IMPACT OF PARTICLES ON UV DISINFECTION OF WATER AND WASTEWATER EFFLUENTS: A REVIEW

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ABSTRACT

Particles in water sources may be classified in various groups such as living vs. non-living, nano vs. sub-micron to micron sized and mineral vs. organic. Living particles for example comprise of bacteria, viruses, protozoa and algae while non-living particles include mineral particles, organic particles, cell debris and macromolecules. Particles in water sources may be dispersed as single entities or associated with multiple particles in flocs or aggregates. Particles may be further characterized by their chemical nature, size distribution, density, absorbance, scattering and typical gross measurements such as turbidity and suspended solids. Although ultraviolet (UV) irradiation is principally accepted as a primary disinfection technology for use in water and wastewater effluent, still research is needed to understand the extent to which particles in water may hinder the UV treatment efficacy by interacting with microbial pathogens. Both of the phenomena: (1) particles physically associated with microorganisms in a clump or aggregate and (2) particles not physically associated with microorganisms however interact by shielding, absorbing, scattering or blocking UV light, are integrated in the term “particle-microbe interactions”. This review covers the impact of the interaction between microorganisms (mainly bacteria) which are the target for UV disinfection and particles in water and wastewater effluents on the efficacy of UV disinfection.

Keywords: Particles, Ultraviolet, Disinfection, Water, Aggregation, Light, Floc

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I. OVERVIEW OF THE PROBLEM

Ultra-violet (UV) light is used to disinfect various water types such as secondary and tertiary wastewater effluents, stabilization ponds, combined sewer overflows as well as filtered and unfiltered surface waters and ground waters. Research has shown that biological flocs formed in wastewater treatment processes have extremely high UV absorbance and may harbor coliform organisms and shield them from UV light (Emerick et al. 1999) by decreasing light through an aggregate mostly due to absorption (Loge et al., 1999; Mamane and Linden, 2006a; Mamane et al. 2006). In water supplies, however, particles can protect microorganisms from UV inactivation mainly by shielding, scattering, absorbing or blocking UV light and not necessarily by particle association as with effluents. For example, the presence of goethite particles in drinking water protected Escherichia coli and Pseudomonas putida from UV inactivation (Wu et al. 2005).

A pathogen target in wastewater effluents may be either non-particle associated (free swimming) or particle associated (enmeshed within a floc). If the target pathogen is non-particle associated, then the disinfectant can potentially kill or inactivate the microorganism by direct path to the target. However when a particle is harboring a target pathogen, the disinfectant must first pass through associated particles or floc before reaching the target.
microbial cell membrane (with chlorine disinfection) or the DNA (with UV disinfection).

Consequently the effectiveness of any chemical based disinfection technology such as chlorine, chlorine dioxide, ozone (Berman et al. 1988; Sobsey et al. 1991; Stewart and Olson, 1996; Dietrich et al. 2003; Barbeau et al. 2005; Dietrich et al. 2007), and UV based disinfection technology (as will be discussed herein) drops significantly as pathogens are enmeshed in flocs. Water reuse is feasible only when providing reliable disinfection treatment of wastewater to meet strict water quality requirements according to the intended application, and thus protecting public health. Major concerns for water reuse are, for example, the elimination of enteric viruses and emerging bacterial and protozoan pathogens from the wastewater effluents. The outcome of particle-microbe association is critical as it restricts the maximum log reduction that is achievable, thus reusing disinfected reclaimed wastewater may introduce potential pathogens that were protected within particles.

2. ULTRAVIOLET DISINFECTION

2.1. Natural UV light - Solar spectrum

UV radiation is usually classified into three wavelengths ranges: UVA (320-400 nm), UVB (280-320 nm) and UVC (<280 nm). Most of the UV radiation reaching the earth surface belongs to UVA (99%), since the ozone layer of the atmosphere absorbs the UVC and most of the UVB. The sun’s electromagnetic radiation contains a wide range of wavelengths. The infrared (IR, wavelength above 700 nm) and ultraviolet (UV, below 400 nm) are best known for their capability to destroy or inactivate cells by thermo-chemical and photo-chemical reactions, respectively. IR and UV radiation cause structural and/or functional changes of nucleic acids, proteins and alteration of other cellular components, ultimately damaging the cells in a reversible or irreversible manner (Patterson and Gillespie, 1972; Béliveau et al. 1992; Yoon et al. 2000; Pfister et al. 2005; Berney et al. 2006, 2007).

