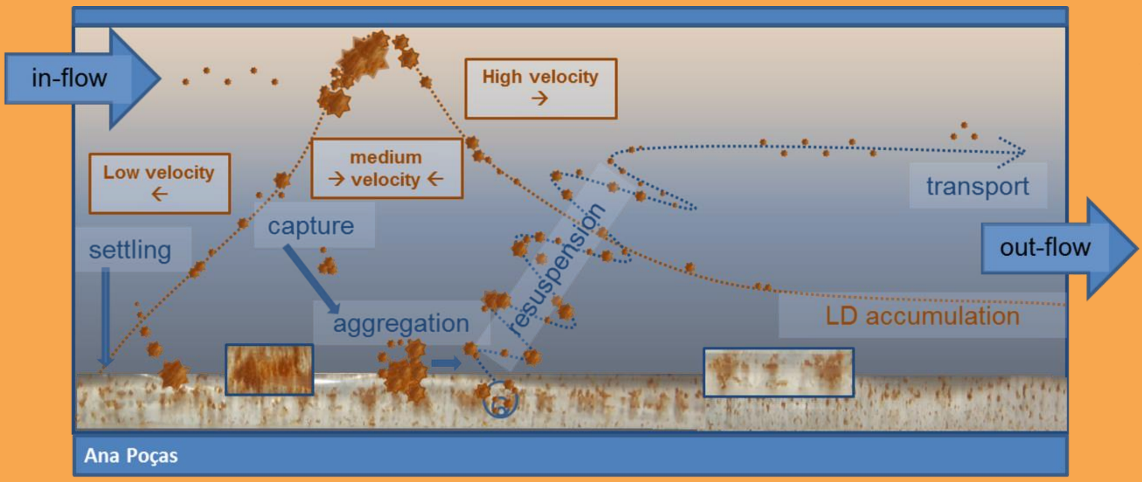
Discolouration loose deposits in distribution systems: composition, behaviour and practical aspects

Ana Poças



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Propositions

accompanying the thesis

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Ana Poças, 25 November 2014

1. For water companies, the low-velocity sampling is an opportunity to analyse the behaviour of loose deposits, investigate the possible origins of their constituents and interpret their build-up rates and profiles (this thesis).
2. Pipe cleaning frequencies need to be outlined with loose deposits’ online monitoring, rather than being mostly based on the historical data of consumer complaints (this thesis).
3. The apparent random accumulation of loose deposits in distribution pipes, including the transitory effects of discolouration, may be explained by their aggregation-disaggregation abilities. These allow loose deposits to travel (accumulate/resuspend) throughout drinking water networks whenever there are velocity changes (this thesis).
4. The loose deposits’ “terminology” needs to be revised, since “deposition” may not be the main process contributing for accumulation (this thesis).
5. The lack of standard methodologies in loose deposits control makes it difficult to compare literature data as well as to interpret data collected within the same network, but from different sampling sites and at different periods (Zacheus *et al.* 2001; Barbeau *et al.* 2005; Carriere *et al.* 2005; Vreeburg 2007).
6. Discolouration customer complaints may be used to track locations with recurrent problems for loose deposits’ resuspension. However, these locations may be different from those with enhanced potentials for loose deposits’ accumulation (experience in sampling loose deposits during an internship at Oasen Drinkwater, Gouda, The Netherlands, in 2007).
7. Drinking water systems need to be looked at as whole infrastructures, rather than as a number of different assets. For example, a pipe that is replaced upstream may lead to water quality deterioration at downstream parts in the network (recent experience in Asset Management, the iGPI project, 2013-2014).
8. Methods proposed by researchers need to be reproducible for data collection to be reliable, should be designed to test initial hypotheses, and should serve the objectives of those who will use them in the first place (research experience at LNEC, 2009-2014).
9. “Whenever a theory appears to you as the only possible one, take this as a sign that you have neither understood the theory nor the problem which was intended to solve.” (Karl Popper).
10. What appears to be an outlier in the present may turn to be the trend in the future.

These propositions are considered opposable and defendable and as such have been approved by the promotor and copromotor: Prof.Dr.ir. L.C. Rietveld and Dr.ir. J.H.G. Vreeburg.

Stellingen

behorende bij het proefschrift

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1. Voor de waterbedrijven is, "lage snelheid monsterneming" een kans om het gedrag van sedimenten te analyseren, de mogelijke oorsprong van hun componenten te onderzoeken en de accumulatiesnelheden en -profielen te interpreteren (dit proefschrift).
2. De frequentie van het reinigen van leidingen moet worden vastgesteld op basis van online monitoring van sedimenten in plaats van het gebruikelijke analyseren van historische gegevens van klachten van consumenten (dit proefschrift).
3. De schijnbare willekeurige accumulatie van sedimenten in distributieleidingen, inclusief de tijdelijke effecten op de kleur van het water, kan worden verklaard door hun vermogen tot aggregatie-desaggregatie. Dit zorgt ervoor dat sedimenten zich kunnen verplaatsen (accumuleren / opwervelen) door het gehele drinkwater netwerk telkens wanneer er snelheidsveranderingen optreden (dit proefschrift).
4. De terminologie "sedimenten" moet worden herzien, omdat "bezinking" misschien niet het belangrijkste proces is dat bijdraagt aan accumulatie (dit proefschrift).
5. Het ontbreken van standaardmethoden in het beheersen van sedimenten maakt het moeilijk literatuurgegevens te vergelijken en om gegevens, verzameld binnen hetzelfde netwerk maar op verschillende locaties en in verschillende periodes,te interpreteren. (Zacheus et al 2001;. Barbeau et al. 2005; Carriere et al 2005;. Vreeburg 2007).
6. Klachten van klanten over de kleur van het drinkwater kunnen worden gebruikt om locaties te identificeren met terugkerende sediment opwervelingsproblemen. Echter, deze locaties kunnen verschillen van de locaties met een verhoogde kans op accumulatie van voor sedimenten (ervaring in het bemonsteren van sedimenten tijdens een stage bij Oasen Drinkwater, Gouda, Nederland, in 2007).
7. Drinkwatersystemen moeten worden gezien als complete infrastructuren, en niet als een geheel van de delen. Zo kan een leiding die stroomopwaarts wordt vervangen, stroomafwaarts leiden tot een verslechtering van de waterkwaliteit in het distributie netwerk. (recente ervaring in Asset Management, de iGPI project, 2013-2014).
8. Door onderzoekers voorgestelde methoden moeten reproduceerbaar zijn om verzamelde gegevens betrouwbaar te maken, moeten beogen de oorspronkelijke hypothesen te toetsen en moeten de doelstellingen dienen van degenen die ze in de eerste plaats gaan gebruiken. (onderzoekservaring op LNEC, 2009-2014).
9. "Wanneer een theorie de enige mogelijke lijkt, is dit een teken dat je noch de theorie begreep , noch het probleem dat je wilde oplossen." (Karl Popper).
10. Wat in het heden op een uitschieter lijkt, kan in de toekomst de maatstaf worden.

Deze stellingen worden opponeerbaar en verdedigbaar geacht en zijn als zodanig goedgekeurd door de promotor en copromotor: Prof.Dr.ir. L.C. Rietveld en Dr.ir. J.H.G. Vreeburg.

Discolouration loose deposits in distribution systems: composition, behaviour and practical aspects

Proefschrift

ter verkrijging van de graad van doctor
aan de Technische Universiteit Delft,
op gezag van de Rector Magnificus prof. ir. K.C.A.M. Luyben,
voorzitter van het College voor Promoties,
in het openbaar te verdedigen op
dinsdag 25 november 2014, om 10:00 uur

door
Ana Isabel de Figueiredo Alves dos Reis POÇAS

Master Degree in Environmental Engineering (Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa)
geboren te Lisbon, Portugal.

Dit proefschrift is goedgekeurd door de promotor(en):

Prof. Dr. ir. L.C. Rietveld
Dr. ir. J.H.G. Vreeburg (copromotor)

Samenstelling promotiecommissie:

Rector Magnificus,	voorzitter
Prof. Dr. ir. L.C. Rietveld,	Technische Universiteit Delft, promotor
Dr. ir. J.H.G. Vreeburg*,	Wageningen University, copromotor
Dr. J. Menaia*,	Laboratório Nacional de Engenharia Civil (LNEC)
Prof. Dr. ir. J. Boxall,	University of Sheffield
Prof. Dr. G. Medema,	Technische Universiteit Delft
Prof. Dr. H.C. Flemming,	University of Duisberg-Essen
Prof. Dr. Ir. J.S. Matos	Instituto Superior Técnico (IST)
Prof. Dr. H. Vrouwenvelder,	Technische Universiteit Delft, reservelid

Dr. José Menaia and Dr.ir. Jan Vreeburg hebben als begeleider in belangrijke mate aan de totstandkoming van het proefschrift bijgedragen.

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Author: Ana Poças
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Ao meu filho João

Contents

1 Discolouration and loose deposits from drinking water distribution systems

1.1 Preamble

Water companies are keen on providing drinking water with quality levels complying with regulations while guaranteeing customers' confidence and satisfaction. For these reasons, online measurements and grab samples are collected, and the outcome results reported on a routine basis. The sole compliance with national or international regulations, however, may not be sufficient for guaranteeing that the water quality is always provided at the highest service levels, particularly when considering the lifespan of drinking water distribution systems (DWDS). In addition, water companies can hardly react, other than in a corrective manner, against some of the inconsistencies showing up during monitoring of DWDS (*e.g.*, pipes with different sediment build-up, places with recurrent discolouration episodes). For these reasons, and to enable water companies to act more preventively, a better understanding of the processes occurring behind such inconsistencies is required.

Discolouration events (Figure 1-1) are a problem that water utilities face on a daily-basis, as consequence of the resuspension of loose deposits (LD) that accumulate in DWDS. Current measures to prevent LD accumulation and resuspension include routine pipe cleaning and on-site intervention, *e.g.*, pipe replacement. These approaches, although useful to counteract extensive LD accumulation, are insufficient to understand LD characteristics at more detailed levels and, in turn, to always prevent tap water discolouration.

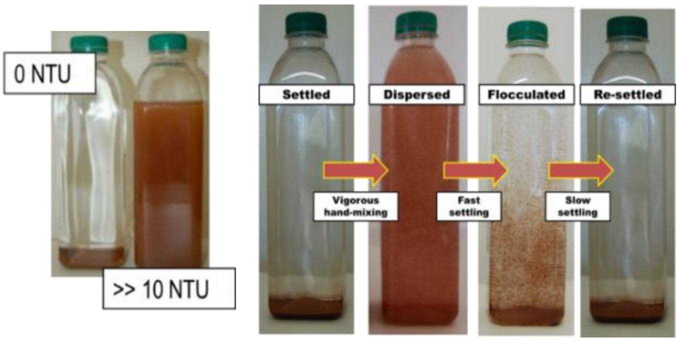

Figure 1-1. Discolouration events: settling and resuspension.

1.2 Background

1.2.1 LD composition and accumulation

Tap water discolouration incidents may occur due to the resuspension of LD that accumulate in DWDS, in case of velocity increases or changes (Vreeburg 2007). Such incidents may be the first reason of customer complaints (Boxall et al. 2003, Husband and Boxall 2011), especially if recurrent. In addition, they may be related with degradation of the microbiological quality of the water (Zacheus et al. 2001, Lehtola et al. 2006) and to the transport of contaminants (Peng and Korshin 2011) while being reported in all DWDS, including those where cast-iron pipes are absent (Vreeburg et al. 2008) and in "particle-free" waters, (Vreeburg and Boxall 2007), either if chlorinated or not. Despite that, discolouration occurrence has been associated with cast-iron pipes' corrosion, and LD to its derivatives (Smith et al. 1997, Benson et al. 2012).

Apart from corrosion, LD may be originated (Figure 1-2) in the catchment water, be added at the treatment plant, be produced at the distribution network, due to pipe scouring (Smith et al. 1997, Gauthier et al. 1999, Gauthier et al. 2001a) and through biofilm release (Zacheus et al. 2001, Batté et al. 2003), or enter DWDS during pipe rehabilitation or repair (Vreeburg 2007).

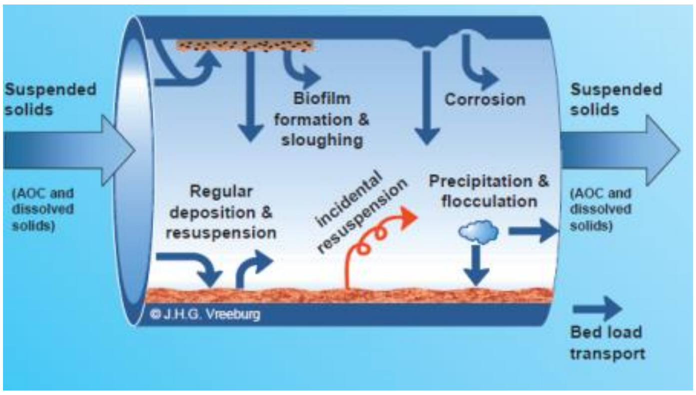

Figure 1-2. Particle-related processes in network pipes (Vreeburg 2007).

In addition, and despite of the multiple sources (Figure 1-2), or the presence of residual disinfectants, LD composition is typically composed of oxidized iron forms and organic compounds, *i.e.*, volatile solids-VS (Gauthier et al. 1999, Vreeburg and Boxall 2007), which may sum up to 70% and 65% of LD dry weight, respectively (Gauthier et al. 1999, Gauthier et al. 2001b, Zacheus et al. 2001, Vreeburg 2007, Husband and Boxall 2011).

Furthermore, oxidized iron forms may occur due to the oxidation of Fe^{2+} by dissolved oxygen and disinfectants and to post-precipitation of Fe^{3+} (Stumm and Morgan 1981), or be added downstream, at the distribution network, as result of cast-iron pipes' corrosion (Benson et al. 2012). Relatively to VS components, these may be released during growth in treatment filters or pipe-wall biofilms (Batté et al. 2003, Lehtola et al. 2004), including extracellular polymeric substances-EPS (Douterelo et al. 2012, Liu et al. 2013a). Alike in pipe-wall biofilms (Simões et al. 2007, Ginige et al. 2011) or in iron-rich sediments from freshwater environments (Gerbersdorf et al. 2008, Plach et al. 2011, Elliot et al. 2012), EPS may be added to LD-VS and contribute to LD accumulation. Likewise, through LD accumulation, EPS may get involved in organic-mineral associations (Liu et al. 2013b), such as iron-binding (Knicker and Hatcher 1997), and provide sorption (Gerbersdorf et al. 2009) or flocculating abilities (Flemming and Wingender 2001, 2010) to the LD.

Given the EPS properties and structural role of hydrogel-flocs of EPS-Fe occurring in freshwater environments, other unknown features of LD from DWDS are foreseen, including their water holding capacity, fractal structure (Johnson et al. 1996, Vahedi and Gorczyaca 2012) and flocculent behaviour (Plach et al. 2011, Elliot et al. 2012).

1.2.2 LD microbial significance

The guarantee of the microbial stability of the water may be achieved by managing chlorine levels throughout the networks or by controlling the assimilable organic carbon

(AOC) levels in the water. The microbial quality of drinking water may, however, deteriorate from source to the tap as consequence of many factors, such as depletion of residual disinfectants, extended residence times (Manuel et al. 2007), increased AOC levels (Flemming et al. 2002, van der Kooij 2003, Lehtola et al. 2004) or LD accumulation (Zacheus et al. 2001, Batté et al. 2003, Liu 2013).

Owing to the their richness in VS, LD may be considered as prime-sites for microbial colonization and growth (Gauthier et al. 1999, Batté et al. 2003, Lehtola et al. 2004, Liu 2013), including to the hosting of pathogens, *e.g.*, Legionella spp. (USEPA 2001). According to Zacheus et al., (2001), LD bacterial counts in old deposits may be 220 times higher than in the bulk water, and in new deposits 80 times higher than in old ones. Similarly, Liu, (2013) observed that the type of bacteria present within the LD may vary during accumulation while highly dependent on the amount of LD used for characterisation. Accordingly, a conceptual model highlighting the ecological importance of LD, including the possible interactions within particle associated bacteria, was proposed (Figure 1-3).

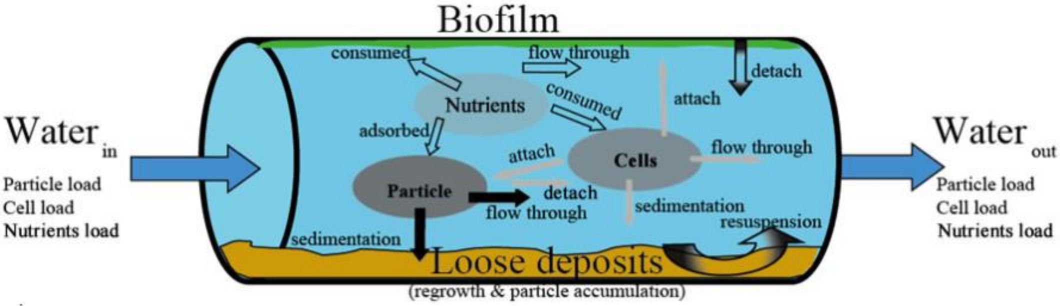

Figure 1-3. Particle accumulation and growth in network pipes (Liu et al. 2013a).

Surface-associated bacteria may represent 95% (Flemming et al. 2002) to 98% (Liu 2013) of the total bacteria in DWDS. This may be due to the favourable conditions for microbial survival and growth at/near the pipe-wall, including protection against residual disinfectants and shear stresses, to LD accumulation (Lehtola et al. 2004), as well as vary with pipe material (van der Kooij and Veenendaal 2001). Within the EPS that are released by bacteria during growth (Flemming et al. 2007, Manuel et al. 2007, Flemming and Wingender 2010), pathogenic bacteria and indicator microorganisms may also grow protected from residual disinfectants or detachment, and further contribute to restrain LD resuspension. Furthermore, anoxic and anaerobic microenvironments may favour the accumulation and growth of different types of bacteria (Li et al. 2010), including iron oxidizing bacteria-FeOB (Emerson et al. 2010). These bacteria, that produce stalks or sheaths with incrusted iron oxides during growth (Ridgway et al. 1981, de Vet et al. 2011) may, therefore, contribute to increased iron and EPS levels in DWDS (Shair 1975, Fredrickson et al. 1998, Li et al. 2010). FeOB, *e.g.*, chemolithoautrotrophic Galionella spp., apart from having a role in discolouration (Ridgway et al. 1981), may also produce AOC, thus allowing for higher potentials for microbial growth and regrowth in DWDS (Lehtola et al. 2004).

Unlike their pipe-wall counterpart, studies on LD microbiology are scarce. Similarly, the affinity of bacteria towards LD with different characteristics, such as LD age

(Zacheus et al. 2001), needs to be further investigated, as well as the interpretation on potential health hazards of tap water discolouration, in order to outline possible measures to counteract microbial proliferation in DWDS.

1.2.3 LD build-up and formation mechanisms

Higher accumulation levels of LD in network pipes are usually associated to low-flow circumstances, which may occur due to the oversized diameters and looped designs that typically characterise DWDS (Vreeburg and Boxall 2007). Extensive LD accumulation could be effectively restrained by guaranteeing daily peaks of *ca.* 0.4 m/s or higher, in accordance with the "self-cleaning" principles (Vreeburg et al. 2009b, Blokker 2010, Husband and Boxall 2011). However, these criteria may be difficult to guarantee in most of the existing networks, owing to the oversized diameters and looped designs. Alternatively, pipe cleaning programmes may need to be monitored over time (Vreeburg et al. 2009) by using, for example, online methodologies such as the resuspension potential method (RPM) or particle counting (Verberk et al. 2007, Vreeburg and Boxall 2007). Taking into account the difficulties for the interpretation of LD in DWDS (Vreeburg 2007, Husband et al. 2008, van Thienen et al. 2011, Liu 2013), pilot scale measurements may be also used to study LD accumulation and build-up profiles. Even if flow conditions occurring in DWDS are difficult to replicate at pilot scale, experiments have suggested that LD accumulation may be reported over just a few weeks of supply and that it may be faster at steady-state flow conditions (Husband et al. 2008, Husband and Boxall 2010). Relatively to LD build-up profiles, rather than only occurring at the pipe bottom, studies have also shown that LD may accumulate at the overall pipe surfaces (Husband et al. 2008, van Thienen et al. 2011).

Overall, the processes leading to differences in LD accumulation in pipes from different district meter areas (DMAs) are not completely understood yet. Wider knowledge on LD accumulation profiles and rates in DWDS, as well as on the effects of cleaning in the mid and long terms, may be helpful to interpret and prevent tap water discolouration.

1.3 Research topic

The main objective of the thesis is to contribute to a better understanding of the possible origins and development processes underlying tap water discolouration. With that purpose, qualitative and practical approaches were used for collecting LD samples from distribution networks, *i.e.*, methodologies that may be used together with daily routine procedures by the water companies to prevent and interpret LD accumulation. To investigate the origins of LD, samples were subjected to physical-chemical characterisation, not only for their typical components (Fe, VS), but also for some organic constituents (*i.e.*, EPS). To study LD behaviour, LD observations were followed by stereoscope and microscopy visualizations, as well as turbidity decrease measurements. Studies on LD build-up were assessed with pilot scale experiments, for better knowledge on the LD formation processes taking place at DWDS. The microbiological relevance of discolouration LD was also evaluated, including the possible role of bacteria in LD accumulation, in order to appraise the possible effects on water quality upon LD resuspension.

