated. On that note, due to the rapid decomposition of O_3 through reaction with water matrix constituents in combination with its off-site addition, it might be technically difficult to maintain a consistent dosage. Therefore, O_3 might be better suited as reconditioning technique and less for maintaining a controlled residual in the washing bath. PAA has the least potential of producing DBPs, whereas chlorine, ClO_2 and O_3 can form primarily THMs, chlorite and bromate respectively. In cases where restrictions are placed on presence of DBPs in the washing water, the DBPs generation by chlorine will be impacted greatly by organic matter, which is high in this case. The same is valid for ClO_2, where reduction of ClO_2 leads to chlorite and chlorate formation. For O_3, the primary concern is the amount of bromide in the tap water. Applying the knockout selection criteria leads to four possible disinfection technologies. PAA seems to possess the least additional disadvantages related to worker safety, related issues, and DBPs formation. O_3 has the most issues from an operational point of view, and based on the criteria it seems to be the least suited for avoiding cross-contamination of the four technologies. **As no weighing factors are present to quantify the impact of the criteria, ultimately a solution will have to be found to a problem for which no single optimal solution is available because multiple criteria are involved. Multiple-criteria decision analysis techniques can be used to deal with this type of problem.**

7.8. FUTURE PERSPECTIVES

In the past five years, research concerning wash water disinfection has greatly increased. This because of the realization that produce decontamination does not seem to be a reliable tool to assure food safety, as well as the fact that avoiding cross-contamination via water during washing is a viable goal. Also, whereas the last two decades a lot of studies focused on the produce and did not incorporate the organic load of the wash water matrix, only recently the issues of disinfectant demand and DBPs production have come to the forefront of this research topic.

The research in this PhD thesis focused on the use of water disinfection in postharvest washing practices. To assess the suitability of water disinfection techniques for fresh produce washing processes, there is a need to close the existing data gap. This does not imply simply accumulating data concerning this research topic, but there is a need for comparative data, as there is virtually no comparative data on the cost-effectiveness of disinfection techniques in fresh produce washing processes. In addition, this comparative research needs to be executed from a holistic approach, i.e. assessing the parameters of most importance during a selected fresh produce washing practice, as have been identified in this PhD thesis. A possible setup for such research would be in a full-scale industrial washing process, assessing both fresh-cut and whole produce washing with free chlorine, PAA, ClO_2 and O_3, under conditions as similar as possible for each disinfectant. As such, factors related to cost-effectiveness could be quantitatively compared among the different disinfectant treatments. Issues related to managerial criteria should be documented, impacts on the produce quality observed and DBPs formation and possible transfer to the produce monitored. **By no means does the author imply that the search for and assessment of new efficient disinfection techniques is of no importance. However, it should not be the sole focus. A clear understanding of the cost-effectiveness of the currently implementable technologies that show potential in these washing processes is of paramount importance for industrial application.** Experiments such as those proposed here, require investment of a fair amount of time and resources and cooperation between the academic world and the industry. However, the author believes that the sum of these individual, interdependent experiments would yield a greater value for industry and governmental agencies than would the same number of individual studies, each executed by an independent research group, with unique experimental setups.

The results in this thesis insinuate that water refreshing has no significant impact on the microbial wash water quality. Virtually no research has been published on the accumulation of pesticides and mycotoxins in and possible cross-contamination via the wash water during fresh produce washing and this is an issue that should be addressed in future research. As water refreshing could be a strategy to mitigate the spread of these contaminants during washing processes, the outcome of this research would be one of the determining factors to evaluate the added value of water refreshing.

Chlorination has shown to produce considerable amounts of THMs in the wash water. However, transfer of significant amounts to the produce, when rinsed with tap water afterwards, has not been observed (chapter 3). As the constitution of fresh produce wash water is different from the natural waters that are used for drinking water and different from municipal wastewater, the types/amounts of produced DBPs could be quite different in fresh produce wash water. Up till know, only information about the formation of TTHMs due to chlorination in wash water from leafy vegetables and carrots has been published. Ultimately a detailed identification of the formed DBPs in these waters will be needed, not in the least because the possibility of harmful DBPs formation in fresh produce wash water is a highly debated issue and a barrier for allowing the use of water disinfectants in these postharvest practices. Concerning other water disinfectants that produce DBPs, such as ClO_2 or O_3, even less is known in the context of fresh produce washing and this should be researched. Even if it should be proven that there is no risk for the fresh produce consumer, the environmental considerations cannot be simply discarded. Whether a large and long-term DBPs production by this and other industries could lead to accumulation of DBPs in the ground water reserves (Bouwer, 2000), or lead to additional removal requirements when producing drinking water (e.g. from contaminated ground water) is largely unknown and complex to study and evaluate. Regardless (and as mentioned in section 6.7.5), although scarce at the moment, legal limits of DBPs in wastewater exist in the USA state of Florida for protection of the surface water quality and to counter the accumulation of DBPs in the environment (Hua & Yeats, 2010). If this is a prelude for DBPs restriction in wastewater, it is conceivable that sewage treatment levies will be invoked on DBPs in industrial effluents once such restrictions are imposed. The issues concerning the relation between DBPs and the environment that were discussed here are mostly based on speculation. However, this does not disprove the importance of these issues and environmental scientists should remain vigilant.