Photochemical reactions for inactivation are most efficient with wavelengths close to the maximum absorbance (~260 nm) of pyrimidine (thymine, cytosine, and uracil) and purine (adenine and guanine) nucleobases (Ravanat et al. 2001). UVB radiation is a minor part of the solar spectrum; however it has a disproportional metabolic effect on microorganisms (Jansen
et al. 2001). Two possible mechanisms of microbial inactivation in the UVB wavelength range were proposed: (a) direct photolysis (dimer production), and (b) natural occurring indirect oxidative damage through production of intercellular reactive oxygen species (ROS). ROS include species such as superoxide radical, hydroxyl radical, hydrogen peroxide, peroxy, and peroxynitrite. These species are mostly short lived and unstable, and can cause lipid per-oxidation, DNA strand breakage, and oxidative damage in cells (He and Hader, 2002). The toxic effects of ROS results in cellular damage, and not in direct photonic absorption into DNA/RNA (which is negligible in the absence of low-dose UV-B and UV-C radiation) (Khaengraeng and Reed., 2005; Pfeifer et al. 2005; Reed, 2004; Taghipour, 2004; Yoon et al. 2000). Under UVB radiation, the formation of ROS depends on, but is not proportional to, the UVB dose. At high doses of UVB, formation of pyrimidine dimers and other photoproducts that are lethal result in direct cell death (He and Hader, 2002). Due to these issues of atmospheric attenuation and penetration into the water, solar UV radiation by itself is considered to be a weak disinfectant.

This review will cover the impact of particles on inactivation of microorganisms (primarily bacteria) in water by mainly utilizing UVC wavelengths (not UVA/UVB or solar UV). A detailed review on the inactivation of viruses, spores, bacteria and protozoan (oo)cysts in water by UV is given by Hijnem et al. (2006). Templeton et al. (2008) recently published a review on particle associated viruses in water.

2.2. UV lamps

UV disinfection has gained growing acceptance as a primary disinfection process for water since it was found to be very effective for inactivating Cryptosporidium (Clancy et al. 2000; Craik et al. 2001; Shin et al. 2001) and Giardia lamblia (Linden et al. 2002) without forming residual disinfection byproducts produced with chlorine disinfection. Controlling these two protozoan parasites are of major importance for the safety of drinking water and wastewater effluents. The mechanism of disinfection by UV light differs from chemical disinfectants such as ozone or chlorine. Gates (1929) discovered that the relative biological effect of microorganisms towards various UV wavelengths matched the absorbance of the nucleotide bases, with peak absorption close to 260 nm. Ultraviolet light damages the DNA by
dimerizing adjacent thymine molecules, forming a thymine dimer, and inhibiting further transcription of the cell’s genetic code and consequently prevent reproduction of the organism (Setlow, 1967), while the organism itself is not damaged. Thus the energy that is absorbed by the microorganism from the UV source can cause a photochemical effect leading to inactivation. The UV disinfection is only effective when the UV is at a wavelength at which the DNA will absorb the ultraviolet light. This absorption does not occur above wavelengths of approximately 300 nm and the fraction of UV with wavelengths below 200 nm cannot penetrate the water. Thus, the portion of the UV spectrum which is effective in UV disinfection is between 200 and 300 nm and is called the “germicidal range”.

Current technology for water and wastewater disinfection by UV include two basic types of mercury lamps: low pressure (LP) UV mercury vapor lamps that emit single monochromatic wavelength that peaks at 253.7 nm and medium pressure (MP) UV mercury lamps with a broad polychromatic spectrum with output at multiple wavelengths throughout the 220 to 300 nm germicidal UV range and beyond. Full-scale drinking water applications generally use LP, low-pressure high output (LPHO), or MP mercury vapor lamps (USEPA, 2006). Both LP and LPHO lamps operate at lower temperatures and have lower mercury vapor pressures than MP lamps, however LPHO typically use amalgams (alloys of mercury in the solid phase) while LP and MP contain liquid elemental mercury. Figure 1 shows the spectrum output from the LP and MP UV lamp, respectively, between 200 and 400 nm. LP lamps are used in water treatment plants due to the high efficiency at the microbicidal wavelength without producing unwanted photochemical changes in other water constituents (Chiu et al. 1999; Haider et al. 2002). MP lamps in some cases can be advantageous over LP lamps because they have exceptionally high energy output and therefore require less space and fewer lamps in water treatment plants (Marshall, 1999).