Thus, this thesis aims to improve knowledge on the “whys” and “hows” of discolouration events in DWDS. More specifically, emphasis was given to understand how such light particulates may accumulate and resist to the shear stresses occurring in DWDS, before LD resuspend at one time and cause tap water discolouration. Accordingly, the research questions of this research were the following:

- to develop a methodology for LD representative and practical sampling in DWDS, to enable LD collection by water companies on a routine basis;
- to apply the developed methodology for LD physical-chemical characterisation and behaviour studies, in order to interpret LD occurrence in DWDS;
- to study the influence of physical-chemical characterisation in LD build-up and formation processes, in order to better understand LD accumulation rates and profiles;
- to investigate the potential of LD to harbour and transport bacteria, including the possible role of bacteria in LD formation, as well as the affinities of bacteria to LD leading to tap water discolouration;
- to develop a conceptual model to study LD dynamics, build-up processes and resuspension behaviours in DWDS, for helping water companies to prevent LD accumulation.

1.4 Thesis’ outline

Next to this introductory chapter, the thesis was divided in seven main chapters.

In Chapter 2 - *Methodology for sampling drinking water discolouration loose deposits at low-velocities*, the developed methodology for sampling and characterising LD from DWDS was introduced and described. The generally used methodologies to collect LD from drinking water networks were discussed within the scope of sample feasibility and representativeness. LD characterisation results were then compared with those published elsewhere. While assumed to be more representative of LD that may lead to water discolouration, despite looking more at the qualitative aspects of LD accumulation, the applied methodology enabled extensive sample analysis and testing, and may be used for LD collection by water companies on a routine basis.

In Chapter 3 - *Hydrogel floc nature and biogenic constituents of drinking water discolouration deposits*, the genesis and role of LD main constituents – organic fraction and iron, were examined. The possible origins of LD in DWDS were identified. Comparison of LD with those occurring in freshwater environments, and that resemble LD in composition, was performed. The presence of biogenic constituents as relevant constituents of LD flocs was demonstrated. Overall, it was suggested that LD leading to tap water discolouration are better described as fractal hydrogel flocs than as “true particles”, *i.e.*, with defined limits and sizes.

In Chapter 4 - *Pilot studies on discolouration loose deposits build-up*, the test rigs used to monitor the development and build-up rates of LD were described. The usefulness of monitoring LD occurrence and formation mechanisms to better understand discolouration was explored. By using transparent test rig pipes in a flow-through mode supplied with drinking water, LD build-up rates and profiles were followed and reported. It concluded by suggesting LD capture, followed by aggregation, may be an important process for LD build-up in DWDS, apart from LD settling.

In Chapter 5 – *Total heterotrophic bacteria in loose deposits from drinking water distribution systems*, the possible effects on the microbiological quality of drinking water upon LD resuspension were investigated. The characteristics that identify LD as prime-sites for microbial attachment and growth were described. The affinities of heterotrophic to discolouration LD with different behaviour were studied. Viable FeOB were found in LD, thus supporting a possible biogenic role in LD formation. Likewise, FeOB growth may support microbial growth/regrowth in DWDS, *e.g.*, in the presence of chemolithoautrotrophic bacteria. While the affinity of bacteria to LD may be different for LD with different characteristics, it concluded by showing some of LD particularities that can favour their affinity towards bacteria.

In Chapter 6 – *Discolouration loose deposits – balancing views and practices*, a conceptual model for LD build-up and resuspension behaviours was proposed. While resulting from the balance between settling, capture/aggregation and resuspension, LD may be formed and accumulate at different rates in different parts of the networks. It concluded by showing LD build-up and resuspension in DWDS may be governed by dynamic processes that may change over LD accumulation, rather than localised events and causes. Possible hints on LD control and prevention, including discolouration LD minimisation were, then, suggested and discussed in terms of applicability and practicability.

In Chapter 7, the overall conclusions of the thesis were presented.

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2 Methodology for sampling drinking water discolouration loose deposits at low-velocities

Abstract

The occurrence of discoloured water at the tap is one of the most frequent reasons for customers to complain. Water discolouration arises from the resuspension of loose deposits (LD) that accumulate in drinking water pipes, due to velocity increase. Because of its typical brownish/reddish colour, discoloured water is traditionally perceived as a consequence of cast-iron pipes corrosion, even in networks devoid of cast-iron pipes. Current approaches for LD sampling imply the use of high flow velocities within the range of those used for pipe self-cleaning (0.40 m/s) and/or pipe cleaning (1.50 m/s). Although useful for quantitative identification of critical zones and diagnosis of LD accumulation, the application of such high-velocities as routine methodologies for LD sampling may be difficult to perform on-field and provide samples with materials other than discolouration causing LD. In this study, sampling velocities from 0.02 m/s to 0.52 m/s yielded relatively large samples (up to 2 g dry weight), thus allowing for extensive qualitative LD analysis and testing. Sample representativeness was analysed through the characterisation of LD key-constituents total iron (Fe^t) and volatile solids (VS). Turbidity decay trends showed the flocculent behaviour of discolouration causing LD.

*Adapted from:

Poças, A., Rebola, N., Cordeiro, B., Rodrigues, S., Benoliel, M.J., Vreeburg, J. and Menaia, J., 2013. Methodology for sampling drinking water loose deposits at low velocities. Water science & technology: water supply 13(4), 1116-1122.

2.1 Introduction

The appearance of discoloured water at the consumers tap is one of the most frequent causes for dissatisfaction and customer complaints (Boxall et al. 2003, Husband and Boxall 2011), especially if recurrent. Consumer dissatisfaction arises from the perception of changes in the water aesthetics, namely the appearance of turbidity or yellow-brownish colour and, in some cases, taste and odour. LD accumulation occurs in every drinking water distribution system (DWDS) and may lead to discoloured water appearance at the tap of the consumers, in case of LD resuspension due to hydraulic perturbations, namely flow increases (Vreeburg and Boxall 2007). Tap water discolouration may also be accompanied by degradation of the microbiological quality of the water (Zacheus et al. 2001, Lehtola et al. 2006) and to the transport of contaminants (Peng and Korshin 2011).

LD in DWDS may have different origins (Vreeburg 2007), including particulates that escape treatment or those released from pipe corrosion, scaling and/or biofilm sloughing, or that result from intrusions of soil or dirty water during pipe repair or rehabilitation. Particles that form and grow in DWDS bulk water by chemical precipitation and flocculation of dissolved and colloidal materials may also be included (Smith et al. 1997, Gauthier et al. 2001, McNeill and Edwards 2001, Batté et al. 2003, Husband and Boxall 2011). Nevertheless, and regardless of the sources and/or the upstream particle-generating processes, LD are mainly, and typically, constituted by volatile solids (up to 65% in dry weight) and oxidized iron forms (up to 70% in dry weight). For this reason, discolouration incidents are generally attributed to cast-iron pipes corrosion (Smith et al. 1997, McNeill and Edwards 2001, Vreeburg and Boxall 2007, Benson et al. 2012), even if discolouration occurs in networks devoid of cast-iron pipes (Vreeburg et al. 2008), irrespectively of the upstream water characteristics or the presence of residual disinfectants (Gauthier et al. 1999, Zacheus et al. 2001).

Accordingly, LD oxidized iron forms may arise from origins other than cast-iron pipes corrosion. The organic fraction composition has not been clearly characterised yet, but it may also originate from different sources. Therefore, the analysis and identification of LD constituents are necessary for understanding LD sources and for the interpretation/mitigation of the discolouration phenomena. The mass and rate of LD accumulation in drinking pipes may be approached for many DWDS locations by using sampling methods such as the Resuspension Potential Method – RPM (Vreeburg and Boxall 2007) or dedicated flushing (Lehtola et al. 2004, Carriere et al. 2005). These methodologies usually imply the use of high flow velocities for sediment removal, *i.e.*, within the range of those used for pipe self-cleaning (0.40 m/s) and/or pipe cleaning (1.50 m/s). These methodologies, although useful for quantitatively identifying critical zones for LD accumulation, solely, do not provide information on the physical-chemical and rheological characteristics of discolouration-causing LD, which may be indicative of their origin and behaviour.

The qualitative characterisation of LD is required for understanding their genesis, transformations and transport, which, in turn, are crucial for the conception and implementation of measures to mitigate their occurrence, accumulation and resuspension. Looseness and, concomitantly, the potential for being easily resuspended and transported along DWDS are distinctive properties of discolouration causing sediments (*i.e.*, LD), thus, fixed and not easily disturbed sediments must be excluded from LD samples. Accordingly, no extreme hydraulic (*e.g.*, flow velocities above normal circumstances) or mechanical (*e.g.*, pigging) conditions should be applied for

LD collection and, when unidirectional flushing is applied, water velocities need only to be high enough to resuspend and transport LD as selectively and extensively as practicable, *i.e.* within the range of the daily-flow velocities that lead to tap water discolouration. However, no established methodology has been developed satisfying such sampling conditions (Vreeburg 2007) and, with a few exceptions (Gauthier et al. 1999, Barbeau et al. 2005, Carriere et al. 2005), published studies do not fully describe the methodology used for LD sampling.

In this chapter, the development, evaluation and application of a low-velocity (*i.e.*, < 1.0 m/s) methodology for representative qualitative sampling of LD, at the amounts required for physical-chemical characterisation, is described. Results on total iron (Fe^t) and volatile solids (VS), the LD major constituents (Gauthier et al. 1999, Gauthier et al. 2001, Zacheus et al. 2001, Carriere et al. 2005), in LD samples from the Lisbon DWDS are presented and discussed in the scope of sample collection suitability and representativeness.

2.2 Materials and methods

2.2.1 Distribution network

Thirty (30) LD samples were obtained from the Lisbon DWDS, which is owned and run by Empresa Portuguesa de Águas Livres, S.A. (EPAL). The system supplies *ca.* 520 000 inhabitants at a daily rate of *ca.* 160 000 m^3, through 1 427 km of main pipes with *ca.* 93 000 connections. Pipes materials include asbestos cement (AC ~29 %), ductile iron (DI ~28%), cast-iron (CI ~19 %), high-density polyethylene (HDPE ~17%) and ~7% of the length is not identified in material. The DWDS water is mostly of surface origin (86%), with a minor contribution from wells. Surface water undergoes conventional treatment, with pre-chlorination, coagulation using aluminium sulphate as coagulant, rapid sand filtration, and chlorination. Water from wells undergoes chlorination only.

2.2.2 Sampling locations

Samples were obtained from unidirectional pipe routine discharges of hydrants during routine pipe cleaning, along with the water company maintenance procedures. Sampled locations were randomly distributed throughout the DWDS and included looped and dead-end pipes of different materials (Table 2-1).

Table 2-1. Sampled pipes location, material and age.

Pipe characteristics	**Number of pipes per material**			
	Asbestos Cement	Cast-iron	Ductile iron	High density polyethylene
Dead-end	4	2	5	10
Looped	1	3	2	3
Total	5	5	7	13
Age (min to max years)	30 to 40	58 to 73	13 to 20	2 to 9

2.2.3 LD sampling

Relatively large volumes (30 or 63 L) of the discharged waters were collected for LD sampling to assure sufficient material for analysis. Volumes were chosen prior to sampling and based on the location's discolouration background, *e.g.*, 30 L for the pipes with more sediment and 63 L for those with less, and on results from preliminary experiments. Sampled volumes were those needed to provide sample representativeness and transport and storage (7 days at 4ºC) practicability (< 100 L).

For collection, a connector pipe was attached to the hydrant and at some locations calibrated flow-meters were used, whilst at other instances, pipe water velocities during sampling were calculated upon flow rate estimates obtained by measuring of the collecting vessel filling rates. To prevent sampling of the stagnant water from the hydrants and the connection pipes, waters discharged during the first 10-30 s were rejected (approximately 2 to 4 times the hydrants plus the connection pipes' volumes). As a rule, these rejected volumes corresponded to >15-20 L of stagnant water.

This methodology has been used during routine pipe discharges for LD removal. These routine discharges are used to drain the pipes out for the time period necessary to get a clear water front, stabilized low turbidities and free chlorine levels within the targeted ranges (in this case: 0.2 - 0.6 mg/L). The pipe discharge sites are usually chosen with basis on the historical data of complaints, recurrent LD accumulation and online monitoring results. Since flow velocity is not a controlled parameter, but function of the hydrant opening, the levels of perturbation at different locations can be different. The objective was to collect as many discolouration samples as possible at the daily flow velocities, regardless of the imposed velocities needed to reproduce discolouration. Samples collected under these circumstances may be more representative of the daily discolouration events than with traditional cleaning because of excluding material that can be collected but it is not contributing to discolouration.

2.2.4 Discharged waters

For characterisation of the discharged waters, total suspended solids (TSS), turbidity and conductivity were determined in aliquots of homogenised discharges water in the laboratory.

2.2.5 LD separation

For LD separation, discharged water samples were decanted for 7 days at 4ºC in the cylindrical containers used for sampling. The 7 day settling period was not random, but obtained from observations made during the settling of sampled waters with the highest turbidity levels. Then, after outdrawing the supernatant water with a peristaltic pump at flow rate of 6 L/h without disturbing the settled material, 1 L to 3 L of LD enriched slurry were obtained. The slurry was used for LD characterization directly or, in some cases, after further concentration by extended settling. The solids' dry weight in the analysed slurries was *ca.* 300 times that of the corresponding discharges, *i.e.* total solids' in samples were concentrated within 91% and 99%. The use of gravitational separation, instead of other physical methods like filtration, was chosen in order to preserve LD shape integrity and size distribution, which could affect the deposits behaviour in the settling pattern studies. Gravitational settling was only used for LD separation, as LD unlikely accumulate by gravitational forces under the turbulence regimes of DWDS.

2.2.6 Turbidity measurements

The turbidity of water discharges and slurries was measured in 25 mL borosilicate glass cells, with a pre-calibrated nephelometer (Turb 555 – 600100, WTW). The study of LD settling behaviour was done with LD slurries suspensions, with turbidity decays registered at every 5 s. To study the reversibility of LD settling patterns, samples were re-dispersed three times by vigorous hand-shaking.

2.2.7 Physical characterisation

Conductivity was determined with a multi-parameter potentiometer (Consort, model C863) and TSS were determined gravimetrically by the membrane filtration method, according to Standard Methods (APHA 1998). Dry weight mass (total solids, TS) and VS contents were determined by drying (110ºC) and igniting to 550ºC for 1 h, until constant value was reached (APHA 1998).

2.2.8 Chemical characterisation

Fe^t was analysed in HCl (6 M) digests of the ashes obtained from VS determination (Räisänen et al. 1992). 6 mL of acid were added to the ashes (~5 mg) and let to react at 121ºC for 1 h. Samples were then filtered in 0.20 µm pore size polycarbonate membranes (Millipore, GTTP04700). Fe^t was determined by the phenantroline method (APHA 1998) in dilutions of the filtrate in distilled water. Hydroxylamine (hydroxylamine ammonium chloride, Merck, p.a.) was used to reduce the Fe^{3+} ion to Fe^{2+}. Fe^t was determined at 510 nm with a UV/VIS spectrophotometer (Jasco, V-630 LSE-701).

2.3 Results and discussion

2.3.1 Sampling velocities and discharges turbidity

Results obtained from the physical-chemical characterisation of LD samples from locations distributed throughout the Lisbon network are summarised in Table 2-2.

Table 2-2. Discharges characteristics, total mass sampled from the pipes and LD composition. (NA=not analysed).

Site	Material	Ø	Vel.	Turb.	pH	Conduct.	TSS	$\text{Total}_{\text{mass}}$	VS	Fe^{t}
		mm	m/s	NTU		µs/cm	mg/L	g	mg/g	mg/g
1	AC	200	0.03	91	7.7	247	51.0	1.530	110.0	576.9
2	AC	200	0.05	12	7.0	117	2.3	0.068	130.0	593.2
3	AC	125	0.16	1	7.8	291	2.3	0.070	101.4	366.6
4	AC	100	0.25	31	8.0	248	28.4	0.851	127.5	610.0
5	AC	125	0.37	28	7.4	354	10.8	0.325	129.6	484.2
6	CI	300	0.02	31	8.0	248	28.4	0.851	90.0	734.0
7	CI	125	0.03	7	7.6	NA	4.7	0.141	183.3	536.4
8	CI	200	0.35	7	7.5	360	6.0	0.179	173.1	499.9
9	CI	80	0.35	47	7.4	449	41.3	1.238	170.0	452.4
10	CI	125	0.52	7	7.5	429	5.0	0.150	83.3	805.1
11	DI	200	0.02	39	7.4	375	31.4	1.978	161.5	276.1
12	DI	150	0.04	18	7.7	265	13.0	0.390	155.6	270.3
13	DI	200	0.06	6	11.0	541	2.6	0.077	180.0	284.7
14	DI	125	0.12	1	7.9	304	31.1	0.933	256.4	567.4
15	DI	150	0.36	10	7.6	429	2.1	0.135	148.1	445.5
16	DI	150	0.36	6	7.2	NA	12.1	0.763	108.4	272.2
17	DI	150	0.36	8	7.1	283	5.8	0.364	95.2	419.7
18	HDPE	200	0.03	10	7.3	217	4.1	0.259	40.8	660.2
19	HDPE	160	0.03	32	7.9	457	8.6	0.258	387.1	365.3
20	HDPE	160	0.03	1	7.4	217	0.5	0.031	111.1	NA
21	HDPE	160	0.04	4	7.7	259	2.3	0.147	33.7	636.0
22	HDPE	160	0.05	53	7.4	290	13.2	0.396	183.3	637.3
23	HDPE	160	0.06	15	7.4	280	4.3	0.128	95.2	489.7
24	HDPE	160	0.06	6	7.8	166	3.8	0.113	140.0	376.3
25	HDPE	110	0.07	51	7.9	466	19.2	0.576	152.8	476.9
26	HDPE	200	0.08	3	7.3	112	1.8	0.053	270.0	326.2
27	HDPE	110	0.09	2	7.6	272	0.8	0.052	440.0	NA
28	HDPE	200	0.20	13	7.6	406	13.7	0.861	90.9	375.0
29	HDPE	110	0.21	1	7.9	304	31.1	0.933	411.8	376.3
30	HDPE	160	0.32	3	7.5	428	6.6	0.413	208.3	466.6
		average		18	8	315	13	0.48	166	478
		stdev.		21	1	108	14	0.49	99	144

Although samples were collected from waters differing in their origins makeup, as indicated by the spanning of their conductivity values (112 µS/cm to 541 µS/cm), discharged waters turbidities did not relate to the sampling flow velocities (Figure 2-1). In fact, high turbidities were observed at every tested velocity range, irrespectively of the water or pipe characteristics. Therefore, even if the perturbation levels were different within the sampled pipes, relatively low velocities (≥ 0.02 m/s) were effective in resuspending LD during sampling from DWDS pipes and may, likewise, trigger discolouration.

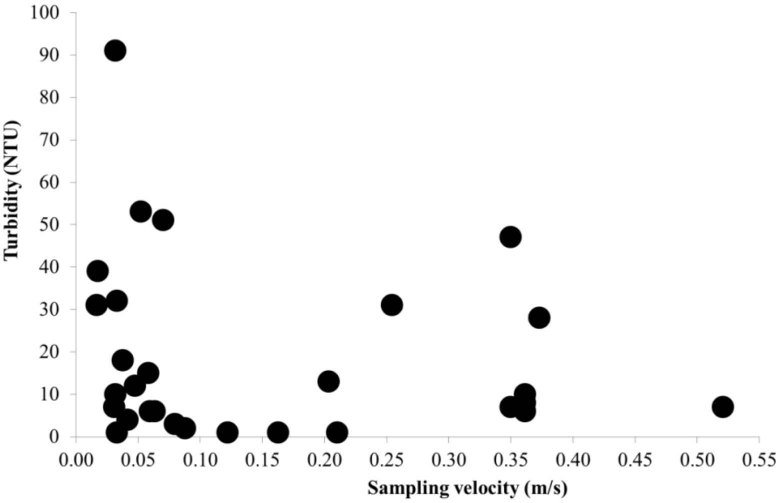

Figure 2-1. Discharged water turbidities versus pipe water velocities during sampling.

Most discharges with clearly perceptible discolouration (>10 NTU) yielded LD samples (Table 2-2) at amounts (up to 2 g dry weight) and allowing for extensive LD physical and chemical characterisation. Except for two samples with TSS levels lower than 1 mg/L, even the less discoloured waters (< 10 NTU) yielded samples (0.294 g ± 0.322 g) suitable for Fe^t and VS determinations (Table 2-2).

On average, VS and Fe^t were about 17% and 48% of the total LD dry weight mass, respectively. The importance of VS and Fe as the prevailing components of LD closely resembled those reported for LD from other chlorinated and non-chlorinated DWDS (Gauthier et al. 2001, Zacheus et al. 2001, Vreeburg and Boxall 2007).

2.3.2 Flow velocity and LD composition

As Figure 2-2 shows, LD contents in Fe^t and VS exhibited significant similarities, even if sampled at flow velocities that spanned over more than one order of magnitude (0.02 m/s to 0.52 m/s). This may be explained by the fact that relatively low velocities are effective in LD sampling, at least in pipes with comparable perturbation levels during pipe routine discharges.

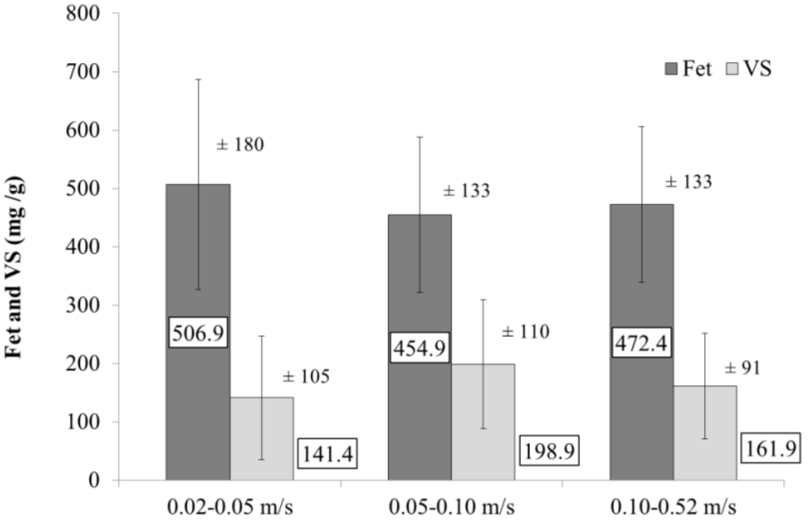

Figure 2-2. Fe^t and VS contents in sampled LD (mg/g). Average values in boxes and error bars representing standard deviations.