In the PhD thesis, washing processes have exclusively been focused on immersion washing baths. However, non-immersion washing systems (by spraying/rinsing with water) are also widespread. Some washing systems are preferred to others dependent on produce type. For example, high-pressure washers (i.e. spraying water at high pressure on produce) have been introduced to the citrus industry as a tool to effective remove insects, mold and soil from the fruit. In this way, insects are removed by the physical impact of the sprays, as such reducing the pesticide use in the field (Smilanick et al., 1999; Walker et al., 1999). In other cases, alternatives to immersion washers show potential of being more effective when compared with immersion washers. Combined chemical spraying with ClO_2 and physical brushing showed higher removal of *Salmonella* on tomatoes than immersion wash water disinfection with ClO_2 (Pao et al., 2009). It is conceivable that spraying techniques could have a lower contact time of water with produce before being collected and recycled, and that a volume of sprayed water potentially comes into contact with comparatively less produce. This could increase the feasibility of integrating process water recycling/reconditioning techniques into the washing system compared to immersion washing systems and could be an interesting research topic. On the other hand, as the amount of brushers, conductors and other food contact surfaces increases, cross-contamination via these surfaces could be a factor of more importance in the overall cross-contamination issue. It would be interesting for industrial users that more comparative research is conducted on the washing efficiency (microbial, chemical, removal of soil) of different washing setups for target produce types, focused on both the decontamination and wash water disinfection efficiency and with the goal of mitigating the overall cross-contamination. Finally, as all washing systems seem to have pros and cons, the search for new, ingenious washing systems should not be abandoned.

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CURRICULUM VITAE

CURRICULUM VITAE

Sam Van Haute, geboren op 19 februari 1985 te Kortrijk, studeerde af in 2010 als industrieel ingenieur: biochemie aan de Hogeschool West-Vlaanderen in Kortrijk. In september 2010 startte hij als doctoraatsbursaal binnen de Associatie UGent op het laboratorium voor Levensmiddelenmicrobiologie en –Conservering aan de Universiteit Gent en het Laboratorium voor Voedingsmicrobiologie en Biotechnologie aan de Hogeschool West-Vlaanderen (nu UGent Campus Kortrijk) onder het promotorschap van Prof. dr. Ir. Mieke Uyttendaele en Prof. dr. MSc. ing. Imca Sampers. Zijn onderzoeksproject kaderde in werkpakket 5: 'Impact of water quality and water treatment systems on the safety of fresh produce (pre- and post-harvest)' van het EU FP7 Veg-i-Trade project (Grant agreement no.: 244994).

Hij is auteur en co-auteur van wetenschappelijke publicaties en nam deel aan (inter)nationale symposia en congressen. Hij begeleidde bachelor-, en masterstudenten tijdens hun stage/eindwerk. Hij was betrokken bij enkele lessen inzake het 'International Training Program in Food Safety, Quality Assurance and Risk Analysis' georganiseerd door het laboratorium voor Levensmiddelenmicrobiologie en –Conservering en enkele lessen binnen de module 'Bioprocestechnologie' in de masteropleiding industrieel ingenieur: biochemie aan de Universiteit Gent Campus Kortrijk inzake water en waterdesinfectie in de voedselindustrie.

CURRICULUM VITAE

Sam Van Haute, born in Kortrijk February19th 1985, graduated in 2010 as industrial engineer: biochemistry at University College West Flanders. In September 2010 he started his PhD research at the Laboratory of Food Microbiology and Food Preservation of Ghent University and the Laboratory of Food Microbiology and Biotechnology of Ghent University Campus Kortrijk under the supervision of Prof. dr. Ir. Mieke Uyttendaele and Prof. dr. MSc. ing. Imca Sampers. The research was part of work package 5: 'Impact of water quality and water treatment systems on the safety of fresh produce (pre- and post-harvest)' of the EU FP7 Veg-i-Trade project (Grant agreement no.: 244994).

He is author and co-author of scientific publications and participated at (inter)national symposia and congresses. He was tutor of bachelor and master students during their thesis. He was involved in a few lectures concerning the 'International Training Program in Food Safety, Quality Assurance and Risk Analysis' organized by the Laboratory of Food Microbiology and Food Preservation of Ghent University and a few lectures in the course of 'Bioprocess technology' at Ghent University Campus Kortrijk.

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