Research has also shown that, to ensure permanent inactivation and prevent the recovery of microorganisms following exposure to UV, a broad, “polychromatic” spectrum of UV wavelengths is necessary as they damage not only on cellular DNA, but also enzymes and other molecules responsible for DNA light repair (Oguma et al. 2002; Zimmer and Slawson 2002; Kalisvaart, 2004). Thus it may be advantageous to use polychromatic UV sources in wastewater effluents discharged to the environment or to reservoirs which are further exposed to light.
Fig. 1: Spectra of low pressure (LP) Hg and medium pressure (MP) Hg UV lamps
Mercury free UV technologies are not included in this review, but they may provide effective disinfection such as pulsed lamps and excimer UV lamps. Some disadvantages of mercury based lamps include the time it takes for the lamps to turn on and also the environmental concern of mercury discharge. Pulsed UV (PUV) lamps can be flash-lamp or surface discharge type. A high power electrical pulse is discharged in a micro-second burst to achieve an intense pulse, with a shift in the average spectrum towards shorter wavelengths (compared to MP spectrum). The discharge gas is usually xenon or krypton which is a non-toxic gas. Several theories postulate enhanced inactivation by pulsed compared to continuous-wave multi-spectral light sources per fluence as a result of the high photon flux that overwhelms cellular repair mechanisms, greater photon energy in the pulse and production of hydroxyl radicals (McDonald et al. 2002). The inactivation of Bacillus subtilis spores on polystyrene surfaces showed no differences in the inactivation efficacy when comparing a continuous wave MP lamp to pulsed lamp on fluence based measurement based on the UV-C photon flux. A study by Bohrerova et al. (2008) concluded that the PUV lamp was more effective for inactivation of the phages T4 and T7 and E. coli in buffered water, as compared to continuous-wave LP and MP UV lamps. Thus pulsed UV may have a potential to compete with MP lamps for water disinfection. A recent review on application of modern excimer lamps for water is provided by Oppenlaender (2007).

2.3. Measurement of UV dose (fluence)

A quantifiable and controlled UV dose (fluence) is obtained using a quasi-parallel beam bench scale UV apparatus emitting UV radiation directed through a circular opening onto a horizontal surface to provide incident radiation normal to the surface of the test suspension. The microorganism suspension (in a petri dish) to be irradiated is placed on the horizontal surface below the bottom of the collimator and completely mixed as illustrated in Figure 2.
Fig. 2: Example of bench scale device
(Source: Bolton and Linden (2003).)

In a completely mixed batch reactor the average UV irradiance (fluence rate) is calculated from the measured UV incident irradiance on the surface of the microbial suspension, the UV absorbance of the water, the sample depth and other correction factors (Bolton and Linden, 2003). The UV dose (fluence) that the microorganisms receive is equal to the volume average irradiance multiplied by the exposure time. The radiometer that is used with a collimated beam apparatus measures the incident irradiance. Advantages of the radiometer are fast measurements and high sensitivity, but they cannot resolve the detailed spectrum, thus appropriate for LP systems, and not sufficient for MP lamps. When using polychromatic light, the UV dose calculation in a batch system is more complicated and will also include the intensity at each wavelength (spectral intensity) and the relative germicidal effectiveness at those wavelengths. Thus a spectrometer can be used for obtaining relative spectral irradiance or a spectroradiometer for obtaining absolute intensity (y-axis) of the spectrum by the transformation of measured raw data into the absolute spectrum.