The levels of Fe^t and VS were similar in sampled LD, irrespectively of sample location within the network (*i.e.*, travelling distance to the treatment plant, water conductivities) and/or water origin makeup. Nevertheless, significant levels of Fe^t and VS were for samples from CI and HDPE pipes, respectively. However, no relationship was found between LD contents in Fe^t and VS and sampled pipe materials (Table 2-3).

Table 2-3. Average iron (Fe^t) and volatile solids (VS) results per pipe material.

LD composition	**Pipe material**							
	AC	n	CI	n	DI	n	HDPE	n
VS	119.7	5	143.5	5	157.9	7	197.3	13
Fe^t	526.2	5	605.6	5	362.3	7	471.4	11
Total (VS+Fe^t)	645.9		749.1		520.2		668.7	

The organic contents of LD were given by the VS masses determined in samples obtained after 7-day settling concentration at 4ºC. The similarity of the VS results to those published for other DWDS, as well as the fact that LD are likely to remain in DWDS for much longer periods, suggest that LD organic fraction is mostly of low-biodegradability and that did not change significantly during the concentration period.

2.3.3 LD aggregation and settling behaviour

As Figure 2-3 illustrates, an apparent relationship between the turbidity and TSS in well homogenised discharged waters was found.

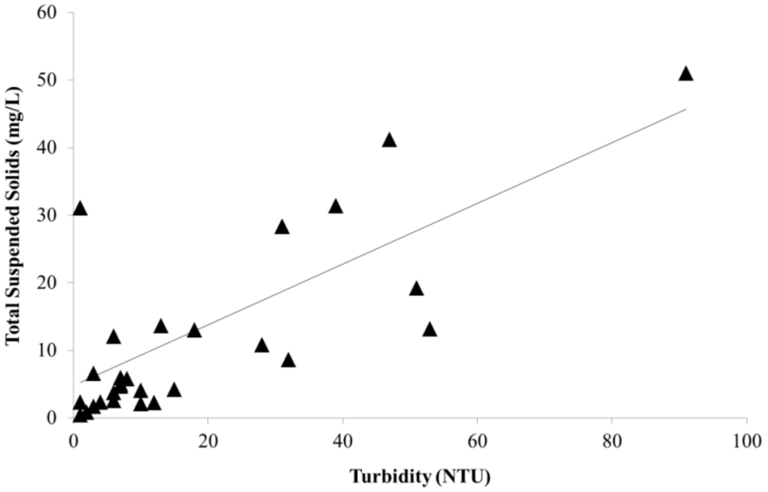

Figure 2-3. Relationship between total suspended solids (TSS) and turbidity (NTU).

As turbidity measurements may be influenced by particles' size and shape, nephelometric studies were conducted to investigate turbidity evolution in homogenised slurries during LD settling. Accordingly, in LD slurries with initial turbidities of 80-100 NTU, clarification to low levels (10-20 NTU) occurred in *ca.* 24 hours and close to undetectable levels (< 2 NTU) after the 7 day-settling. Moreover, after re-homogenisation, samples showed repeatable turbidity decay patterns and rates (Figure 2-4), which reversibility may be indicative of the flocculent behaviour of the LD slurries (Winterwerp and van Kesteren 2004).

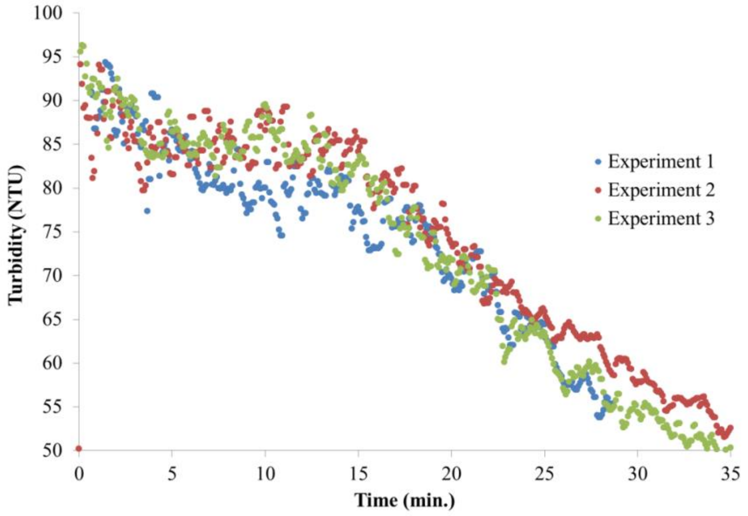

Figure 2-4. Turbidity evolution pattern in LD sample (3 repeated experiments) settled over 7 days.

Likewise, turbidity decay trends were also reversible and similar within discharged waters (DW) and LD slurry samples from the same location (Figure 2-5).

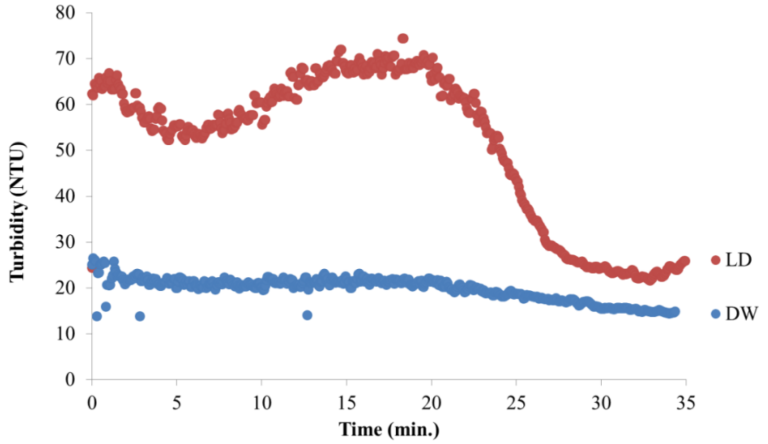

Figure 2-5. Turbidity evolution patterns in the discharged water (DW) and in the LD slurry (concentrated over 24 h).

The naked-eye visualization of LD aggregation and the influence of clustering on settling were made apparent by studying turbidity decays of discharged waters (Figure 2-5) and LD suspensions. The observed trends from Figure 2-4 and Figure 2-5 are similar to those for the “settling processes for cohesive sediment”, described by Migniot (1968) and Morris and Fan (1998). In the first phase (0-5 min) the deflocculated LD units became larger due to the increased levels of pore water caused by turbulence; in the second phase (5-15 min), LD individual units became floc settling assemblies; that, in the third phase (> 15 min) settled at the same rate (Migniot 1968, Morris and Fan 1997). In the third phase, settling rates first approximated to lineariality and to a logarithmical scale in a fourth phase. Most likely the extension of each phase depended of the number of particles from each sample, as shown by the differences between the turbidity decay patterns of discharged waters and LD slurries. In addition, the observed results suggest that, in the absence of mixing, complete clarification of most discoloured waters may occur in 2-4 days.

The fact that LD flocs could be easily aggregated and disaggregated may explain why these could also be easily resuspended and, thus, sampled at low-velocities. This also indicates that the flocs will be transported easily through the system and may accumulate in quiescent zones, commonly found in looped distribution pipes.

In spite of the very low number of discoloured water complaints, results showed LD accumulate in Lisbon DWDS pipes, where they can resuspend and cause tap water discolouration, and can be obtained and characterised from discharged waters with imperceptible levels of discolouration (< 5 NTU). Despite not providing data on accumulated LD amounts, as dedicated flushing does, low-velocity sampling from hydrants (prior to routine pipe cleaning) could be used for LD collection and monitoring. In addition to being less inconvenient for the water companies, as it is economic, does not require special equipment or water supply interruptions, LD obtained with low-velocity sampling may be more representative of tap water discolouration sediments while leaving behind coarser materials that hardly reach the consumers’ tap.

2.4 Conclusions

The obtained results support the following main conclusions:

- The simple, practical and low-cost methodology developed in this study allows for the representative qualitative sampling of discolouration causing LD from DWDS, at amounts enabling for an extended physical-chemical characterisation;
- Relatively low velocities (≥ 0.02 m/s) are effective in resuspending LD during sampling from DWDS pipes and may, likewise, trigger discolouration;
- LD exhibit aggregative- and flocculent abilities that are likely to favour the transport of the deposits through the system, as well as their settling and accumulation in quiescent zones of the DWDS.

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3 Hydrogel floc nature and biogenic constituents of drinking water discolouration deposits

Abstract

Loose deposits (LD) develop and accumulate in drinking water distribution systems' (DWDS) pipes and often lead to discolouration incidents, which are a major reason for customers' complaints. In addition, LD may accumulate contaminants, while discolouration may be accompanied by degradation of the water's microbiological quality. Along with oxidized iron forms, organic compounds are well established as predominant constituents of LD. Even so, the composition and role of the latter remain unknown. Since microbial extracellular polymeric substances (EPS) are components of iron-rich flocs from many freshwater environments, the presence of EPS in LD was investigated. This was confirmed by the systematic occurrence of polysaccharides and proteins in LD samples. In addition, microscopic observations and measurements of LD water contents showed that, alike their freshwater counterparts, LD have a hydrogel floc nature and fractal morphology. By providing insights on LD origin and behaviour, these findings may be important for the understanding of DWDS water discolouration phenomena.

*Adapted from:

Poças, A., Miranda, A., Paiva, J., Benoliel, M.J., Vreeburg, J. and Menaia, J., 2013. Hydrogel floc nature and biogenic constituents of drinking water discolouration deposits. Water science & technology: water supply 13(6), 1486-1494.

3.1 Introduction

Loose deposits (LD) are ubiquitous in drinking water distribution systems (DWDS), where they accumulate and, upon resuspension, may lead to water discolouration events. Discoloured tap water, namely the appearance of yellowish to brownish colour and turbidity, and, in some cases, taste and odour, may diminish the consumers' confidence in drinking water safety, thus being a major reason for complaints (Vreeburg and Boxall 2007). In addition, LD may accumulate and transport contaminants while discolouration may be accompanied by degradation of the water's microbiological quality (Zacheus et al. 2001). Consequently, and because LD may occur in water with no apparent discolouration, mitigation of LD formation and accumulation in DWDS is advisable, but still requires a better understanding of LD origin and behaviour, which LD composition may reflect.

Typically, in addition to minor levels of some inorganic compounds (*e.g.*, Mn, Ca, Mg, Al species), LD have oxidized iron forms (up to 70% of dry weight) and organic materials (volatile solids up to 65% of dry weight) as main constituents (Gauthier et al. 1999, Vreeburg and Boxall 2007). Nonetheless, LD are mostly seen as particulate derivatives of cast-iron pipes' corrosion (Benson et al. 2012), even if discolouration occurs in networks devoid of metallic pipes (Vreeburg et al. 2008). Apart from corrosion, in DWDS, oxidized iron forms may arise with raw water or from post-precipitation of Fe^{3+}, due to Fe^{2+} oxidation by dissolved oxygen or disinfectants (*e.g.*, ozone, chlorine) at the treatment plant and downstream in the distribution network (Benson et al. 2012). Other possible sources include Fe^{3+} salts, whenever these are used as coagulants in drinking water treatment, and oxidized iron forms metabolically produced by iron oxidizing bacteria (FeOB), *e.g.*, Galionella spp., which involvement in drinking water discolouration has been suggested for long (Ridgway et al. 1981).

Although omnipresent in LD, to the best of our knowledge, only Gauthier et al. (1999) and Zacheus et al. (2001) examined the organic fraction of this type of deposits. Based on LD relative contents in C and N, those authors (Gauthier et al. 1999, Zacheus et al. 2001) suggested the occurrence of biogenic materials as LD constituents. Accordingly, and taking into account the importance of microbial extracellular polymeric substances (EPS) in flocs that are common in many aquatic environments (Gerbersdorf et al. 2009) and, like LD, have volatile solids (VS) and oxidized iron forms as important components (Elliot et al. 2012), it is likely that EPS are also LD constituents.

In DWDS, EPS may enter with raw water, may be released by treatment filters and pipe-wall biofilms, and may be metabolically produced during growth of FeOB in the form of EPS stalks or sheaths with encrusted Fe oxides (Ridgway et al. 1981). Such type of iron-rich EPS debris may be released during detachment of DWDS pipe-wall biofilms, as these may host iron depositing bacteria (Ginige et al. 2011). The occurrence of biogenic EPS as important constituents of the LD organic fraction is, therefore, probable. The assessment of such hypothesis was the main goal of this work.

Given the EPS properties and structural role in EPS-Fe^{3+} hydrogel-flocs that develop in freshwater environments, other unpublished features of LD may be foreseen, including high water contents, fractal structure (Johnson et al. 1996, Vahedi and Gorczyaca 2012) and flocculating behaviour (Plach et al. 2011, Elliot et al. 2012). As such properties may be important for the understanding and modelling of LD behaviour in DWDS, these were also addressed in this study.

In this chapter, 29 samples of LD collected from random locations of the Lisbon drinking water network were investigated. The occurrence of EPS was examined by determining LD polysaccharides and total protein, *i.e.* the predominant EPS' constituents (Flemming et al. 2007, Gerbersdorf et al. 2009), as well as by LD specific staining. The morphology of LD was visualized by microscopic observations. In addition, samples' representativeness was evaluated by comparing their Al, Mn, Ca, iron (Fe^t) and VS contents with those published for DWDS.

3.2 Materials and methods

3.2.1 Distribution network

The Lisbon DWDS supplies *ca.* 520 000 inhabitants, through 93 000 connections and *ca.* 1 427 km of main pipes. The water is mostly of surface origin (86%) and undergoes conventional treatment through pre-oxidation (ozonation or chlorination), aluminium sulphate coagulation, flotation, rapid sand filtration, and chlorine disinfection; water from other sources undergoes conventional treatment (Tagus river), or chlorination only (groundwater). Pipe materials comprise asbestos cement (AC~29%), ductile iron (DI ~28%), cast-iron (CI ~19 %), high-density polyethylene (HDPE ~17 %) and the remaining ~7% of unknown materials.

3.2.2 LD sampling

Samples were collected from waters discharged from hydrants during programmed routine pipe discharges at unidirectional flow. Velocities used in sampling were those provided by the hydrants' opening, which are relatively low (≤ 1 m/s) when compared to conventional pipe cleaning (Poças et al. 2013). Sampling locations were randomly distributed throughout the network and included mains and dead-end pipes of different materials and age (Table 3-1).

Table 3-1. Sampled pipes' material and age.

Material		Age (years)	
	n	min.	max.
AC	9	18	73
CI	6	16	73
DI	5	9	17
HDPE	9	2	8

After rejecting two to four times the capacity of the hydrants and connections, 30 L or 63 L of the discharged water were collected during the flushing velocity peak, *i.e.* that delivered by the hydrants' valve maximum openings. These volumes were chosen prior to sampling, based on the expected LD loads and feasibility of sample transport and storage (*i.e.*, 30 L and 63 L for the pipes where more and less sediment was expected, respectively). The collected waters were then decanted for seven days at 4ºC and, after outdrawing the supernatant with a peristaltic pump at flow rate of 6L/h, the obtained slurries (1-3 L) were used for LD characterization, directly or after extended settling (total solids concentrated by *ca.* 300 times).

3.2.3 LD water and VS contents

LD water contents were determined in LD (0.5-1.0 g) centrifugates (10 min at 17500 × g). These were dried at 60ºC to constant weight (Robertson and Eastwood 1981). The weight loss during drying was taken as a measure of LD free-water contents.

Total solids (TS) and VS in LD slurry samples (> 5 mL) were determined by drying (110ºC) to constant weight and by ignition (550ºC), respectively (APHA 1998). Results for VS were used as a measure of LD organic fraction.

3.2.4 LD polysaccharides and protein contents

Total polysaccharides and protein were quantified in centrifugates of LD slurries (> 5 mL) separated at 10 956 g for 10 min. Polysaccharides were determined by following the protocol described by Daniels et al. (1994) for the determination of total carbohydrates by the anthrone method (Daniels et al. 1994), with D^+ glucose (Sigma G-6 152) as standard. Total protein was determined by using the Bradford method (Bradford 1976). Bovine serum albumin (BSA) fraction V (Merck, p.a.) was used as standard. Protein was extracted by resuspending and boiling the centrifugates in 3 mL of sodium hydroxide (1 M) for 15 min, and determined at 595 nm with a UV/Vis spectrophotometer (Jasco, V-630 LSE-701).

There can be errors from the used colorimetric methods used for sugar and protein analyses, particularly when using glucose and bovine serum albumin as standards – these may not fully represent the polysaccharides and proteins of LD. Other drawbacks of the methodology include possible errors associated with sample treatment or handling. The volumes used for LD characterisation were low (5 mL of the concentrated slurries) and, due to the floc aggregation abilities there can be some variation in mass.

3.2.5 LD mineral contents

Total iron (Fe^t) was determined by the phenantroline method (APHA 1998) in LD ashes' digests. These were obtained by digesting ashes (>5 mg) of ignited (550ºC) LD in HCl (6 M) for 1 hour at 121ºC (Räisänen et al. 1992). The digests were filtered through 0.20 µm pore polycarbonate membranes (Millipore, GTTP04700) to remove possibly occurring undigested particulates (*e.g.*, Si compounds). Hydroxylamine (hydroxylamine ammonium chloride, Merck, p.a.) was added to the filtrates (APHA 1998) to reduce Fe^{3+} to Fe^{2+}. Determination of Fe^t was done at 510 nm with a UV/Vis spectrophotometer (Jasco, V-630 LSE-701) in dilutions of the filtrates in Milli-Q water.

Digests of dried LD samples (> 300 mg), obtained by using the EN 13 346 standard (2000) closed microwave method (EN13346: 2000), were used for determination of Al, Mn and Ca. Samples were resuspended in 3 mL of HCl (37%) plus 9 mL HNO_3 (65%) in Teflon closed containers and digested in a microwave Milestone Ethos Pus. The digested samples were then resuspended in ultrapure water and analysed by inductively coupled plasma mass spectrometer (Thermo XSERIES II) for Al and Mn; and by inductively coupled plasma optical emission spectrometer (Thermo IRIS Intrepid II) for

Ca. As controls, standards of sewage sludge (Calitax Labaqua) and freshwater river sediments (LGC 6 187 and RM 8 704) were used as matrix reference materials.

3.2.6 Microscopic observations

A stereomicroscope (Olympus SZ-PT) and an optical microscope (Olympus BH2) were used to observe wet mounts of fresh LD slurry samples on glass Petri dishes and slides, respectively. Microphotography and LD size analyses were done with a digital camera (Olympus, XC 30) and by using the Olympus Cell-A software.

Ferric iron was specifically visualized in LD by the Perls' Prussian Blue staining reaction. Slurry samples on glass slides were sequentially covered with drops of $K_4[FeII(CN)_6]\cdot 3H_2O$ (Merck, p.a.) in distilled water (0.2%) and of HCl (1 M). The stained samples were then observed and photographed under the optical microscope.

The Alcian Blue staining was used for the visualization of EPS (Passow and Alldredge 1995) in LD samples. Slurry samples (1 mL) were centrifuged in 1.5 mL Eppendorf tubes at 12 500× g for 5 min and resuspended in 1 mL of an Alcian Blue 8GX (Sigma A5268-10g) solution in distilled water (2%, w/v). After 5 min. of staining, the samples were centrifuged and resuspended in distilled water. This washing procedure was repeated for three times and the obtained resuspensions of stained LD were used for microscopic observations and photography.

3.3 Results and discussion

3.3.1 LD composition

The effect of discolouration was reproduced by resuspension of all LD concentrated slurries, even if from water discharges with no perceivable turbidity (< 5 NTU). The representativeness of the samples collected from the Lisbon DWDS was confirmed by results observed for LD composition. Fe^t (489.3 ± 173.4 mg Fe^t/g LD dry weight) and VS (155.8 ± 93.7 mg VS/g LD dry weight) were the predominant LD-components (Table 3-2), while other inorganics, namely Al, Mn and Ca, were found at lower levels (26.0 ± 19.5 mg Al/g; 14.3 ± 14.6 mg Mn/g; and 17.4 ± 9.4 µg Ca/g LD dry weight). Overall, the relative concentrations observed for the analysed constituents (Fe, Al, Mn, Ca, VS) varied alike and fell within the ranges described for chlorinated (Gauthier et al. 1999, Zacheus et al. 2001) and non-chlorinated networks (Vreeburg et al. 2008).

With respect to the analysed organic compounds, either polysaccharides or total protein were detected in all analysed samples. Polysaccharides were found in 81% of the 26 samples analysed (excluding interferences), and at D+ glucose equivalents ranging from 2.5 mg/g to 106.4 mg/g. Protein was detected in 97% of the samples (n = 29) at BSA equivalents of 0.6 mg/g to 150.0 mg/g. The amounts of polysaccharides and protein found in LD exceeded those reported (Gerbersdorf et al. 2009) for riverine flocs' EPS (*i.e.*, 1.3 mg/g to 6.7 mg/g in glucose equivalents and 0.4 mg/g to 12.9 mg/g in BSA equivalents). Accordingly, the observed results evidenced EPS as a meaningful constituent of LD. In addition, measured water contents averaged 85%, thus showing that water is the dominant constituent of discolouration-causing LD.

Table 3-2. Results from LD composition analyses.