In addition, the use of a radiometer is not suitable for measuring the irradiation in a UV reactor, from an array of UV sources, nor with scattering suspensions, because the radiometer measures irradiation normal to the planar surface of the detector (Rahn et al. 1999). Proper fluence rate measurement – the total radiant power from all directions onto an infinitesimally small sphere – can be approximated using an experimental tool that receives UV photons from different directions (Rahn et al. 1999; Bolton, 2001). One extensively used alternative to traditional radiometry is
chemical actinometry, which is a chemical method that measures a chemical change produced by radiation. The decrease in concentration of the actinometer upon exposure to either monochromatic or polychromatic UV sources is utilized to directly calculate the UV fluence. Examples for actinometers are the iodideiodate process based on a photochemical reaction sensitive to 254 nm (Jortner et al. 1961; Rahn, 1997) and the potassium ferrioxalate actinometer sensitive to variable wavelengths between 200-300 nm (Hatchard and Parker, 1956); however polychromatic UV irradiation is usually not totally absorbed by chemical actinometers. In general, any defined photochemical reaction can be used as a chemical actinometer provided the formation of the photoproduct is straightforward with the number of absorbed photons, and the quantum yields (QY) are accurately known for a large number of wavelengths (Kuhn, 1989).

In the research by Sharpless and Linden (2003), ferrioxalate actinometry was used to quantify the incident photon irradiance. The incident irradiance over the 200 -300 nm range was measured in separate experiments by a chemical actinometer in conjunction with spectral relative measurements with a spectrometer. Shanshan et al. (2006) used uridine and iodide-iodate actinometers in order to compare to the radiometer readings of the MP lamp. Bohrerova et al. (2006a) combined radiometer readings with both iodideiodate and ferrioxalate actinometers.

3. PARTICLES IN WATER

3.1. Origin and nature of particles

One of the most fundamental characteristics concerning impurities in the aquatic environment is the distinction between dissolved and particulate forms, where particles may be of inorganic and organic nature. Dissolved ionic components such as bicarbonate, calcium, magnesium, hydroxyls and sulfates are not considered particles; however they may impact flocculation of particles through complexation and precipitation with components within the floc. Inorganic particles result mainly from natural weathering processes and include iron and alumina oxides and clays, such as kaolinite and silica. Biological-organic living particles include viruses, bacteria, and algae while non-living biological particles include cellular debris (Wilkinson et al. 1997), or dead microbial cells.
Particles may range in size from a few nanometers on the border between dissolved and colloids, sub-micron, micron range and up to millimeter dimension as with sand particles. A distinction is being drawn between colloidal and suspended particles, with an arbitrary boundary of 1 \( \mu \text{m} \) or of 0.45 \( \mu \text{m} \) defined as boundary to suspended solids measurements in wastewater characterization. Colloidal particles vary between 0.001-1 \( \mu \text{m} \), and dissolved constituents are typically smaller than 0.001 \( \mu \text{m} \), however the distinction may depend on quantification method of colloids and dissolved constituents. Organic macromolecules (colloidal and dissolved) mainly originate from biological degradation of plant and animal remains such as humic and fulvic compounds, polysaccharides and proteins. Collectively these substances are known as natural organic matter (NOM). NOM is considered dissolved (i.e. dissolved organic matter - DOM) when filtered thorough 0.45 \( \mu \text{m} \), even though colloids may be in the DOM fraction. The distinction between dissolved, colloids and suspended particles is illustrated in Figure 3.

![Fig. 3: Distinction between solutes, colloidal and suspended particles.](image)

3.2. Particle measurements

3.2.1. Sizing and counting

There are a variety of methods to measure particle size in water such as: (a) microscopy methods including imaging techniques (light, transmission
electron (TEM), scanning electron (SEM)), (b) particle counters (light interaction methods, electrical property methods; automated image analysis) and (c) separation methods (sedimentation, centrifugation, serial membrane filtration, fractionation and classification). Comparison of particle sizing obtained by different methods is difficult. Because the effectiveness of chlorine and UV disinfection is dependent on particle size, this determination has become very important especially with wastewater effluents for reuse purposes (Metcalf and Eddy, 2004). Microscopic methods including image analysis generally correlate the particle morphology to its 2-D equivalent spherical diameter (ESD) or its 3-D volume. While electrical property method correlates a particle's electrical properties to its volume, laser diffraction systems correlates a particle's intensity and angle of scattered light to the volumetric properties of the whole particle population, and laser counters correlate light blocked to particle diameter. Colloids are difficult to size with the standard particles sizers due to their small size range.