		Inorganic constituents				Organic constituents		
Pipe material	Water	Fe^t	Al	Mn	Ca	VS	Polysaccharides	Protein
	%	mg/g	mg/g	mg/g	ug/g	mg/g	mg/g	mg/g
AC	39%	938.5	8.76	3.71	<	54.2	4.8	1.1
AC	NA	484.2	15.03	5.99	29.20	197.2	ND	20.9
AC	NA	884.0	NA	NA	NA	84.9	INT	0.6
AC	NA	420.9	NA	NA	NA	132.0	12.5	1.2
AC	NA	523.1	NA	NA	NA	101.3	50.0	5.1
AC	81%	437.6	NA	NA	NA	36.1	21.0	1.9
AC	92%	593.2	NA	NA	NA	129.6	53.3	32.2
AC	94%	366.6	NA	NA	NA	101.4	30.4	7.3
AC	87%	567.4	NA	NA	NA	127.5	90.0	52.9
CI	NA	454.5	6.20	1.81	<	65.2	30.5	6.6
CI	NA	499.4	6.57	2.19	5.51	95.2	INT	1.1
CI	NA	452.4	29.04	6.04	14.50	168.3	ND	3.8
CI	NA	443.6	NA	NA	NA	188.7	27.2	14.8
CI	NA	284.3	NA	NA	NA	148.1	ND	25.6
CI	79%	734.0	NA	NA	NA	87.3	106.4	60.7
DI	64%	276.1	44.52	34.53	17.80	161.5	2.5	ND
DI	NA	272.2	37.20	25.62	33.20	108.4	20.8	4.7
DI	NA	419.7	52.55	33.30	15.40	121.6	13.4	12.2
DI	94%	284.7	NA	NA	NA	181.8	51.4	26.1
DI	97%	613.0	NA	NA	NA	256.4	32.4	5.1
HDPE	NA	466.6	56.43	35.99	22.30	129.6	3.2	4.1
HDPE	NA	637.3	25.69	5.09	11.60	83.3	5.5	9.4
HDPE	86%	476.9	3.78	2.55	7.01	152.8	INT	1.2
HDPE	NA	NA	NA	NA	NA	234.0	66.1	10.1
HDPE	NA	NA	NA	NA	NA	440.0	ND	14.8
HDPE	NA	NA	NA	NA	NA	111.1	ND	43.3
HDPE	99%	326.2	NA	NA	NA	272.7	36.8	145.9
HDPE	99%	NA	NA	NA	NA	411.8	67.1	150.0
HDPE	96%	376.3	NA	NA	NA	136.4	15.7	10.1
Avg.	85%	489.3	26.0	14.3	17.4	155.8	35.3	24.0
Stdev.	17%	173.4	19.5	14.6	9.4	93.7	28.8	38.4
Min	39%	272.2	3.8	1.8	5.5	36.1	2.5	0.6
Max	99%	938.5	56.4	36.0	33.2	440.0	106.4	150.0

Note: AC-asbestos cement; CI-cast-iron; DI-ductile iron; HDPE-high-density polyethylene; INT-interferences; NA-not analysed; ND-not detected; avg.-average; stdev.-standard deviations; Fe^t*-total iron; VS-volatile solids.*

3.3.2 LD composition vs. pipe material

Results from Table 3-2 were then analysed with respect to pipe material (Table 3-3). Fe^t contents found among LD samples were comparable, irrespectively of the sampled pipes' characteristics. Nonetheless, the highest Fe^t contents occurred in LD from AC pipes and not from CI ones. Therefore, and in accordance with other authors (Vreeburg et al. 2008), results suggest that, in the bulk, LD which accumulate in pipes are not *in-situ* formed corrosion debris, but LD oxidized iron forms that can originate from upstream sources.

With regard to LD organic fraction, the maximum VS contents found were generally higher in samples from high-density polyethylene pipes (Table 3-3) and the same applies to polysaccharides and protein. This suggests that a selective LD accumulation might have occurred in plastic pipes, possibly due to increased LD adhesion driven by the pipes and EPS hydrophobicity.

Table 3-3. LD composition vs. pipe material.

Pipe material	**Water (%)**			**Fe^t (mg/g)**			**VS (mg/g)**			**Poly. (mg/g)**			**Prot. (mg/g)**		
	n	**min.**	**max.**	**n**	**min.**	**max.**	**n**	**min.**	**max.**	**n**	**min.**	**max.**	**n**	**min.**	**max.**
AC	5	39%	94%	9	366,6	938,5	9	36,1	197,2	7	4,8	90,0	9	0,6	52,9
CI	1	79%	79%	6	284,3	734,0	6	65,2	188,7	3	27,2	106,4	6	1,1	60,7
DI	3	64%	97%	5	272,2	613,0	5	108,4	256,4	5	2,5	51,4	4	4,7	26,1
HDPE	4	86%	99%	5	326,2	637,3	9	83,3	440,0	6	3,2	67,1	9	1,2	150,0

Note: Fe^t-total iron; VS-volatile solids; ; Poly-polysaccharides; Prot-protein; AC-asbestos cement; CI-cast-iron; DI-ductile iron; HDPE-high density polyethylene.

The occurrence of possible linear correlations between the relative amounts of Fe^t, VS, polysaccharides and protein contents were examined, including those relating LD composition to pipe's material and age. However, no statistically significant correlations could be found[1]. In addition, the relative contents of LD constituents were dispersed within a relatively large range, thus suggesting that, on the whole, LD composition developed non-uniformly.

3.3.3 LD structure and behaviour

The Prussian Blue and Alcian Blue positive-staining (Figure 3-1), which were consistently observed for all the examined samples (*ca.* 20), evidenced Fe^t and EPS as meaningful LD components, thus corroborating the results observed for polysaccharides and protein.

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[1] See Annex 1 – supporting information on statistics.

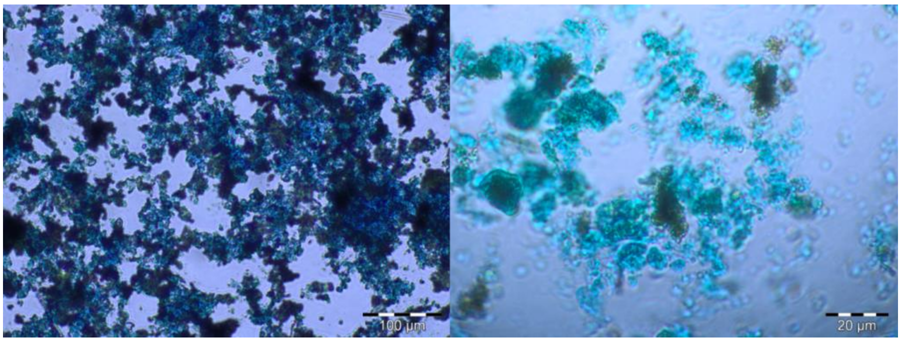

Figure 3-1. Microphotograph of Prussian Blue (left) and Alcian Blue (right) stained LD.

The morphology and dimension of LD were visualized by observations of fresh mounts under the stereomicroscope and the optical microscope (Figure 3-2). At both magnifications (*ca.* 40 x and *ca.* 400 x) LD invariably appeared as flocs with fractal-like morphology.

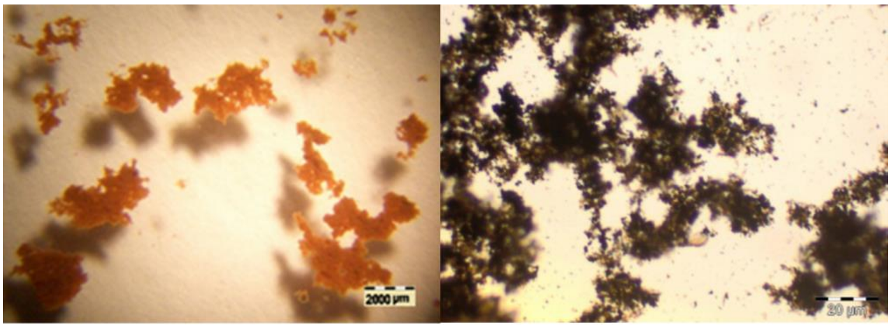

Figure 3-2. Fresh mounts of LD under stereomicroscope (left) and optical microscope (right).

These results contrast with the common assumption of discolouration-LD as “true particles”, thus with defined surface(s), ascribable sizes (< 10 µm) and densities, but agree with the results of van Lieverloo et al., (2012). The stereomicroscope observation of sediments from 34 Dutch DWDS (van Lieverloo et al. 2012) led to the conclusion that DWDS’ sediments are predominantly composed of floc materials.

In addition, spontaneous aggregation of previously dispersed LD could be observed under stereo- and optical microscopes (Figure 3-2), as well as with the naked-eye. Such flocculent behaviour agrees with the previously reported flocky and cohesive-like properties of LD (Vreeburg and Boxall 2007).

The fractal structure and morphological arrangement, the staining characteristics, as well as the flocculent behaviour, were observed for the seven day settled LD and for samples stored for more than one year in the laboratory at 4ºC. These observations suggest LD to be stable both in structure and in composition. While iron oxide stability may be expected in water at circum-neutral pH, provided that O_2 is present, EPS’ polysaccharides and protein are generally seen as more labile, due to their potential for biodegradability. Alike EPS polysaccharides and proteins in aquatic sediments that often exhibit a remarkable and long stability, LD-EPS constituents may be essentially stable, due to various protective mechanisms, including the entrapment or binding of iron oxides (Knicker and Hatcher 1997).

Flocs with biogenic EPS as structural components are common in freshwater environments (Plach et al. 2011, Elliot et al. 2012), including iron oxide rich flocs resembling those herein described LD, particularly with respect to fractal morphology and behaviour (Liss et al. 1996). In such flocs, EPS occur as colloids or associations of fibrils of relatively large sizes, thus providing large surfaces for the bridging of ferric iron (Brown et al. 1998). In addition, those flocs' fibrilar-matrices exhibit high water holding capacity (Flemming et al. 2007), which are a defining property of hydrogels (Rosiak and Yoshii 1999). Even though the tested samples (n = 13) were diverse in their EPS contents and composition (Table 3-2), results showed unbound water as the predominant LD constituent (~85%). Hence, along with the observed fractal morphology and flocculent behaviour, these results indicate that LD are hydrogels in their nature, rather than "true" particles. On the other hand, colloidal amorphous iron oxides that often occur in flocs from aquatic systems may also form hydrogels (Plach et al. 2011, Elliot et al. 2012). Therefore, it can be expected that LD hydrogel flocs are also formed upon the association of oxidized iron forms with EPS.

While the capacity of EPS for adsorption, complexation and binding of iron oxides has been established, the origins of oxidized iron forms and EPS in DWDS remain to be explained, as well as the mechanisms underlying their association to generate LD. In addition to the possibility of being corrosion derivatives, oxidized iron forms may arise with upstream water or be formed within the distribution network. Likewise, EPS may come with upstream water or be released by pipe biofilms. In addition to the association of EPS and iron oxide particulates, LD may also result from debris of EPS with encrusted Fe oxides, like those that are released by FeOB of pipe-wall biofilms (Ridgway et al. 1981).

The herein suggested role of biogenic constituents in LD structure and hydrogel floc behaviour may help the interpretation of discolouration phenomena, then allowing for improvements on its control. Likewise, LD may exhibit densities close to the water, which can explain how they can be easily transported through the networks at the daily flows. The fact that LD may be formed and resuspend reversibly allows them to occur, be transported and either re-accumulate or cause discolouration, in different ways, at different parts of the networks. Even so, and considering the possible negative impacts on water quality, it should be emphasized that discolouration events are generally restricted to critical locations of DWDS. This is the case of the studied DWDS, where discoloured water is the exception. In the Lisbon DWDS the rule is by far the supply of good quality water, as compliance with the applicable EU Directive confirms.

3.4 Conclusions

In this study, 29 samples of LD collected from random locations of the Lisbon drinking water network were examined. The samples composition was similar to that reported for LD collected worldwide. In addition to evidencing the occurrence of biogenic EPS as LD constituents, results suggest that LD have hydrogel floc nature and fractal morphology. These findings contrast with the current assumption that discolouration-causing LD are particles with defined limits and sizes, and with the density of their solid constituents. That assumption underlies present approaches in describing and simulating LD behaviour in DWDS, namely their accumulation, resuspension and transport by direct application of conventional gravitational settling theories (*e.g.*, Stokes' Law). However, such approaches seem inappropriate (van Thienen et al. 2011), given the herein reported hydrogel floc nature of LD, *i.e.* fractal dimension and density approaching that of water (Johnson et al. 1996, Vahedi and Gorczyaca 2012).

From considering LD as hydrogel flocs with biogenic EPS as meaningful constituents, important scientific and practical impacts can be expected concerning the understanding of LD origin and behaviour, and therefore of the phenomena associated to water discolouration in DWDS.

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4 Pilot studies on discolouration loose deposits' build-up

Abstract

Tap water discolouration occurs due to resuspension of loose deposits (LD) that accumulate in drinking water distribution systems. Strategies for discolouration control involve network pipe cleaning and replacement of cast-iron pipes. However, the sole application of such measures is not generally effective. Therefore, a deeper understanding on processes associated with LD origin and development is required. Transparent (but covered) test rig pipes (Ø=23 mm) continuously supplied with drinking water at steady flow through mode (7-9 months) were used to investigate LD build-up under laminar flow (0.2-4.1 cm/s) conditions. Rather than continuously and homogeneously, LD developed as spots, and predominantly at the pipes' bottom. Results suggested that particle attachment may be added to settling as a LD build-up process. This is consistent with LD cohesive-adhesive properties, as shown by LD repose angle tests, as well as to the occurrence of flocculent extracellular polymeric substances-EPS in LD.

*Adapted from:

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4.1 Introduction

Loose deposits (LD) develop and accumulate in drinking water distribution systems (DWDS) and may lead to discoloured water events whenever hydraulic changes cause LD resuspension (Vreeburg and Boxall 2007, Husband and Boxall 2011). Tap water discolouration, namely the appearance of yellow to brownish turbidity, weakens the consumers' confidence in the supplied water safety, thus being a major reason for complaints (Vreeburg and Boxall 2007, Husband and Boxall 2011).

Possible origins for LD in DWDS include particles released by treatment plants, waterborne precipitates, pipe-wall biofilm debris, cast-iron corrosion derivatives and particulates entering the networks during pipe rehabilitation or repair (Gauthier et al. 1999, Carriere et al. 2005, Vreeburg and Boxall 2007). Despite so many possible origins, owing to their high contents in iron-oxides (up to 70% in dry weight), LD are commonly seen as corrosion particulates (McNeill and Edwards 2001, Benson et al. 2012). However, LD typically have organic contents (volatile solids-VS) at levels comparable to those of iron oxides and water as the main constituents (Poças et al. 2013a).

Apart from pipe cleaning, discolouration control strategies traditionally involve the replacement of old metallic pipes. Far from being effective, as LD are formed and discolouration occurs and persists in networks devoid of cast-iron pipes (Vreeburg et al. 2008), such reactive measures are insufficient to control tap water discolouration. Alternatively, extensive LD accumulation in DWDS' pipes would be more effectively prevented by assuring that pipes' flow velocities reach a daily peak of *ca.* 0.4 m/s or higher (Vreeburg et al. 2009b, Blokker 2010, Husband and Boxall 2011). However, in most existing DWDS, such "self-cleaning" conditions are not easily guaranteed, since networks frequently have oversized pipe diameters and looped designs. On the other hand, as discolouration events are of short duration, unpredictable (Vreeburg and Boxall 2007, Husband and Boxall 2011) and occur in buried infrastructures, LD generation and accumulation in DWDS are difficult to follow or predict. Thus, a deeper insight on LD build-up underlying mechanisms is required to understand and better control drinking water discolouration.

Experiments have been conducted to study the impacts of hydraulics (*e.g.* residence time, shear stress) on the build-up of discolouration materials (Vreeburg 2007, Husband et al. 2008). Although useful to investigate LD occurrence, the used experimental conditions may be difficult to replicate elsewhere and, with few exceptions (Husband et al. 2008), do not fully reproduce the conditions occurring in DWDS (Douterelo et al. 2012). This also applies to studies with LD surrogate particles (*e.g.*, kaolinite) in closed-loop systems with water recirculation (Husband et al. 2008, van Thienen et al. 2011). As DWDS flows may range from non-flow to laminar and turbulent regimes (Manuel et al. 2007), and are far from being steady (Husband et al. 2008, Blokker 2010), such flow patterns are hard to reproduce in laboratory experiments.

Due to their size distribution (< 20 μm), and density close to that of the water (Poças et al. 2013a), it is unlikely that LD build-up is driven by gravitational settling forces solely (Vreeburg and Boxall 2007, Husband et al. 2008, Husband and Boxall 2011, van Thienen et al. 2011). Most probably, LD "cohesive-like" (Boxall and Prince 2006, Vreeburg and Boxall 2007) and flocculent (Poças et al. 2013b) behaviours also play a role in the formation and stability of LD, particularly those that are easily resuspended, thus leading to DWDS' water discolouration. Such type of behaviour, which most likely results from the cohesive/flocculent (Vreeburg and Boxall 2007, Poças et al. 2013b) properties of LD extracellular polymeric substances-EPS (Poças et al. 2013a), may influence LD build-up rates and profiles. Similarly to biofilms (Abe et al. 2012), EPS may confer cohesiveness and thickness to LD, thus influencing their formation and stability processes (Poças et al. 2013a).

Herein, test rigs with transparent pipes were developed and operated in flow through mode to investigate LD build-up and formation under different drinking water flow velocities. The main objective was to study the influence of flow velocities in LD loads and composition. Repose angles tests – conventionally used to determine the threshold slope for non-cohesive sediments sliding (Morris and Fan 1997) - were used to appraise the relative influence of gravity and cohesive-adhesive properties on LD behaviour. Sand beds were introduced in the test rigs' pipes to allow faster LD development (*i.e.*, sand bed colonization by biofilms). The use of laminar flows to study LD accumulation was chosen to permit the installation to operate in a flow through mode. The low velocities were based in previous observations that these may be in the order of few cm/s (Vreeburg 2007, Blokker 2010). Formed LD were analysed for total iron (Fe^t) and volatile solids (VS), as well as for EPS main constituents (Flemming and Wingender 2001), namely polysaccharides (PL) and total protein (PT).

4.2 Materials and methods

4.2.1 Test-rigs

Each test rig comprised transparent polyvinyl chloride (PVC) pipes (internal diameter: 23 mm; length: 2 m). These were continuously supplied by a manifold connected to a constant head reservoir fed with tap water (Figure 4-1). Each pipe was operated in a flow through mode at a pre-set velocity controlled by downstream valve manoeuvring. Except wherever indicated, and before starting the experiments, a uniform siliceous sand bed (10 g; D50=0.2 mm; length = 1 m; depth *ca.* 2 mm) was introduced in the pipes by using a rectangular gutter. The beds ends were 50 cm distant from the pipes' edges.

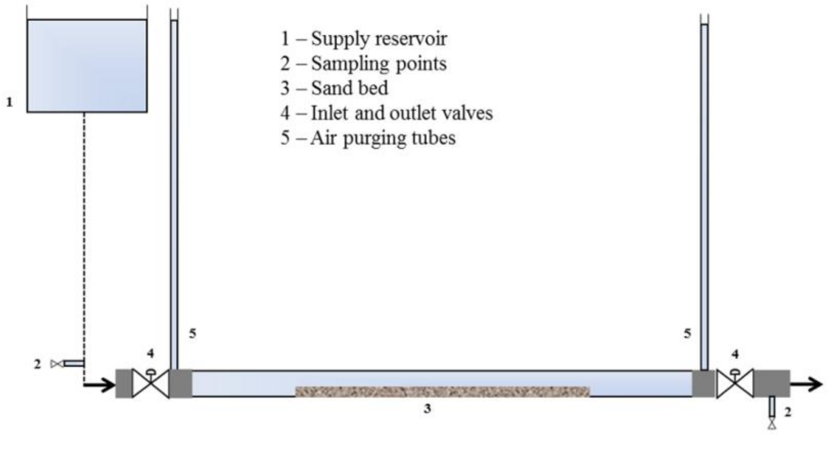

Figure 4-1. Test-rigs' layout.

The setup was used in two separate experiments. In the first one, four parallel pipes were used. In the second experiment, a fifth pipe with no sand bed was added to the setup to allow the appraisal of the effect of sand on LD accumulation rates and profiles.

After filling the pipes with tap water and purging the air bubbles out by tilting them slightly, flows were gradually tuned to the target values. Weekly, each pipe flow was determined by measuring the filling rate of a graduated vessel. During operation, pipes were kept horizontally, covered for light protection and at controlled room temperature (20ºC ± 1ºC). The first experiment started in October 2010 and finished in July 2011. The second started in September 2011 and finished in March 2012.

4.2.2 LD build-up profiles (visual observations)

In the second experiment, top-down photographs were taken to record and follow LD build-up profiles in the pipes, at two week intervals. The number and size of distinguishable aggregates occurring at every 10 cm lengths were taken as a measure of LD build-up levels. Sizes were scored from 0 to 5, *i.e.*, from non-visible to the larger size observed. LD build-up scores were the number of countable aggregates times the respective size ranks.

4.2.3 LD characterisation

For LD collection and pipes' complete drainage by the end of the experiments, rig pipes were tilted up to 90º inclination for allowing their contents to flow through the outlet valve. Collected sand was separated by decantation and washed three times with tap water. Supernatants were added to the collected pipe contents and LD were concentrated by settling over seven days at 4º C.

Dry weight masses of LD accumulated in each pipe during the whole experiments were determined. The obtained LD slurries were used for characterisation, as described in Poças et al., (2013a,b). Total solids (TS) and volatile solids (VS) were determined by drying and igniting (550ºC) LD (5 mL) samples (APHA 1998). Total suspended solids (TSS) were determined gravimetrically by the membrane filtration method (APHA 1998). Water contents were determined in LD slurry (0.5 g) centrifuges (10 min at 17500 × g) dried at 60ºC until constant weight (Robertson and Eastwood 1981). The weight loss during drying was taken as a measure of LD free-water contents. The phenantroline method (APHA 1998) was used for Fe^t determination in dilutions of LD ashes' digests. Total protein (PT) and polysaccharides (PL) were determined by the anthrone (Daniels et al. 1994) and Bradford (Bradford 1976) methods, respectively.

4.2.4 LD repose angles

At the end of the second experiment repose angles assays were done before LD collection. The assays were performed by pipes' stepwise tilting, with the repose angle being the critical slope at which sand or LD started sliding. The tests were done with the pipes still filled with water, after flow interruption by smooth closing of the outlet and inlet valves. Repose angle assays were also performed for control pipes with virgin sand beds (t=0 days).

4.2.5 LD build-up rates

Total loads of influent water particles (TS water) were estimated from averages of the determined flows assuming a constant influx of TSS of 0.6 mg/L, *i.e.*, the mean value found for inflow and outflow waters over the whole duration of the experiments. The build-up of LD masses in the rigs' pipes was determined as TS accumulated during the course of the experiments.

4.2.6 Network LD

Results from the application of the resuspension potential method (RPM), with adaptations (Vreeburg and Boxall 2007, Vreeburg et al. 2009a), were used to approach the rate of LD accumulation in seven network pipes located nearby the laboratory facilities. RPM involved an increase in flow velocity by 0.35 m/s during a five-minute disturbance and the measuring of turbidity evolution in the flushed waters. During the disturbance peak, large volumes (> 30 L) of flushed waters were collected for TSS analysis. In estimating the levels of LD accumulated in network pipes (mg/m), constant TSS levels were assumed for the flushed waters over the five-minute disturbance.

4.2.7 Shear stresses

The effect of wall friction shear stresses on accumulation was considered to estimate LD build-up under laminar (test rigs' pipes) and turbulent flows (network pipes). Shear stresses were calculated using the Hagen-Poiseuille and Chézy-Manning formulations[2] for water at 20ºC. For network pipes, shear stresses were estimated by assuming low-flow (0.06 m/s) and average flow velocities (0.20 m/s).

4.3 Results

4.3.1 LD build-up profiles

Inflow water turbidity was low (0.3 ±0.2 NTU) during the course of the experiments. At the end, and after sand bed falling, resuspension of the remaining LD test rig pipe contents reproduced discolouration. While pipe-wall biofilm formation was not perceptible to the naked eye during the whole course of the experiments, pipe fouling occurred appreciably on the pipes' bottom as LD. In pipes operated at the same flow velocity range, no visible differences in LD build-up profiles could be distinguished. However, at different velocity ranges, LD build-up profiles were clearly dissimilar (Figure 4-2). For the same time length, LD aggregates formed under the medium velocity range (*ca.* 2 cm/s) were visibly larger than those formed at lower rates (*ca.* 0.2 cm/s), possibly due to the larger loading of TSS and to the resuspension of smaller aggregates at higher flows. However, at velocities close to 4 cm/s, lesser accumulation was observed, possibly due to LD resuspension. Contrarily to what was initially hypothesized, sand bed surfaces were not starting or preferential locations for LD build-up, *e.g.*, biofilm colonization. In pipes operated at the medium-velocity range (*ca.* 2 cm/s), LD aggregates could be observed after *ca.* three weeks of continuous water supply. At low (*ca.* 0.2 cm/s) and high flow velocity ranges (*ca.* 4 cm/s) these could only be observed after *ca.* eight weeks (Figure 4-2) and *ca.* twelve weeks, respectively.

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[2] See Annex 2 – supporting information on hydraulics.

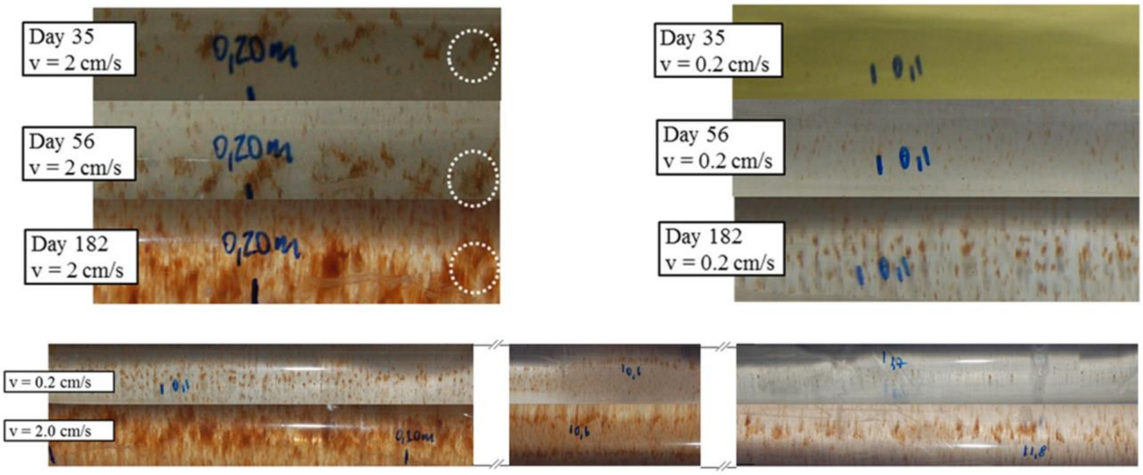

Figure 4-2. LD build-up and in-situ growth: in the first 10-30 cm of pipe lengths at the medium-velocity (top left) and at the low-velocity (top right) ranges; and upstream, sand bed and downstream by the end of the experiments (bottom).

In addition, LD did not accumulate on the pipes bottom as a continuum (Figure 4-3), rather developed as spots, and at larger numbers and sizes at the upstream part of the pipes. During the whole course of the two experiments, LD accumulation was more intense at the initial 50 cm lengths, *i.e.*, upstream the sand beds, but decreased through the pipe length. As so, the timescale (7-9 months) of the experiments was apparently insufficient for LD build-up to have reached a steady-state over the pipes' full lengths (Figure 4-2 and Figure 4-3).

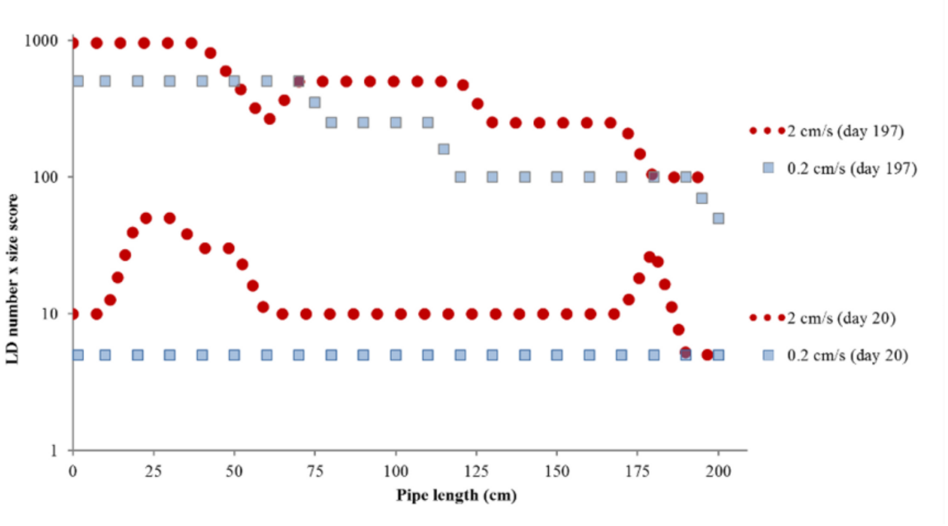

Figure 4-3. LD build-up scores for days 20th and 197th.

Contrarily to what had been firstly hypothesized the sand grains did not get colonized by biofilms. Similarly, no influence of the sand bed in LD accumulation was observed. However, due to the sand bed roughness, higher turbulence and, possibly, LD accumulation, could be expected in the pipes where sand beds were placed.

4.3.2 LD build-up rates

Ran at laminar flow regimes (Reynolds' numbers < 2000), tests covered flow velocities differing by more than one order of magnitude, *i.e.,* from *ca.* 0.2 cm/s to 4 cm/s (Table 4-1) in the two experiments. Despite that the first experiment was *ca.* 1.4 times longer than the second; the masses of LD which accumulated during the first experiment were only *ca.* 1.1 times higher than those during the second one. In all test rig pipes the masses of accumulated LD were a small fraction (<5%) of the estimated global TSS loads in the influent water.

Table 4-1. Experimental conditions, estimated water incoming loads (TS water), masses of LD accumulated per meter of pipe (TS total) and percentage of LD which accumulated in the pipes (TS retained).

	Flow-velocity (avg. ±stdev. cm/s)	**Time (days)**	**Reynolds (Re = v . Ø/ v)**	**TS water (mg)**	**TS total (mg/m)**	**TS retained pipe (%)**	
1st experiment	0.53 ±0.12	273	121	17.921	145	1.6%	
	0.74 ±0.31	274	169	25.798	169	1.3%	
	2.29 ±1.72	267	524	86.945	187	0.4%	
	4.09 ±0.84	275	935	168.932	151	0.2%	
avg. ±stdev.	1.9 ±1.7	272 ±4	437 ±377		163 ±19		
2nd experiment	0.18 ±0.08	202	41	7.657	70	1.8%	*
	0.20 ±0.11	202	46	6.858	88	2.6%	
	0.22 ±0.13	202	50	7.482	92	2.4%	
	1.70 ±0.56	203	389	58.301	251	0.9%	
	1.80 ±0.51	203	412	63.036	264	0.8%	
avg. ±stdev.	0.8 ±0.9	202 ±1	188 ±194		153 ±96		
Overall (avg. ±stdev.)	1.3 ±1.3	233 ±37	299 ±299		157 ±69		

*average=avg.; stdev=standard deviation; Reynolds (v=1.00e-6, at 20°C); TS=total solids; *pipe without sand bed.*

The accumulated LD masses were influenced by flow velocities (Figure 4-4). Within the range of 1 to 2 cm/s (box 2 in the figure), LD build-up masses increased with flow velocity, while for velocities lower than 1 cm/s (box 1 in the figure) and higher than 2 cm/s (box 3 in the figure), lesser accumulation was observed. On the other hand, and despite the low number of results (n=9), a strong relationship (r^2=0.97) was observed between the estimates of the retained fraction of influent water particle loads and the tested flow velocities. Hence, within a certain range of velocity, LD build-up was promoted and increased with flow. The contrary may have occurred at velocities which shear stresses surpassed threshold values for LD disaggregation and detachment. In addition, given that LD build-up rates and accumulation did not accompany the incoming particles' loads (Figure 4-4) particle formation, aggregation and growth may have played a role in LD build-up. Therefore, settling may have been the prevalent process in LD build-up at low velocities; whereas particle attachment and LD disaggregation/resuspension may have been predominant at the medium and high velocity ranges, respectively.

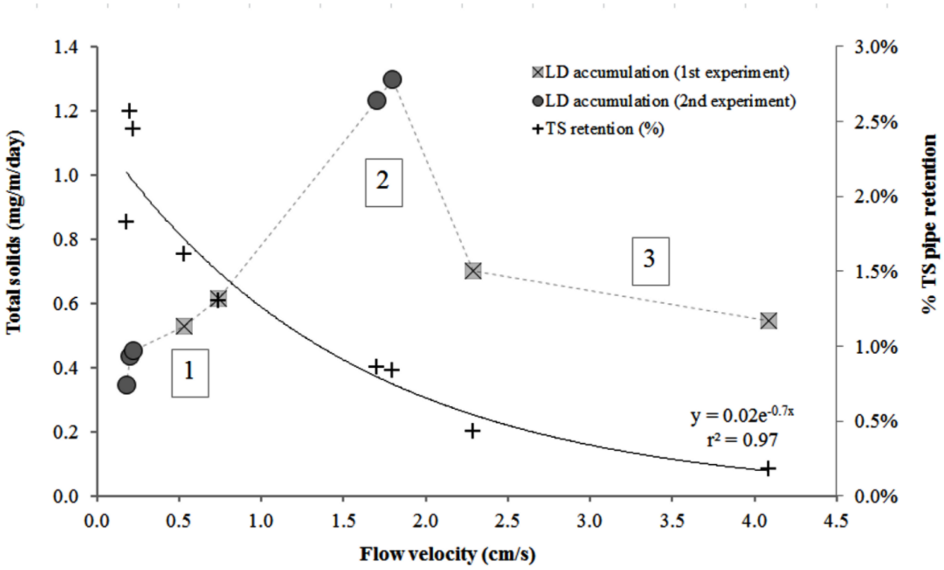

Figure 4-4. Accumulated LD masses (mg/m/day) and TS pipe retentions (%) at the tested velocities (cm/s). Boxes: 1, 2 and 3 label the low (< 1 cm/s), medium (1-2 cm/s) and high (> 2 cm/s) velocity ranges.

The relative LD build-up rates (mg/m) occurring in the test rigs were then compared to those estimated for the network (n=7 pipes) and obtained with the RPM method. On average, total LD build-up masses in the test rigs (157 ±69 mg/m) were comparable to those found for the network pipes (148 ±72 mg/m). Then again, to approach LD accumulation levels at laminar and at turbulent regimes, shear stresses occurring in the test rigs and in network pipes were estimated. Accordingly, given the quotients between τ values in network pipes (τ=0.0172 and τ=0.0133, at 6 cm/s) and in test rigs (τ=0.0064, at 1.8 cm/s) from Table 4-2, and only considering shear stress effects on accumulation, LD build-up rates could have been two to three times faster in the test rigs than in the network pipes. Likewise, LD build-up rates could have been similar at 4 cm/s and at 6 cm/s, respectively.

Table 4-2. Estimated coefficients, head-losses and shear stresses for network LD and test-rigs' pipes.

	Velocity (cm/s)	**Ø (m)**	**δ (m)**	**Chézy coefficient**	**τ (N/m²)**	**Reynolds (Re = v . Ø/ ν)**
Network LD	6.0	0.080	2.94E-03	45	0.0172	4.772
		0.200	3.32E-03	52	0.0133	11.930
	20.0	0.080	1.04E-03	53	0.1376	15.907
		0.200	1.15E-03	60	0.1100	39.767
	Velocity (cm/s)	**Ø (m)**	**Length (m)**	**Head loss (m)**	**τ (N/m²)**	**Reynolds (Re = v . Ø/ ν)**
Test-rigs' LD	0.5	0.023	2	6.73E-05	0.0019	114
	1.8			2.29E-04	0.0064	412
	4.0			5.20E-04	0.0147	915

Ø=pipe diameter; δ=boundary layer; τ=shear stress; Reynolds (ν=1.00e-6, at 20°C).

4.3.3 LD composition

Overall, the organic fraction and iron contents exhibited similarities within the developed LD at all tested velocities (Table 4-3). On average, LD contents had 330 mg ±63 mg VS/g and 247 ±58 mg Fe^t/g in the first experiment (*ca.* 9 months long) and 246 mg ±38 mg VS/g and 133 ±27 mg Fe^t/g in the second experiment (*ca.* 7 months long). While flow velocity influences on LD composition were not observed, different VS and Fe^t contents in LD were found in the two experiments, thus suggesting that LD composition may change during accumulation. As inflow water temperature was 18.3°C ± 1.8°C in the first experiment and 19.1°C ± 2.4°C in the second one, the influence of water temperature in the experiments could not be observed.

Table 4-3. LD composition and repose angles.

	Flow-velocity (avg. cm/s)	**Water contents (%)**	**VS (mg/g)**	**Fe^t (mg/g)**	**PT (mg/g)**	**PL (mg/g)**	**Repose angle °**
1st experiment	0.53	N.A.	333.3	310.5	21.9	13.9	N.A.
	0.74	N.A.	375.0	271.3	20.4	20.4	N.A.
	2.29	N.A.	371.0	175.3	25.3	14.9	N.A.
	4.09	N.A.	240.0	230.9	5.6	25.4	N.A.
avg. ±stdev.	1.9 ±1.7		330 ±63	247 ±58	18 ±9	19 ±5	
2nd experiment	0.18	92%	257.1	122.7	76.8	65.5	33 *
	0.20	92%	295.5	108.7	82.5	94.1	51
	0.22	93%	262.3	135.4	32.5	50.2	54
	1.70	87%	203.6	178.2	24.1	32.3	61
	1.80	90%	213.7	119.4	30.3	56.9	52
avg. ±stdev.	0.8 ±0.9	91 ±2%	246 ±38	133 ±27	49 ±28	60 ±23	50 ±10
Overall (avg. ±stdev.)	1.3 ±1.3		284 ±64	184 ±72	35 ±26	42 ±27	

*average=avg.; stdev=standard deviation; VS=volatile solids; Fe^t=total iron; PT=total protein; PL=polysaccharides; *pipe without sand bed; N.A.=not analysed.*

With respect to EPS constituents, PT and PL contents in LD were apparently not influenced by flow velocities, but were higher in younger (49 mg ±28 mg PT/g and 60 mg ±23 mg PL/g) than in older LD (18 mg ±9 mg PT/g and 19 mg ±5 mg PL/g). In the second experiment, irrespectively of LD flow velocity, water contents averaged 91% of the collected LD masses.

4.3.4 LD repose angles

Repose angle assays were initially devised to appraise possible increases in cohesiveness within the sand bed grains due to LD coating. However, no differences could be observed between sand bed repose angles of control pipes (t = 0 days) and those of the experiments (*ca.* 33°). During pipe tilting, LD aggregates deposited on sand beds' surfaces were carried by these, whereas those accumulated downstream were dragged by the falling sand. Upstream, even if sand movements may have caused flow currents in the pipe, LD did not fall or disaggregate. Instead, LD started falling in "chunks" (see: video, supplemental file) at tilting angles from 51° to 61° (Table 4-3). Sand beds complete removal occurred at angles from 46° to 52°, while upstream LD removal occurred at 61° to 69° angles. In the pipe without sand, LD accumulated downstream (last 50 cm of the pipe length) started falling at *ca.* 33°. Alike in the other pipes, LD complete removal over the pipe whole length was only observed at *ca.* 65°.

Repose angles were then plotted against the levels of analysed LD components[3]. Despite few results (n=4 pipes), the coefficient of correlation ($r^2 > 0.65$) found between

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[3] See Annex 1 – supporting information on statistics.

LD repose angles and their contents in Fe^t and polysaccharides suggested that some level dependency may have occurred between these two variables (Figure 4-5). Therefore, it can be hypothesized that iron contents may have contributed to increases in the stickiness levels of LD, due to the coagulant proprieties of iron, while polysaccharides may have contributed to lower particle densities, owing to higher water retention. However, no correlations were found between repose angles and LD contents in VS or PT (results not shown).

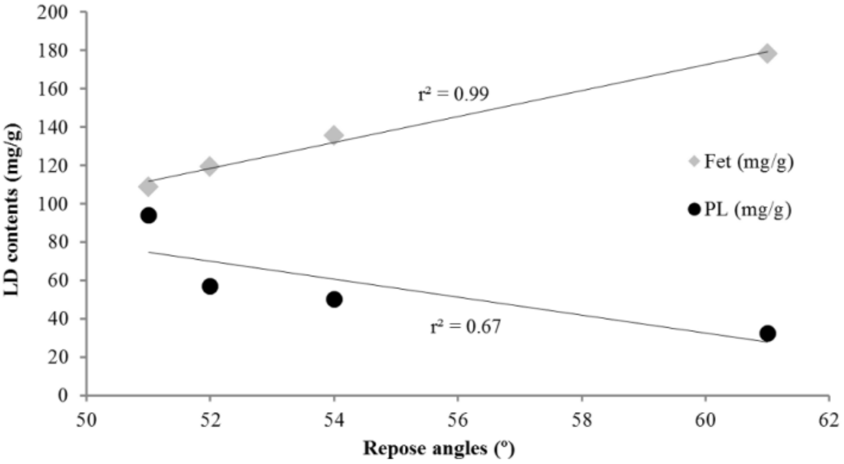

Figure 4-5. Repose angles against LD iron (Fe^t) and polysaccharides (PL) contents.

4.4 Discussion

The flocky appearance and spotted accumulation of LD aggregates (Figure 4-2 and Figure 4-3) differs from what has been described for turbulent flows (Vreeburg and Boxall 2007, van Thienen et al. 2011), namely that LD accumulate evenly in “layers” over the pipe surfaces. However, it agrees with the reported “cohesive-like behaviour” of LD (Boxall and Prince 2006, Vreeburg and Boxall 2007, Husband et al. 2008), which was made evident by the LD repose angle assay (supplemental file).

Despite that the pipes used in the experiments were operated at close to steady laminar flow conditions, where viscous forces may predominate, flow conditions in DWDS also lead to LD floc assemblages of dissimilar sizes (Poças et al. 2013a). In fact, this, together with the occurrence of spotted accumulation patterns in DWDS, may explain some of the unsteadiness occurring in water turbidity profiles observed during routine pipe cleaning (Vreeburg and Boxall 2007), or during normal operation (Blokker 2010, van Thienen et al. 2011), where accumulation is hindered beyond certain flow velocities.

The similarities in accumulation levels of LD in the test rigs and those estimated for the network pipes were not expected, as total TSS loads going through the test rigs were less than in the network pipes. However, at steady-state (Husband et al. 2008) laminar flow conditions, LD can accumulate faster, while at intermittent “self-cleaning” velocities LD can resuspend easier. Then again, smaller surface areas are available for LD deposition in the test rigs (Ø=0.023 m) than in the network pipes (Ø≥0.080 m), thus allowing for thicker layers of LD in the former.