The distinction in particle size between solutes, colloidal and suspended particles (Figure 3) relates to particles that are stable and disperse; however this is not the case for all particles in natural and engineered systems. Flocculation of particles, in an aggregation process, results in formation of larger particles from the smaller suspended particles (Droppo et al. 2005). Particles and flocs in wastewater have irregular shapes that exist simultaneously and differ in size (Shubert and Gunthert, 2001). Flocs or aggregates are extremely difficult to size and shape due to their fragile nature that may result in break-up upon sampling, their open architecture, their poorly defined association of particles within the floc, their irregular shape and their 3-D characteristics (Droppo et al. 2005).

In most applications of particle analysis the size is determined and not the shape. Microscopy and image analysis are common methods to characterize also the shape of particles by incorporating a camera with an image analysis processing. The shape of particles as observed by Scanning Electron Microscopy (SEM) may be correlated to the processes wastewater effluents undergo and may correspond to different origins of the wastes, as reflected via the distribution of elements in different flocs and detected by SEM-EDX (energy-dispersive X-ray spectroscopy) analysis (Adin et al. 1989). Results point out that the particles from the open reservoir are characterized be gelatinous shape and dominated compositionally by Si, although particle content may change with the season. Particles from the activated sludge plant
are dominated by Cl, Si, and Ca, while particles from the aerated lagoons maturation ponds are dominated mostly by P, K and Ca, which is typical for an algal environment (Adin et al. 1989). SEM-EDX can also be a tool to study the non-homogeneity of the aggregate or floc elemental surface composition (Mamane and Linden, 2006a).

Particles in wastewater can be enumerated microscopically by counting individual particles and stains can be added to aid in differentiation. However analysis of particles by microscopy is limited to a small number of images; thus data is not statistically representative, data analysis is laborious and results can be subjective. Recent developments of dynamic digital image analysis of particles are used to measure the 2-D area of a particle directly to obtain size, shape and count by capturing direct images of each particle in flowing liquids through automated sample introduction, image acquisition and analysis (Douglas et al. 2004; Thomas and Moore, 2004). Dynamic particle image analyzers have a potential in evaluating treatment process efficiencies such as addition of coagulants to granular filtration by coupling size and shape parameters into a meaningful tool (Mamane et al. 2008). Figure 4 illustrates images of filter backwash samples, in a tertiary treatment for wastewater effluents. The right image represents the image itself, while the left image represents the perimeter of the particle as analyzed for size and shape parameters.

Fig. 4: Images of filter backwash samples of a wastewater floc as captured by image analysis (right); perimeter of the particle as analyzed for size and shape parameters (left) (rev Mamane et al. 2008).

It is clear that there are two different particles in the floc imaged; one of biological organic origin (Rotifer is near to a floc) and the other is a
wastewater floc (Mamane et al. 2008). There are issues to consider with image analysis such as the pixel area where higher resolution is obtained with smaller pixels (see grid Figure 4) and the threshold which differentiates between a darker pixel that represents a particle, versus a brighter pixel that represents the carried fluid. This image is an example of microorganism associated with particles that may be protected from disinfection.

Information on the colonization of microorganisms with particles can be revealed by using direct fluorescent staining and visualizing by epifluorescent or confocal microscopy. Confocal laser microscopy can be especially useful for studying floc structure and allows optical sectioning of the aggregate and 3D reconstruction (Liss et al. 1996; Schmid et al. 2003). Recent developed methods use flow cytometry in combination with florescent antibody staining and direct viable counts for rapid and quantitative detection of pathogens (Dietrich et al. 1991; Tanaka et al. 2000; Yamaguchi et al. 2003). Fluorescence in-situ hybridization (FISH) methods can be utilized to detect and quantify microbial community within wastewater aggregates by using group-specific gene probes for broad groups of bacteria (Emerick et al. 1999; Schmid et al. 2003; Linden et al. 2004) and also determine the strength that different groups of bacteria bind to the floc surface (Wilen et al. 2008). The relative abundances of individual bacterial groups assessed by FISH in the activated sludge supernatant after secondary clarifier support the idea that the microbial community structure of the biomass effluent from the activated sludge process is different from that of the activated sludge remaining in the plant. A large number of bacterial groups in supernatant were not free swimming (60–70%), but associated with small flocs (Morgan-Sagastume, 2008), which has implication on UV disinfection efficacy of secondary effluents. Electron microscopy provides higher resolution images but requires elaborate sample preparation including drying, that may result in structural damage but may allow revealing nanometric structures of the floc.