Overall, results suggested that particle settling and attachment are important processes for LD build-up, at least under low velocities and at laminar flows. At higher velocities, due to disaggregation and resuspension/transport of LD floc assemblages, lower accumulation may occur. The extent to which each process occurs and, thus, LD accumulation, may be governed by flow regimes and patterns. As LD build-up progresses, waterborne particles may attach or LD may resuspend and further re-accumulate or reach the consumers' tap. Accordingly, results agree that cohesive-adhesive forces play an important role in LD build-up rates, profiles and behaviour. Despite being operated at laminar flows, test-rigs allowed to evidence that discolouration LD may develop and accumulate continuously, rather than only occurring due to single and localised events.

4.5 Conclusions

The build-up of discoloration LD in transparent pipes supplied with tap water in flow through mode was followed and quantified. Ran at close to steady laminar regimes, test rig pipes covered different velocity ranges. On the whole, results evidenced that:

- flow velocities did not influence LD composition;
- LD may develop and accumulate in spots, rather than as continuous layers;
- LD build-up rates may be favoured at certain flow velocity ranges;
- the nucleation type of growth observed during the experiments and the adhesive cohesive properties of EPS suggest that, apart from settling, other phenomena (*e.g.*, capture, aggregation) may have contributed for LD accumulation.

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5 Total heterotrophic bacteria in loose deposits from drinking water distribution systems

Abstract

Loose deposits (LD) accumulate in drinking water distribution systems (DWDS) and may lead to tap water discolouration incidents, upon resuspension. While inconvenient for the consumers and the water companies, discolouration may be accompanied by degradation of the microbiological quality of the water, and possibly to the transport of bacteria. The affinity of heterotrophic bacteria towards LD with different characteristics was investigated. Bacterial loads in discoloured water samples and LD concentrated over different settling times were analysed. Total heterotrophic numbers did not relate with turbidity or suspended solids in discoloured water. Higher affinities of heterotrophic bacteria were observed for slower-settling LD (< 24 h), which were also richer in microbial extracellular polymeric substances-EPS. The detection of viable iron-oxidizing bacteria in LD supported their possible roles in LD formation and microbial growth/regrowth in DWDS. Results suggest that LD may exhibit low affinities to hold and transport bacteria, at least heterotrophic bacteria and in chlorinated supplies.

*Adapted from:

Poças, A., Napier, V., Neto, C., Ferreira, E., Benoliel, M.J., Rietveld, L.C., Vreeburg, J. and Menaia, J., 2014. Affinity of heterotrophic bacteria to loose deposits in drinking water distribution networks *(under review)*. Water Science&Technology: AQUA.

5.1 Introduction

Tap water discolouration is a frequent reason for customers to complain (Husband and Boxall 2011), particularly when recurrent. Discolouration arises from hydraulic perturbation and consequent resuspension (Vreeburg and Boxall 2007) of loose deposits (LD) that accumulate in drinking water distribution systems (DWDS). Generally identified as cast-iron corrosion particles (Benson et al. 2012), owing to the yellow to brownish turbidity of discoloured water, LD occurrence is often perceived as an aesthetic problem solely. However, as LD may adsorb substrates and nutrients, these may also be considered as prime-sites for bacterial growth (Batté et al. 2003), including the hosting of pathogens, *e.g. Legionella* spp. (USEPA 2001). In fact, many problems in DWDS may be related with microbiological growth/regrowth, *e.g.*, biofilm formation, LD accumulation, microbial influenced corrosion, opportunistic pathogens (Gauthier et al. 1999, Zacheus et al. 2001, Batté et al. 2003, Liu 2013). With respect to LD accumulation and further resuspension, discolouration incidents are often related with the microbial deterioration of drinking waters (Batté et al. 2003, Lehtola et al. 2004).

Possible sources for LD in DWDS include particles coming with upstream waters, or formed within the distribution network, *e.g.*, through precipitation of bulk water colloidal and dissolved materials, pipe scaling and/or scouring, or biofilm detachment or sloughing (Gauthier et al. 1999, McNeill and Edwards 2001, Vreeburg and Boxall 2007, Husband and Boxall 2011). In addition, and apart from pipe corrosion, LD may enter the DWDS during pipe repair or rehabilitation. Nonetheless, despite these so many LD origins, and even if their formation mechanisms have not been comprehensively identified yet, discolouration LD typically have oxidized forms of iron and organic compounds (volatile solids) as main constituents (Gauthier et al. 1999, Barbeau et al. 2005, Vreeburg and Boxall 2007, Poças et al. 2013b).

Microbial extracellular polymeric substances (EPS) have also been reported as important LD constituents (Poças et al. 2013a). These, among a diversity of possible origins (*e.g.*, raw water, pipe biofilms), may be produced by iron-oxidizing bacteria (FeOB) growing on appendages impregnated with iron oxides. These bacteria, apart from being associated with discolouration (Ridgway et al. 1981, Li et al. 2010), may also produce organic substrates for microbial regrowth (Lehtola et al. 2004) during chemolithoautotrophic growth (Emerson et al. 2010). While embedded within the polymeric matrices of released or produced EPS' sheaths, microorganisms may then grow protected against residual disinfectants or other oxidants (Flemming et al. 2007). Furthermore, EPS may contribute to the hydrogel-floc nature of LD (Poças et al. 2013a), including their high water contents and cohesive and flocculant properties (Vreeburg and Boxall 2007, Husband and Boxall 2010, Abe et al. 2012, Douterelo et al. 2012), and provide different sorption-sites for bacterial development and growth. While hydrogel-flocs (Poças et al. 2013a) with high interstitial water contents and permeability, different affinities towards bacteria may thus be observed within the accumulated LD, particularly when compared to "true particles", such as sand grains or corrosion derivatives.

Although described as prime-sites for bacterial regrowth in DWDS (Batté et al. 2003), the ability of LD leading to discolouration events to hold and transport bacteria is not clearly understood yet. With the purpose of evaluating the potential of discolouration LD for bacterial attachment and transport, the affinities of total heterotrophic bacteria towards LD with different characteristics (*e.g.*, settling velocities, composition) were studied. The presence of viable FeOB, due to their role in LD formation (Ridgway et al.

1981) and possible impacts in the bio-stability of DWDS' water (Emerson et al. 2010), was also investigated.

5.2 Materials and methods

5.2.1 LD sampling

The source water is mainly of surface origin (86%) and undergoes conventional treatment or chlorination only. Sampled locations included dead-end and distribution pipes of different materials (asbestos cement-AC, ductile iron-DI and high-density polyethylene-HDPE) and ages (from 1 to 27 years). Cast-iron pipes, although present in the network (~19%), were not part of the sampled LD sites. 14 large volume samples were collected in sterilised cylindrical containers (30 L) from 12 sampling sites[4] from the Lisbon DWDS, along with unidirectional pipe routine discharges (Poças et al. 2013a, Poças et al. 2013b). Sampling velocities, rather than pre-defined, were those achieved by the hydrant's valve opening, for selective and representative LD collection and characterisation.

5.2.2 LD separation

At the laboratory (Figure 5-1), 1 L aliquots of the large volume samples (W_{0h}) were used for turbidity, conductivity, pH, total suspended solids (TSS) and total heterotrophic (TH) numbers' analyses. For LD separation, the remaining volume of samples (*ca.* 29 L) was left to settle at 4ºC for 24 h. After settling (Figure 5-1), 1 L of top-supernatant water (SW_{24h}) and 2 L of the bottom settled concentrates (LD_{24h}) were collected with a peristaltic pump at a flow rate of 6 L/h. The SW_{24h} fraction was then analysed for TSS and TH. The remaining volume (*ca.* 26 L) was left to decant at 4ºC (Poças *et al.* 2013a, b). After 7 days of settling (Figure 5-1), the supernatant volume was removed and *ca.* 1-2 L of the enriched slurries (LD_{7days}) were collected for LD physical-chemical characterisation.

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[4] Samples were collected from pipes targeted by the water company pipe discharge program, designed to respond to discoloured water complaints or reports. At the two sites whose pipes needed to be discharged again, samples were collected twice, in separated times (> 3 weeks).

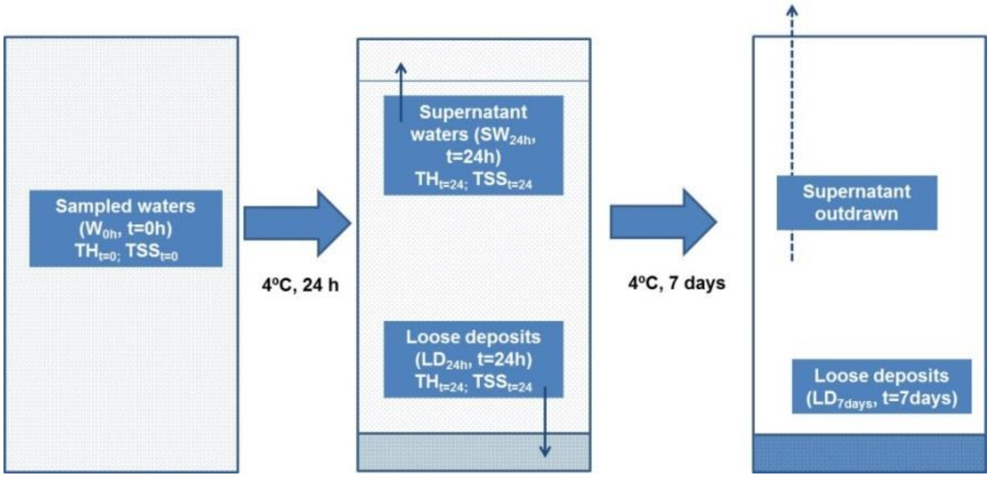

Figure 5-1. Sample separation: initial sampled waters (W_{0h}), supernatant waters (SW_{24h}) and loose deposits (LD_{24h}) fractions over 24 h of settling; and the concentrated slurries (LD_{7days}), over 7 days of settling.

5.2.3 Water and LD characterisation

Turbidity (NTU) was measured in 25 mL borosilicate glass cells in a nephelometer (Turb 555 – 600100, WTW). Conductivity and pH were measured in a multi-parameter potentiometer (Consort, model C863). TSS were determined by the gravimetric method (APHA 1998). Total solids (TS) and volatile solids (VS) were determined by drying at 110ºC to constant weight and ignition at 550ºC (APHA 1998). Total iron (Fe^t) was determined by the phenantroline method (APHA 1998), with adaptations (Poças *et al.* 2013a,b). Polysaccharides (PL) and total protein (PT) were determined by the anthrone (Daniels et al. 1994) and Bradford (Bradford 1976) methods, as described in Poças *et al.*, 2013a.

5.2.4 Microbiological analyses

In W_{0h}, SW_{24h} and LD_{24h}, TH numbers were determined by the most probable number (MPN) method in liquid R2A medium at 22ºC incubated for 7 days. To preserve LD behaviour, and for the comparison of results with previous publications (Zacheus et al. 2001), no sample pre-treatment was used, other than gravitational settling.

An adaptation of the gel-stabilized gradient tubes method (Emerson and Floyd 2005) was used to detect FeOB growth on Fe(II)-EDTA in O_2 gradient cultures (Kumaraswamy et al. 2006). The presence of grown FeOB in the turbidity bands was further confirmed by microscopic observations of Prussian Blue (Pellegrin et al. 1999) stains. Bands' microorganisms were also observed in fresh wet mounts under phase contrast microscopy and tested for Gram staining. Similarly treated smears of fresh *Escherichia coli* (ATCC 25922) cultures were used as controls. Tested inocula (n=7 samples) were obtained during a tap water discolouration event from a household with corroded DI plumbing and from discharges collected from DI (n=4) and HDPE (n=2) network pipes.

5.3 Results and discussion

5.3.1 Sampled waters

Large volume samples (n=14) were collected during routine pipe cleaning at velocities from 0.07 m/s and 0.32 m/s (Table 5-1). Neither turbidity (from 1 NTU to 46 NTU) nor TSS (from 0.3 mg/L to 35.8 mg/L) was influenced by sampling velocity, pipe material, or the water characteristics, as shown by electrical conductivity (from 169 to500 µs/cm) and pH (from 7.5 to 9.9).

Table 5-1. Sampling conditions and results observed for large volume samples.

Pipe material	Pipe age (years)	Velocity (m/s)	pH	Conductivity (µs/cm)	Turbidity (NTU)	TSS (mg/L)	TH counts/L
AC	27	0.25	9.9	169	17	12.6	1.10E+06
DI	10	0.09	8.4	415	21	16.6	2.70E+04
DI	13	0.19	7.9	386	25	11.1	5.40E+03
DI	10	0.21	8.0	386	26	21.5	2.00E+02
DI	11	0.08	8.0	496	6	1.5	3.50E+06
DI	17	0.09	7.5	248	1	1.1	< 2.00+02
DI	10	0.08	7.9	483	43	35.8	1.10E+04
DI	13	0.09	7.9	472	1	0.5	4.90E+03
DI	14	0.09	7.9	500	2	2.0	4.50E+02
DI	8	0.07	7.5	298	5	4.2	< 2.00+02
DI	12	0.32	8.8	253	1	0.3	2.10E+06
HDPE	6	0.18	7.8	438	7	9.0	9.20E+05
HDPE	1	0.18	7.8	373	46	19.0	5.40E+06
HDPE	6	0.20	7.9	392	27	21.0	< 2.00+02
average	11	0.15	8.1	379	16	11.2	1.19E+06
stdev	6	0.08	0.6	102	16	10.6	1.80E+06
max.	27	0.32	9.9	500	46	35.8	5.40E+06
min.	1	0.07	7.5	169	1	0.3	2.00E+02

TH were detected in 11 out of 14 sampled waters at levels from 2.0x10^{2} to 5.4x10^{6} per L. Overall, sampled waters did not carry high bacterial loads, as these were within those typically found for drinking waters, where the average is around 5-10x10^{6} per L (Zacheus et al. 2001, van der Kooij 2003, Allen et al. 2004), either with or without residual disinfectant.

Bacterial loads were not related with samples' turbidity or TSS levels (Figure 5-2). In fact, the highest observed bacterial loads (in the order of 10^{6} per L) occurred both at low (< 10 NTU) and high (> 10 NTU) turbidity levels in sampled waters. This is consistent with previous studies where no clear relationship was found between turbidity and TH counts (Liu 2013). A linear correlation[5] (r^2=0.82 and p-value < 0.05) could be found between turbidity and TSS, which may suggest a similar LD behaviour in the collected samples. Therefore, and taking into account the range of TH numbers in the samples,

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[5] See Annex 1 – supporting information on statistics.

LD bacterial content may have varied, not with water turbidities in samples (Figure 5-2), but possibly because LD were different in composition or age.

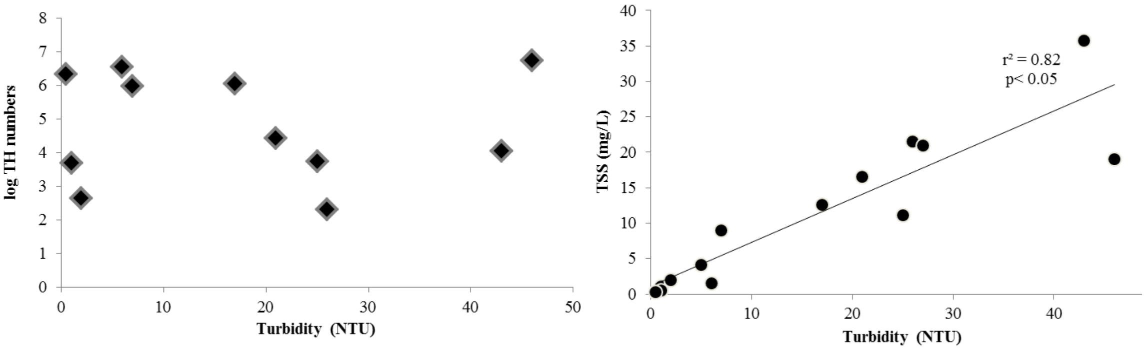

Figure 5-2. Turbidity and: logs of total heterotrophic-TH counts (on the left) and total suspended solids-TSS (on the right) in sampled waters.

5.3.2 Loose deposits

Over the 24h of settling, average turbidity at the supernatant fraction was 6 ± 3 NTU, while that in the settled LD fraction was 91 ± 182 NTU. This shows there were still deposits settling after the first 24 h, which is consistent with previous studies suggesting complete clarification of discoloured water samples may take place over several days (Poças et al. 2013b).

With respect to the bacterial loads in TH/g, values found in SW_{24h} and LD_{24h} were comparable to those measured in the collected water samples-W_{0h} (Figure 5-3).

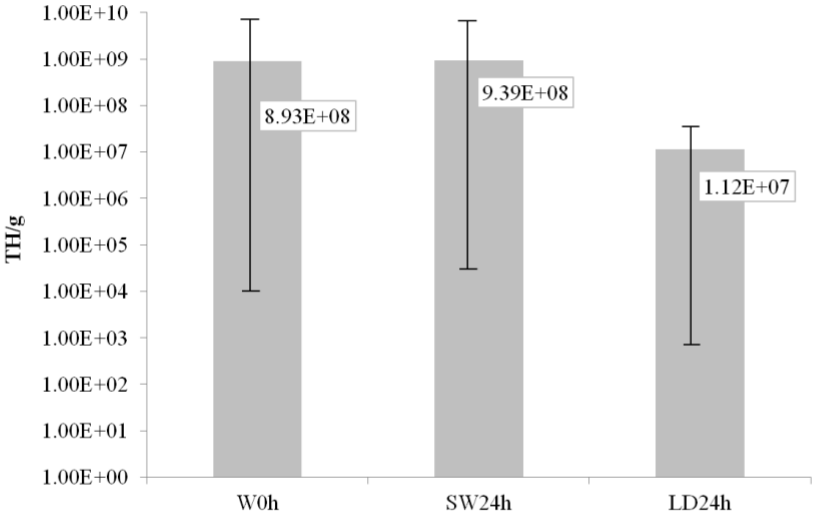

Figure 5-3. Average total heterotrophs (TH) per gram of LD in sampled waters (W_{0h}) and in the unsettled (SW_{24h}) and the settled deposits after 24 h (LD_{24h}). The error bars represent the maximum and minimum, respectively.

The two log difference in the unsettled (SW_{24h}) and the settled (LD_{24h}) LD fractions suggested that the bacterial loads were higher in LD with the slowest settling rates. As TH/g in SW_{24h} and in W_{0h} were also comparable to those reported by Zacheus et al., (2001) in R2A medium ($\leq 10^9$TH/g of LD) done with the same sample treatment and with LD flushed from hydrants, results suggested higher bacterial loads may be found in younger or in "softer" LD. The different LD settling characteristics may have been due to dissimilarities in LD age and, likewise, in LD composition. This is in line with Liu, (2013), that observed that the type of bacteria present in LD differed over accumulation, as well as depended on the amount of LD used for characterisation. Therefore, the differences in bacterial counts per deposit may differ with LD settling rates, or sampling sites, owing to different LD accumulation levels.

After the 7 days of settling, settled LD were used for physical-chemical characterisation. Since only the slowest LD were left to settle after the first 24 h, LD characterisation results mostly referred to the supernatant LD fraction. On average, VS contents were found to be 179 ±103 mg/g and Fe^t 126 ±56 mg/g (Table 5-2). This confirms the occurrence of Fe-rich LD in all pipe types of the DWDS, apart from cast-iron, as sampled pipes were not made of cast-iron. Relatively to the main constituents of EPS (Flemming and Wingender 2001), PT was only detected in four samples and PL found in all samples at considerable amounts (up to 268 mg/g). Although no significant correlations could be observed between LD components and TH numbers (results not shown), higher TH numbers were found in samples which were richer in Fe^t and EPS-PL components (Table 5-1 and Table 5-2).

Table 5-2. Physical-chemical characterisation of LD slurries (LD_{24h}).

Pipe material	VS (mg/g)	Fe^t (mg/g)	PT (mg/g)	PL (mg/g)
AC	131	141	N.D.	135
DI	157	83	N.D.	82
DI	220	141	N.D.	105
DI	133	119	N.D.	141
DI	43	244	N.D.	INT.
DI	263	N.A.	2	268
DI	148	68	N.D.	56
DI	125	N.A.	N.D.	INT.
DI	168	93	4	INT.
DI	67	73	N.D.	74
DI	471	N.A.	16	148
HDPE	250	N.A.	1	35
HDPE	155	192	N.D.	152
HDPE	171	107	N.D.	60
average	179	126	6	114
stdev.	103	56	7	65
max.	471	244	16	268
min.	43	68	1	35

N.D.=not detected; N.A.=not analysed; INT. = interferences.

The different LD settling rates suggested slower settling LD could be closer to soft pipeline young deposits, along with had already been suggested by Zacheus et al., (2001). Likewise, slower LD may be closer to hydrogel-flocs, *i.e.*, flocs with fibrilar matrices and densities approaching that of water (Poças et al. 2013a), apart from the fractal three-dimensional structure. Unlike heavier "true particles" that can settle more rapidly (*e.g.*, pipe derivatives, sand particles), hydrogel-flocs may be easily transported throughout the networks upon resuspension and reach the consumers' tap during discolouration events. Therefore, to analyse the potential health risks upon LD accumulation, not only the microbial identification and quantification are required, but also the probability of sampled LD to reach the consumers' tap needs to be considered, alike in water safety plans methodologies (Bartram et al. 2009).

5.3.3 Iron bacteria

The occurrence of Gram-negative motile rods with the ability for microaerophilic growth coupled to Fe(II) oxidation at circumneutral pH was observed in LD samples from DI pipes, thus suggesting the possible involvement of FeOB in discolouration. Therefore, since chemolithoautotrophic Gallionella spp. are commonly associated with discolouration (Ridgway et al., 1981; Li et al., 2010), carbon fixation products may be formed within the LD and contribute for increases in the water's AOC levels and, consequently, to bacterial regrowth. As detected FeOB grow under low oxygen concentrations (Emerson et al. 2010) it is also possible that, alike within the pipe-wall biofilm EPS matrix, other microorganisms, including anaerobes, survive within the LD floc microenvironments (Liu 2013), where they are protected against residual disinfectants. The assessment of the potential health risks occurring during LD resuspension and tap water discolouration may need, therefore, to be complemented with the enumeration of specific bacterial species, other than TH counts only.

5.3.4 Bacterial affinity towards loose deposits with different characteristics

In this study, bacterial loads were low in the sampled waters ($< 10^7$ TH/L) and did not correlate with discolouration intensity (Figure 5-2). This is consistent with previous studies on LD, where TH counts were not associated with turbidity or TSS, possibly due to particle-associated bacteria and, thus, to TH underestimations (Liu 2013). Likewise, TH counts may only refer to a small fraction (< 0.1%) of the total quantity of microbes present, thus adding difficulties to the finding of correlations.

Overall, these results, although only obtained from a single water supply system, which is also chlorinated, contrast with descriptions of LD as prime-sites for bacterial growth (Zacheus et al. 2001, Batté et al. 2003, Liu 2013). This may be due to the different water characteristics, analytical methods, sample treatment (Liu 2013), or the presence of residual disinfectants (Camper 2004). On the other hand, investigations on the microbiological quality of drinking waters usually disregard LD age in the collected samples. In addition, overestimations on LD potential health hazards may be led by the collection of non-discolouration representative LD. Comparative studies on three-stage samples (*i.e.*, biofilms, LD and the bulk water) should, therefore, address the possible differences in sample residence times or in behaviour (*e.g.*, LD with different settling rates), as well as LD sampling velocities. However, while LD behaviour and sampling velocities may be quantified, sample residence times are more difficult to obtain. As hypothesized herein, lighter LD may refer to the younger LD fraction. If confirmed, the relation between LD behaviour and age could help to estimate sample residence times and, therefore, contribute for a better understanding of the risks associated with tap water discolouration.

5.4 Conclusions

The presence of heterotrophic bacteria to LD was investigated. Bacterial loads in discoloured water samples and LD that were concentrated over different settling times were analysed. Higher affinities were observed for slower-settling LD, which were also richer in microbial polymeric substances-EPS. Overall, results showed that the intensity of discolouration was not related with high bacterial loads, at least for heterotrophic bacteria, in a chlorinated DWDS. Despite this, different bacterial loads, as well as types of bacteria, may be expected in LD with different characteristics (*e.g.*, settling behaviour, composition or residence times) and collected using different sampling methods. Furthermore, in the presence of chemolithoautotrophic iron-oxidizing bacteria, LD can have a role in discolouration and bacterial regrowth in DWDS, including the heterotrophic counts that were found.

Overall, when assessing the microbial health hazards of LD accumulation, not only microbial identification and quantification are required, but also sample residence times and LD behaviour, as well as LD sampling velocities. As lighter LD may correspond to younger LD, better knowledge on the association of LD behaviour with age (*e.g.*, through LD settling velocities) could help to assess the potential health risks arising with tap water discolouration. Similarly, it could provide hints on the necessary pipe cleaning frequencies and support water companies in the designing of LD control programmes.

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6 Discolouration loose deposits: balancing views and practices

Abstract

Tap water discolouration incidents may occur due to resuspension of loose deposits (LD) that accumulate in drinking water distribution systems (DWDS), due to velocity increases or rapid changes. While lowering the consumers' confidence in tap water's safety, they are often a cause of customer complaints, especially when recurrent. Owing to the lack of knowledge on LD dynamics and build-up processes in DWDS, however, LD control and prevention strategies are still ineffective to counteract LD accumulation and prevent tap water discolouration incidents. In this chapter, supported by relevant literature and results from a four year study on LD sampling and characterisation, a conceptual model for LD dynamics and build-up processes in DWDS is proposed. The model description together with the expected LD build-up rates were used to interpret discolouration phenomena. The model's practical implications and recommendations were also discussed, including the potential strategies for LD control and prevention in DWDS. Overall, it was concluded that LD accumulation is most critical at mid-range velocities and that, under this range, it may occur exponentially in DWDS. The possible changes in the relative composition of LDs occurring over accumulation were studied and related with LD resuspension potentials, along with LD ageing and residence times.

*Adapted from:

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6.1 Introduction

Loose deposits (LD) may develop and accumulate in drinking water distribution systems (DWDS) and often lead to tap water discolouration upon resuspension, which can be driven by velocity increases or changes (Vreeburg & Boxall 2007; Husband & Boxall 2011). Alike flow supply interruptions, discolouration is a common reason of consumers' complaints (Seth et al. 2004; van Dijk & van der Kooij 2005) while lowering the confidence on tap water's quality and safety, particularly wherever recurrent. As consequence of their typical yellow to brownish colour, LD are generally associated with cast-iron corrosion particles (McNeill & Edwards 2001; Benson et al. 2012) and mostly seen as an aesthetic problem solely. However, LD accumulation may be associated with bacterial attachment and regrowth in DWDS (USEPA 2001; Batté et al. 2003), as discolouration incidents may also lead to degradation of drinking water quality upon LD resuspension (Zacheus et al. 2001; Lehtola et al. 2006).

Discolouration events may occur in every DWDS, including those devoid of cast-iron pipes (Vreeburg et al. 2008), and irrespectively of the water characteristics or the presence of residual disinfectants (Gauthier et al. 1999; Zacheus et al. 2001). Particles in DWDS (Vreeburg & Boxall 2007; Husband & Boxall 2011) may come with source water (Gauthier et al. 1999; Lehtola et al. 2006) and pass through, or be originated, at the water treatment plant (WTP) (Smith et al. 1997; Gauthier et al. 2001); be produced in the distribution network, being released through pipe scaling, or corrosion (McNeill & Edwards 2001; Benson et al. 2012) and biofilm sloughing (LeChevallier et al. 1987; Batté et al. 2003; Liu et al. 2013), or occur due to chemical precipitation/flocculation of dissolved/colloidal materials (Sly et al. 1990). Alternatively, particles may enter into DWDS during pipe rehabilitation or repair operations. Aiming at controlling LD levels and prevent discolouration incidents, routine pipe cleaning (Carriere et al. 2005; Vreeburg et al. 2009) and on site interventions (*e.g.*, changing connections, pipe replacement) are usually performed. LD monitoring may be approached by using the resuspension potential method – RPM (Vreeburg & Boxall 2007), particle counting or turbidity measurements. These diagnostic approaches, although useful to identify critical zones for LD accumulation, to analyse effectiveness of pipe cleaning strategies and to assess the pipe's potential for discolouration, are short in providing information for the understanding of LD origin and behaviour in DWDS. As so, knowledge on LD's physical-chemical characteristics and behaviour at more detailed levels is required, in order to better interpret LD occurrence in DWDS (Poças et al. 2013c). In addition, more adapted strategies for LD management and control are necessary.

Recent publications on discolouration incidents in DWDS (van Lieverloo et al. 2012, Poças et al. 2013a, Poças et al. 2013b) have focused more on the relevance of light/soft deposits (Zacheus et al. 2001), rather than on heavier particulates (*e.g.*, sand grains). Unlike these, light LD flocs, that have densities close to water (Poças et al. 2013a) and sizes varying between 1 and 25 µm (Boxall et al. 2001, Verberk et al. 2006), accumulate in DWDS through build-up processes other than gravitational forces only (Vreeburg and Boxall 2007). However, little is still known about the mechanisms underlying discolouration and LD build-up processes in DWDS, or the practical implications of having such light floc materials travelling in the networks. On the other hand, the importance of LD main components, *i.e.*, iron and volatile solids (VS), for the development of cohesive discolouration layers in distribution pipes (Vreeburg and Boxall 2007), has not yet been clearly understood.

In this chapter, supported by the relevant literature on the subject, as well as by the main results obtained during a four year study on LD sampling and characterisation in the Lisbon's DWDS, a conceptual model for LD dynamics and build-up processes in DWDS is proposed. In addition, the practical implications and recommendations driven by such model are discussed, with the aim of devising potential strategies for LD control and prevention in DWDS.

6.2 Methods

LD were collected in DWDS from hydrants during pipe routine discharges. For discolouration LD representative sampling, Poças et al., (2013b) describe a low velocity methodology that is based on the collection of large sample volumes ($\geq$ 30 L) at the velocities achievable by the opening of the hydrants' valves. The low-velocity sampling methodology was used to characterise over 70 samples, collected from the Lisbon DWDS. LD physical-chemical characterisation followed the methods described in Poças et al., (2013a,b). The results were compared to relevant literature and translated into a model.

6.3 Results and discussion

6.3.1 Loose deposits characterisation

Results from the sampling in Lisbon DWDS showed that LD composition was similar to that reported elsewhere (Gauthier et al. 1999, Zacheus et al. 2001, Vreeburg and Boxall 2007), not only with respect to the typical main components (Poças et al. 2013a), but also in their relative contents (*ca.* 500 mg of total iron and *ca.* 160 mg of volatile solids per g of LD). In addition, LD's composition was not influenced by the onsite characteristics, such as pipe material or the type of connection (main or dead-end), and showed no relationship with the distance to the WTP. With respect to Fe contents, high levels (> 400 mg/g) were found in pipes of all kinds of materials. Thus, no tendency for more iron-rich LD was observed in cast-iron pipes. This is further supported by the fact that in the Lisbon's DWDS only *ca.* 19% of the pipes are made of cast-iron, as well as by the reported literature, where it is shown that discolouration incidents may occur in DWDS, irrespectively of pipe material (Vreeburg and Boxall 2007).

A number of studies have investigated the interpretation of the behaviour, composition and transport of LD in DWDS (Husband et al. 2008, Vreeburg et al. 2009, Blokker 2010). While some have focused more on LD modelling behaviour and transport (Vreeburg and Boxall 2007, Husband et al. 2008, Husband and Boxall 2011), or on the mechanisms underlying particle accumulation in DWDS, *e.g.*, turbophoresis (Vreeburg 2007, van Thienen et al. 2011); others have focused on the physical-chemical characteristics and behaviour of LD (Gauthier et al. 1999, Boxall et al. 2001, Poças et al. 2013a, Poças et al. 2014) to interpret LD build-up in DWDS. Either way, it is stated that LD accumulation in DWDS does not occur due to gravitational forces alone, but mostly because of the cohesive and adhesive properties of LD (Vreeburg and Boxall 2007, Poças et al. 2014), which, in turn, may be driven by the presence of iron and VS constituents. As suggested by Poças *et al.*, (2013a), LD may be expected to form upon the association of Fe^{3+} with organic components, *i.e.*, extracellular polymeric substances (EPS), thus providing a "hydrogel-floc" nature and behaviour to LD. These hydrogel floc properties, apart from the high water holding capacity (up to 99%), may confer the cohesive-adhesive characteristics which are required for light flocs, such as LD, to assemble, be accumulated and stay accumulated in the turbulent regimes of DWDS. Unlike turbophoresis (van Thienen et al. 2011), which may be limited to deposition of large particles (> 50 µm) in transport mains, LD accumulation through cohesive-adhesive mechanisms is not size dependent or exclusive of the turbulent regimes. In addition, it suggests that LD accumulation patterns in DWDS may be influenced by LD composition, apart from the flow characteristics.

The lack of consistent relations between LD composition and the network characteristics (*e.g.*, pipe material, location) highlights the importance of the total particle load coming from the WTP for LD accumulation in DWDS, comparatively to the network (*e.g.*, cast-iron corrosion). To further illustrate the importance of the WTP, van Dijk and van der Kooij (2005) showed that with a capacity of 5 million m^3 per year and a residual iron concentration of 0.05 mg/L, the treated water coming from the WTP had the potential load of 500 kg $Fe(OH)_3$ per year to the network. The particle load entering the distribution network pipes may thus be minimised by improving the efficiency levels at the WTPs. Likewise, estimations over the inputs of iron and VS compounds in treated water, *e.g.*, EPS and assimilable organic carbon (AOC), may be useful to evaluate the drinking water's potential for LD accumulation and pipe-wall biofilm development. This applies not only for WTPs under high levels of treatment, where LD build-up may occur (Vreeburg et al. 2009), but also for those with lower treatment levels. Similarly, it may be applied to assess LD build-up potential over treatment upgrades or changes, in order to better interpret the discolouration phenomena, including LD planning and monitoring procedures.

6.3.2 The conceptual model

Based on the results obtained with LD characterisation and behaviour studies (Poças et al. 2013a, Poças et al. 2013b), with pilot-scale studies (Poças et al. 2014), and in other literature (Zacheus et al. 2001, Vreeburg 2007, Vreeburg and Boxall 2007, Husband et al. 2008, Vreeburg et al. 2009, Blokker 2010), a conceptual model for LD dynamics in DWDS was developed (Figure 6-1). In this framework, flow velocity was considered to be the main factor influencing LD build-up in DWDS. The indicated thresholds for flow-velocities are the maximum values that may occur for a few minutes (*e.g.*, 10 – 20 minutes) every day.

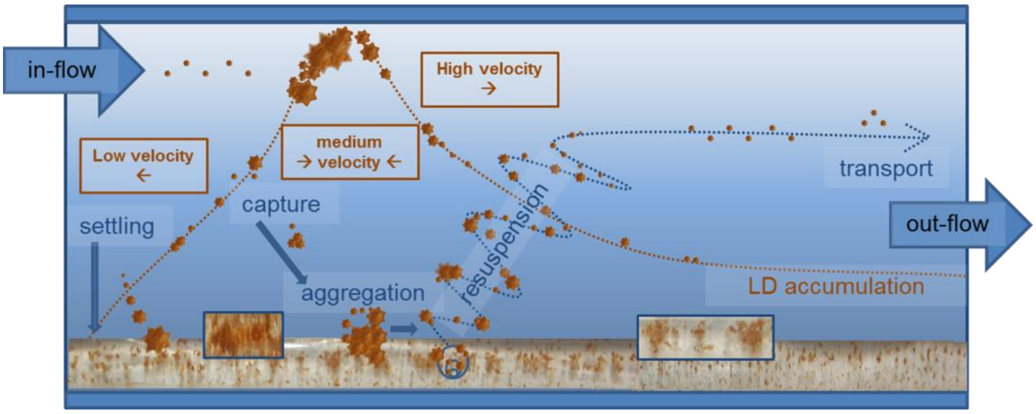

Figure 6-1. Conceptual model for LD dynamics in DWDS. The brown line depicts LD accumulation variation with velocity ranges (low, medium and high). The blue text boxes show the main process occurring at the different velocity ranges. The dotted blue line indicates LD resuspension.

At a low flow velocity range, which may be below 0.01 m/s (Vreeburg 2007, Blokker 2010), the main LD build-up process expected to occur is LD settling through gravitational forces (Poças et al. 2014). In this case, LD build-up rates are low and accumulation depends on the bulk water incoming particle loads, mostly. This may occur in the quiescent zones of the networks or in pipes with large residence times, including connections with no-flow, or whenever the specific-weight of particles (*e.g.*, sand grains) is sufficient for gravitational settling to occur solely. Due to the turbulence that occurs in other parts of the DWDS, however, it is not to be expected that settling through gravitational forces, alone, is the main process for LD accumulation in DWDS (Boxall et al. 2003, Vreeburg and Boxall 2007). This is consistent with the fact that dead-end pips, where steady-low-flows may predominate, are not preferential sites for LD accumulation (Poças et al. 2013b).

At a mid-velocity range, which absolute values may span from 0.01 m/s to 0.3 m/s (Vreeburg and Boxall 2007), LD build-up is unlikely to be linear in time, as it depends on flow patterns, LD properties and occurrence, as well as pipe characteristics. At this velocity range, the main LD build-up process expected to occur is LD capture and further LD aggregation (Poças et al. 2014). This can happen during normal flow operation, due to turbulence and to the cohesive-adhesive properties of LD (Boxall and Saul 2005, Husband and Boxall 2010, Poças et al. 2013a), when the accumulated LD take hold on those still travelling in the bulk water. Thus, provided that there is already material accumulated, LD capture does not depend only on the bulk water loads, but also on the accumulated LD levels, and, as long as these are not at steady-state, LD capture increases with LD accumulation. Because of more inertia at the boundary layers than in the bulk water (van Thienen et al. 2011), LD may then be retained and surpass the flow shear stresses, which extents may be influenced by LD proprieties.

At a high velocity range, where absolute velocities may be above 0.3 m/s (Vreeburg and Boxall 2007), LD build-up may no longer be expected; instead, LD resuspension prevails. The tipping point for LD resuspension, *i.e.*, the critical flow velocity levels at which resuspension may start, depends on: hydraulics, LD physical-chemical characteristics, as well as the levels of LD that have already accumulated. With respect to flow hydraulics, rapid and relative changes in shear stresses (Husband et al. 2008, Poças et al. 2014) may be more effective for LD resuspension than the absolute values, irrespectively of the accumulated LD levels. Relatively to composition, particularly the relative amounts of LD main constituents (*i.e.*, water, iron and VS), interferences may be expected in resuspension potentials, as LD may become denser and more resistant to shear stresses over accumulation (Kjellberg et al. 2009). However, even if resuspension potentials change with LD composition and accumulation levels, and rapid increases in flow velocity are more important than the absolute values, flow velocities at this high range are at self-cleaning values (> 0.3 m/s) (Vreeburg et al. 2009, Blokker 2010).

6.3.3 LD accumulation patterns

The continuous sources for LD build-up in DWDS suggest LD accumulation cannot be described by constant accumulation rates and have a linear development in time, at least at mid-range velocities, where settling is not the main process contributing to LD accumulation. With enhanced LD potentials for aggregation, LD build-up should be closer to an exponential type of development, than to linearity (Figure 6-2, on the left). This has been observed in test rig experiments where LD accumulated faster when there was already material accumulated (Poças et al. 2014) and in RPM measurements done by Vreeburg, 2007, in two different areas: a research area supplied with ultra-filtered water and a reference area supplied with conventionally treated groundwater (Vreeburg 2007). Although there were only few measurements (Figure 6-2, on the right), LD built-up differently at the research area (the more constant profile) and at the reference area (the steeper profile), which was explained by the different particle loads in the supplied waters. Thus, as long as particles with aggregation abilities reach the distribution pipes or stay accumulated, LD build-up rates may no longer approach a linear type of development, but may increase more rapidly over time due to LD aggregation. This suggests that, whenever LD have already accumulated, LD build-up may be critical at places with few cleaning frequencies and lower treatment levels.

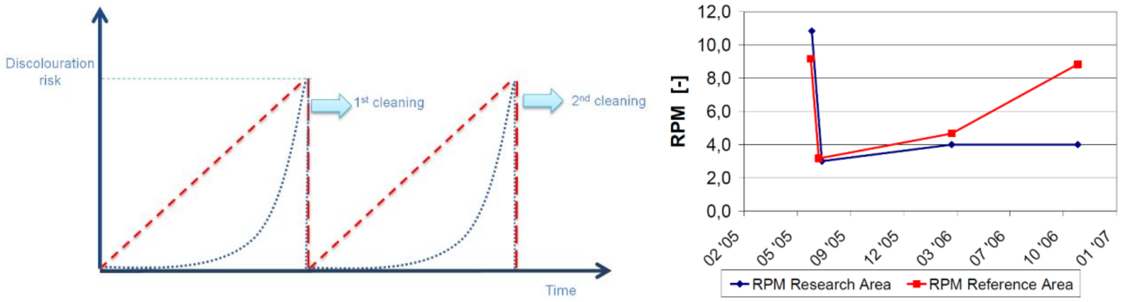

Figure 6-2. Discolouration potential build-up: linear and exponential (on the left; adapted from Vreeburg and Boxall, 2007). Average RPM in a research area supplied with ultra-filtered water - the more constant blue line, and in a reference area with drinking water – the red line with the steep increase (on the right; adapted from Jan Vreeburg, 2007).

Overall, LD build-up may be promoted through smooth flow increments at low velocities/shear stresses, due to LD settling and capture/aggregation, while rapid increases in velocities/shear stresses may lead to LD disaggregation and resuspension and, ultimately, to water discolouration. This is consistent, not only with the cohesive flocculent characteristics that contribute to increased LD aggregation potentials (Poças et al. 2013a), but also with the fact that relatively low velocities (≥ 0.02 m/s) are effective for LD sampling and may, likewise, trigger discolouration (Poças et al. 2013b), as long as shear stress variations are above those occurring on a daily basis. As a result, cleaning frequencies should not be outlined with basis on the absolute outcome of sampling results, but on the relative sharp increases that may occur over LD monitoring. Likewise, instead of waiting for *e.g.*, discolouration complaints or lab monitoring results to trigger pipe cleaning exercises, routine LD monitoring may be used to track LD accumulation patterns and identify the time at which the exponential development really starts. As so, whenever steep increases are observed from one collection to another or the turbidity's threshold value for consumers to detect discolouration (around 10 NTU) is reached, the pipe in study should be marked for priority cleaning. The management of LD build-up development patterns in distribution pipes, *e.g.*, through online turbidity monitoring, may, thus, be used to prevent discolouration incidents, particularly if combined with low velocity sampling methodologies, as these do not require too much of planning or any special equipment (Poças et al. 2013b).

6.3.4 LD characterisation and build-up rates

Due to the aggregation disaggregation features of LD, standard values of LD settling velocities in DWDS may be difficult to find. Since the diameter and density of LD flocs may change over turbulence and depend on the number of available units for aggregation (Poças et al. 2013b) settling velocities may also change over time throughout the DWDS. In addition, because flow velocity is considered to be the main factor influencing LD build-up in DWDS, LD accumulation and resuspension potentials may change with the relative composition of LD. Likewise, the presence of iron may be related to higher stickiness/cohesive levels and densities in the accumulated LD, while VS components, namely EPS, may be related to higher aggregation potentials, along with three dimensional fractal structures (Poças et al. 2013a, Poças et al. 2014a, Poças et al. 2014b). Therefore, with extended accumulation, and due to increased iron contents, LD may get denser and resist more to shear stresses (Kjellberg et al. 2009), thus allowing for resuspension velocities to shift to the upper mid-range values (≈ 0.3 m/s). Increased EPS levels, in turn, may be related with higher flocculation and water holding capacities, thus resulting in bigger and lighter LD flocs (Poças et al. 2014a) that are, probably, more easily resuspended at the lower mid-range velocities (≈ 0.01 m/s). In addition, the relative composition of iron Fe and EPS in LD may be indicative of their age and residence times. While LD with lower EPS/Fe ratios may have accumulated in DWDS for long periods (*i.e.*, months), those with higher EPS/Fe ratios may have accumulated for shorter ones (*i.e.*, weeks).

As resulting from the balance between settling, capture/aggregation and resuspension, LD may then form and accumulate at different rates at different parts of the networks. Therefore, there can be pipes more prone for LD build-up (*e.g.*, pipes with steady flow patterns and operating at mid-range velocities) while others are more for LD resuspension (*e.g.*, pipes with flow fluctuations, velocity changes, daily peaks leading to LD erosion). Either way, wherever LD build-up, LD potentials for further LD accumulation are increased, due to LD capture/aggregation (Poças et al. 2014b). In addition, due to the predominance of velocities taking place at the mid-range velocities (0.01 – 0.3 m/s), LD capture may be the main process contributing for LD build-up processes in DWDS, rather than settling. In order to outline possible strategies for LD control and prevention, the critical flow velocities at which LD capture and aggregation occur in DWDS need, therefore, to be investigated.

6.4 Conclusions

In this research, experiments and observations led to the conclusion that LD accumulation and resuspension in DWDS are driven by continuous (*e.g.*, the particle load from the WTP, pipe-wall biofilm sloughing) and dynamic (*e.g.*, LD capture/aggregation, exponential LD build-up, LD relative composition) processes. More specifically, results evidenced that:

- LD accumulation may follow exponential development in DWDS, due to LD capture and aggregation. Hence, sampling velocities should not be outlined with basis on absolute values, but on the tracking of relative sharp increases over LD monitoring;
- LD accumulation is most critical at mid-range velocities (0.01 – 0.3 m/s), *i.e.*, at the common velocities occurring in DWDS and it is not due to LD gravitational settling. This supports observations that dead-ends are not preferential sites for LD accumulation;
- the combination of minimisation of the particle load coming from the WTP and the application of self-cleaning principles to DWDS may reduce more effectively the overall particle budget and, thus, LD occurrence in DWDS;
- the relative composition of iron and EPS in LD may be indicative of their age and residence times, as well as their potentials to stay accumulated or resuspend in DWDS.

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7 General conclusions

The aim of this thesis was to contribute to a better understanding of loose deposits (LD) build-up, characteristics and behaviour, including the possible effects on the bacteriological water quality upon LD resuspension, in order to improve knowledge on the practical implications of discolouration LD in drinking water distribution systems (DWDS).

The research objectives of the thesis were the following:

- to develop a methodology for representative, qualitative and practical sampling of LD in DWDS;
- to apply the developed methodologies for LD physical-chemical characterisation and behaviour studies;
- to study the influence of physical-chemical characterisation in LD build-up and formation processes;
- to investigate the potential of LD to harbour and transport bacteria, including the possible role of bacteria in discolouration;
- to develop a conceptual model to explain LD dynamics in DWDS and reflect on the practical implications brought out by such a model, including LD monitoring and prevention strategies.

7.1 Practical sampling of LD in DWDS: collection of samples representative of the discolouration phenomena

A methodology for representative, qualitative and practical sampling of LD in DWDS was developed. The methodology was based on the collection of large amounts of discoloured waters (< 100 L) during daily routine operation (< 1.0 m/s) and was used for the collection and characterisation of more than 70 water samples (chapters 2, 3 and 5). Rather than involving too much of planning (other than the background on LD accumulation), special equipment, or valve manoeuvring, the used methodology allowed LD to be sampled along with the water company's routine monitoring plans. With this approach, sample collection may be facilitated, thus allowing for more samples to be used for characterisation and, thus, more data is available for further interpretation, including the practical implications of discolouration LD accumulation. In addition, lower are the chances of having customer complaints post to sampling, in comparison with that done with the usual cleaning velocity standards (*e.g.*, 1.5 m/s).

The low-velocity sampling methodology that herein is proposed is based on the collection of large volumes of drinking water for LD separation and characterisation. Therefore, and unlike the common methods used for LD sampling, methodology limitations may not be the absolute pipe flow velocities, but the collected volumes used for characterisation, or concentration times, especially if microbiological analyses are included for LD investigation (chapter 5). Low-velocity based methodologies for LD collection and characterisation in DWDS' pipes may then be used to assess composition and behaviour of representative discolouration LD, *i.e.*, those that can reach the tap of the consumers, on a routine basis. Samples collected under these circumstances may be more representative of the daily discolouration events than with traditional cleaning, because of excluding material that can be collected but that it is not contributing to discolouration. However, since flow velocity is not a controlled parameter, but function of the hydrant opening, the levels of perturbation at different locations can be different. For LD monitoring and sampling in general, the developed methodology should be adapted, in order to provide the assessment of LD composition more frequently. For better LD control and management, combination of qualitative and quantitative approaches for LD sampling should, therefore, be considered.

7.2 Physical-chemical characterisation of LD: identification of the typical components of discolouration deposits

The used methodologies for representative, qualitative and practical sampling were applied for LD physical-chemical characterisation. Water discharges were concentrated over 7 days at 4ºC (concentration factors of *ca.* 300 times). LD separation through gravitational settling enabled LD shape integrity and size distribution preservation. Unlike filtering or sieving techniques that may be selective for LD size and morphology, gravitational settling does not compromise further LD behaviour observations (chapters 2, 3 and 5). In addition, as LD sizes may vary with different aggregation levels, separation techniques for LD characterisation may be not applicable for discolouration LD studies. Gravitational settling was only used for LD separation, as LD unlikely accumulate by gravitational forces under the turbulence regimes of DWDS.

Overall, sampled LD showed a composition which may be considered as typical for chlorinated and non-chlorinated networks. While mainly composed of iron and volatile solids (VS), LD showed minor contents of other mineral contents (chapters 2 and 3). With respect to VS components, microbial extracellular polymeric substances (EPS) were systematically present in LD samples (chapters 3, 4 and 5). Their high water holding capacities and capture/aggregation potentials were considered to be the key factors in LD occurrence interpretation (chapters 3, 4, 5 and 6). This extended to observations showing that discolouration LD flocs may be easily resuspended throughout the networks (chapter 2), at the same time that their cohesive and adhesive properties can keep them from resuspension (chapters 4 and 6).

For better understanding of LD occurrence and behaviour in DWDS, the actual role of Fe and VS components in LD needs to be analysed in more detail. Likewise**,** LD composition, as it may provide hints on LD age or their potential to stay accumulated, resuspend or reach the consumers' tap (chapter 6), should be addressed in future studies.

7.3 Build-up and formation mechanisms of LD: the influence of composition in the behaviour of discolouration deposits

LD build-up and formation mechanisms were studied with pilot scale systems (chapter 4) and compared to LD samples collected DWDS, through behaviour measurements (chapters 2 and 5), accumulation profiles (chapter 4) and microscopic observations (chapter 3). The nucleation type of growth in LD formed in the test rigs was consistent with their aggregation-disaggregation behaviour observed for the network LD (chapter 2), as well as with their floc and fractal structures (chapter 3), together with the slow-settling properties (chapter 5). On the other hand, LD accumulation showed to be velocity-dependent, even under subtle velocity changes, at least in laminar regimes (chapter 4).

Floc capture/aggregation was suggested as an important process for LD build-up (chapter 4 and 6). This was consistent with the cohesive and flocculent behaviours of LD, which may be related with the presence of iron and EPS (chapters 3, 4, 5 and 6). In addition, it was consistent with observations showing that LD build-up may occur mostly due to continuous and dynamic processes (chapter 6), rather than localised processes and events, such as cast-iron corrosion or pipe repair.

The relevance of capture/aggregation as LD build-up process should be addressed in future studies (chapters 4 and 6), including the main processes for LD build-up in DWDS. This, in combination with more detailed information on flow profiles and residence times, as well as on LD composition, may be used to evaluate LD accumulation profiles and rates in DWDS, in order to better understand the factors promoting LD accumulation.

7.4 Total heterotrophic bacteria in loose deposits in drinking water distribution systems

The potential of LD to harbour and transport heterotrophic bacteria, as well as the possible effects on the bacteriological water quality upon LD resuspension, were investigated (chapter 5). Overall, the examined samples showed heterotrophic bacteria loads were relatively low in sampled discoloured waters, *i.e.*, in the range of the common values found for chlorinated waters (< 10^6 total heterotrophic bacteria per L). Despite this, differences could be observed in LD with different behaviour, namely LD with different settling rates, possibly because these were richer in EPS contents. Likewise, it was hypothesized that LD composition may be related with dissimilar LD accumulation levels (chapters 5 and 6). Overall, results suggested that discolouration LD, *i.e.*, those that may be experienced by the consumers on a regular basis, may exhibit low affinities to heterotrophic bacteria, at least in chlorinated water supplies. On the other hand, detection of viable iron oxidizing bacteria (FeOB) in LD suggested a possible role in LD formation and microbial regrowth in DWDS, provided that chemolithoautotrophic, *e.g.*, Gallionella spp., are present (chapter 5).

Accordingly, when investigating the potential hazards of discolouration events, not only the amounts and the occurring bacterial species should be considered, but also LD with characteristics and behaviour, including their residence times. Taking into account that LD may occur due to continuous and dynamic processes (chapter 6), the potential of LD to harbour and transport bacteria is also likely to change over time, or be different for different types of bacteria through accumulation. As so, to demonstrate that LD are prime-sites for microbial growth (chapter 5), not only comparisons over the bacterial loads in multiple stage samples (*i.e.*, biofilms, LD and bulk water) need to be considered, but also sample residence times. In addition, the used sampling methodologies need to be comparable.

7.5 Discolouration loose deposits: balancing views and practices

A conceptual model for investigating LD dynamics and build-up processes in DWDS was proposed (chapter 6). In this model, flow dynamics was considered to be the main process influencing LD accumulation. The conceptual model practical implications, including the potential strategies for LD control and prevention in DWDS, led to the conclusions that LD accumulation is critical at mid-range velocities (0.01 – 0.3 m/s) and that LD may build-up exponentially in DWDS. In addition, LD build-up may occur mostly due to dynamic processes (chapter 6), other than localised causes or events, like pipe corrosion or repair (chapters 2, 3, 4 and 6). Thus, along with minimisation of LD recharges to the network, pipes operating at self-cleaning values should be considered. Investigations over the potential of drinking waters for LD accumulation in the networks are, therefore, needed to restrict extensive LD accumulation (chapter 6).

The importance of composition in LD build-up was also investigated (chapters 4, 5 and 6). The effects of the typical components of LD (Fe, EPS) in build-up, namely their potentials for accumulation or resuspension (*e.g.*, floc density, stickiness, and water contents) were associated with LD age and residence times (chapters 4, 5 and 6). Overall, the combination of a better knowledge on the occurring flow patterns (chapters 2 and 6), namely at the most critical velocities (chapters 4 and 6), and LD composition and residence times (chapters 3 and 5), may be used by water companies to improve and adapt pipe cleaning programmes and, thus, prevent more effectively tap water discolouration incidents.

7.6 Take-home message

The appearance of discoloured water at the tap is one of the most frequent reasons for consumers to report complaints to the water companies, especially if recurrent. Limitations over the interpretation of the discolouration phenomena include the lack of representative methodologies for LD sampling and understanding of LD behaviour, as well as the relevance of LD constituents in build-up rates and profiles. The herein proposed methodology, even if adaptions are needed (*i.e.*, in terms of practicability and linked to the daily procedures of the water companies), may be used to sample and characterise qualitatively discolouration LD on a routine basis. Likewise, it may be used by water companies to improve their knowledge on LD accumulation processes, as well as to investigate the potential health hazards that may occur at the consumers' tap due to LD resuspension.

The described features of LD, namely their hydrogel nature, fractal structure, aggregation-disaggregation abilities and capture/aggregation proprieties may explain how such light particulates may accumulate and resuspend over the DWDS, irrespectively of the water or pipe characteristics. Likewise, LD may exhibit densities close to the water, which can explain how they can be easily transported through the networks at the daily flows. The fact that LD may be formed and resuspend reversibly allows them to occur, be transported and either re-accumulate or cause discolouration in different ways, at different parts of the networks.

As their main constituents (*i.e.*, iron and VS) may be found for LD samples collected from chlorinated and non-chlorinated systems, estimations over the inputs of iron and VS compounds in treated waters may, thus, be used to evaluate drinking water potentials for LD accumulation and pipe-wall biofilm development. This, in combination with LD routine sampling and characterisation, may be used to prioritise district meter areas or pipes needing to be cleaned soon.

The association of discolouration LD to heterotrophic bacteria was studied. Results showed that high discolouration levels in sampled waters may not lead to degradation of the microbial water quality, at least in chlorinated water supplies. This is in line with the general acceptance that discolouration incidents are aesthetic problems to the most.

However, because VS were systematically present as important LD constituents, including bacterial EPS components, the role of the organic contents and bacteria in LD accumulation and resuspension needs to be examined. In addition, when assessing the microbial health hazards of LD accumulation, not only the microbial identification and characterisation need to be accounted for, but also sample residence times and behaviour, as well as LD sampling methodologies. Thus, on a frequent basis, LD should be investigated for potential hazardous levels occurring upon discolouration incidents, and also for the seeking of relations between the found bacterial species and LD composition and build-up rates.

A conceptual model was used to explain LD dynamics and build-up processes in DWDS, including the influence of LD composition in accumulation and resuspension potentials over LD accumulation. The model highlighted the importance of flow hydraulics in LD build-up and that of LD composition to assess LD accumulation and resuspension potentials in DWDS. In addition, it highlighted the role of LD capture/aggregation as main LD build-up process, rather than gravitational settling, which is consistent with the random LD accumulation patterns that may be found in DWDS. The point that settling/deposition may be not the main process contributing for LD build-up may, in fact, suggest a different terminology for addressing the particles/flocs that accumulate in DWDS, instead of "loose deposits". The model, together with adapted low-velocity sampling, characterisation methodologies and standardized methods, may be used to interpret discolouration LD development and phenomena in DWDS, as well as to improve cleaning procedures and frequencies. As so, LD build-up rates may be followed with routine LD sampling at the daily-flow velocities; the most critical velocities for LD accumulation may be studied with basis on flow consumption pattern and residence time modelling; the validation of self-cleaning practices and network redesigns (*e.g.* new district meter areas) may be evaluated through routine and low-velocity sampling, provided that the levels of perturbation are measured and controlled; the relative composition of LD may be used to indicate LD accumulation times (*i.e.*, age and residence times) and resuspension potentials; and online monitoring may be used to track relative sharp increases in LD build-up, and serve as alarm for immediate pipe cleaning.

Overall, the conclusions brought out by this thesis, as herein are summarised, apart from the proposed methodologies for representative qualitative LD sampling and characterisation, may be useful to assess discolouration LD composition and behaviour, as well as to rethink the design, cleaning frequencies and LD control and prevention strategies in DWDS.

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List of abbreviations

AC – Asbestos Cement
AOC – Assimilable Organic Carbon
CI – Cast-Iron
DI – Ductile Iron
DMA – District Meter Area
DWDS – Drinking Water Distribution Systems
EPS – Extracellular Polymeric Substances
FeOB – Iron-Oxidizing Bacteria
Fe^t – Total Iron
HDPE – High-Density Polyethylene
LD – Loose Deposits
NTU – Nephelometric Turbidity Units
PVC – Polyvinyl Chloride
SW – Supernatant Waters
Re – Reynolds' number
RPM – Resuspension Potential Method
TS – Total Solids
TSS – Total Suspended Solids
TH – Total Heterotrophs
VS – Volatile Solids
WTP – Water Treatment Plant

Annex 1 – supporting information on statistics

Along the thesis, statistics were used to attest the levels of independency between two different variables.

To investigate the significance levels of the obtained linear correlations, the coefficients of correlation and determination (*r* and the squared-*r*) were calculated using the minimum square method and/or Microsoft Office Excel tools.

The levels of significance of the correlations between the measured parameters were then analysed by subjecting the linear regressions coefficient (*r)* to a statistical hypothesis test

[a]. Using the normal distribution, at confidence levels of 95% and 97.5%, and by calculating the p-value under the null hypothesis *r*=0, the levels of independency between two variables were then assessed.

With α as the significance level, the following criteria were used to appraise the significance of the statistical test:

Hypotheses	Result of the statistical test	Decision (rejecting the null hypothesis H_0)
$\begin{cases} H_0: \rho = 0 \\ H_1: \rho \neq 0 \end{cases}$	$z = \dfrac{\frac{1}{2}\log\left(\frac{1+r}{1-r}\right)}{\frac{1}{\sqrt{n-3}}}$	$\|z\| > \phi^{-1}\left(1-\frac{\alpha}{2}\right)$

z – statistic test value; r – coefficient of correlation (adapted from Fonseca, J., 2001).

The used formula of the statistical test was derived from Fonseca, J., 2001, where it is demonstrated that it follows a Normal distribution, $\sim N(0,1)$.

The probabilities were obtained with the z-score normal probability table. The respective p-values were calculated as follows:

$$p - value = 2 \times (1 - \phi(|z|)) = \begin{cases} 2 \times (1 - \phi(z)), if\ z \geq 0 \\ 2 \times \phi(z), if\ z < 0 \end{cases}$$

Alternatively, the GraphPad software, which is available for free in the internet, may be used for calculations or to confirm results.

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[a] These formulas were derived from the book (in Portuguese):
Fonseca, J., 2001. Estatística Matemática, Edições Sílabo, Lisboa.

Annex 2 – supporting information on hydraulics

In chapter 4, shear stresses were calculated to investigate and compare the perturbation levels on sediment deposition/resuspension occurring in the test-rig pipes and in the network pipes. In the test-rigs pipes, which operated under laminar flows, the Hagen-Poiseuille's formulations were used to calculate the shear stress. In the network pipes, which operated under turbulent flows, the Chézy-Manning's formulations were used. Flow regimes were characterised according to the Reynolds' number (*Re*), which was computed as following:

$$Re = \frac{V.D}{\nu}$$

Re –Reynold's number; V – velocity (m/s); v – kinematic viscosity (m^2/s).

Reynolds' numbers below 2000 were assumed to be at laminar flow and above 4000 at turbulent flow.

Test-rig pipes: laminar flows[a]

The head loss effect of the wall-friction was estimated by using Poiseuille's equation:

$$hL = \frac{32.\mu.V.L}{\rho_w.g.D^2}$$

hL - head loss (m); μ - viscosity (kg/m/s); V – velocity (m/s); L – length; p_w – mass density (kg/m^2); g - gravitational constant (m/s^2); D – diameter (m).

The shear stress on the pipe-wall was then be calculated by:

$$\tau_w = \frac{(h_L).\rho_w.g.D}{4.L}$$

τ_w – shear stress (N/m^2); h_L - head loss (m); p_w – mass density (kg/m^3); g – gravitational constant (m/s^2); D – diameter (m); L – length (m).

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[a] For laminar flow calculations, formulas were derived from:

Grefte, A. (2005) Behaviour of particles in a drinking water distribution network, MSc Thesis, Technical University of Delft, Delft.

Network pipes: turbulent flows[b]

The shear stress in the network pipes was calculated using the following equation:

$$\tau = -\rho . R . g . \frac{u^2}{C^2}$$

τ – shear stress (N/m^2); p – mass density (kg/m^3); R – hydraulic radius (m); g – gravitational constant (m/s^2); u –flow velocity (m/s); C – Chézy coefficient,

where the Chézy coefficient was be obtained with:

$$C = \frac{18 . \log\left(\frac{48 . R}{\delta}\right)}{\delta}$$

C – Chézy coefficient; R – hydraulic radius (m); δ – the hydraulic condition;

and the hydraulic condition, considering that hydraulic smooth conditions, was calculated using the Newton-Raphson method to solve the following equation:

$$\delta = \frac{12 . v . 18 . \left(\log \frac{48 . R}{\delta}\right)}{\sqrt{g} . u}$$

δ – hydraulic condition; v – kinematic viscosity (m^2/s); R – hydraulic radius (m); g – gravitational constant (m/s^2); u –flow velocity (m/s).

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[b] For turbulent flow calculations, these formulas were derived from:

Lut, M. (2005) Hydraulic behaviour of particles in a drinking water system, MSc Thesis, Technical University of Delft, Delft.

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Curriculum vitae

Ana Poças is Portuguese and was born in Lisbon, June 5, 1982. She made her high-school studies at Colégio Valsassina, in Lisbon, in 2000. Ana started her Environmental Engineering Sanitary Engineering degree-studies at Universidade Nova de Lisboa in 2000 and graduated in 2006. In the last year, Ana followed her Erasmus internship on the subject of drinking water distribution at the Delft University of Technology. From 2006 to 2009, Ana worked in a project at Instituto Nacional de Engenharia e Tecnologia Industrial (INETI – Lisbon) as a research grantee (2006) on the field of anaerobic digestion; at the company Tecnoplano SA (Lisbon) as an Environmental Engineer (2007-2009), and at Ambirumo as freelancer (Lisbon) for planning and engineering design. During this period, Ana also got her MSc degree on the subject of particles in drinking water distribution at Universidade Nova de Lisboa (2008), in collaboration with Delft University of Technology and Oasen Drinkwater (The Netherlands). In 2009, Ana started working at the National Laboratory for Civil Engineering (LNEC - Lisbon) as Ph.D. candidate of Delft University of Technology.