3.2.2. Particle size distribution

Usually particle size analyzers/counters measure particle size distribution (PSD) for particles larger than 1 μm; thus PSD can provide a measure of removal for the larger sized particles (Adin, 1999a). A particle size distribution is defined as the fraction of particles within a certain size range in terms of a frequency function f(x) where x is generally a measure of particle diameter. The fraction of particles in the infinitesimal size interval between x
and $x + dx$ is given by $f(x)dx$. The fraction of particles between $x_1$ and $x_2$ is given by (Gregory, 2005):

$$\int_{x_1}^{x_2} f(x)dx$$  \hspace{1cm} (1)

The mean size $\bar{x}$ is given by:

$$\bar{x} = \int_{0}^{\infty} x \cdot f(x)dx$$ \hspace{1cm} (2)

A monomodel distribution is a distribution with one peak (called the mode of the distribution) as shown in Figure 5. The median size is where half of the particles have sizes smaller (or larger) than the median. For a symmetric distribution, the mean, median, and mode sizes are all the same, but not for an asymmetric distribution which is the case in natural aquatic environment.

![Particle size distribution of latex beads at a size of 90.7 ± 17.7 μm (left): particle image of the latex beads obtained with automated image analysis, (right) (rev Mamane et al. 2008).](image)

**Fig. 5:** Particle size distribution of latex beads at a size of 90.7 ± 17.7 μm (left): particle image of the latex beads obtained with automated image analysis, (right) (rev Mamane et al. 2008).

Particle size distribution can be described by fractional number, mass or volume of particles in a certain size range. In addition, size distribution can be described as in a continuous function or in the form of a discrete distribution (histogram). In natural environment, particles can be described by power law distribution (Eq. 3). The particle size distribution based on particle number ($N$) follows Pareto’s law for particles larger than 1 μm.
\[ \frac{\Delta N}{\Delta d_p} = A_d p^{-\beta}, \]  

where

\( N \) is the number of particles with size less than \( d_p \) (particle diameter in the size interval); \( A \) and \( \beta \) are empirical constants, where \( A \) relates to the total amount of material and \( \beta \) indicates breadth of distribution. When presenting eq 3 in log-log form, \( \beta \) is the slope and in natural water ranges between 3-5.

In natural waters, plots usually show increase in \( N(x) \) with decrease in particle size. This general validity of Pareto’s law implies that in most water samples the number of small particles may be order of magnitude greater then the larger ones, and combined with larger diffusion coefficient explains the faster coagulation of small particles. It has been demonstrated that PSD in wastewater effluent relates to this power law function (Adin and Elimelech, 1989).

3.2.3. Gross particle measurements

Turbidity is a measure of light transmitting properties of water, and is one of the most common tests to indicate quality of water and evidence of particles. The measurement of turbidity is based on comparison of the intensity of light scattered by a sample at 90 degrees to the path of incident light to the light scattered by a reference sample. Turbidity in water and wastewater is caused by suspended and colloidal particles as clay, finely divided organic and inorganic matter, and microscopic organisms (APHA, 2005). Turbidity provides information on the colloidal particles (<1 \( \mu m \)) thus can be a measure of colloidal removal. Rice et al. (1996) emphasized that turbidity is a reliable measure of particle removal when source water turbidity is above 5 NTU whereas particle counts is a more reliable measure in less turbid waters. However both particle counts and turbidity are not reliable indicators for removal of Cryptosporidium due to the low concentration of Cryptosporidium compared to the concentration of other particles (Edzwald and Kelley, 1998).

Total solids (TS) are obtained after evaporating a sample to dryness and measuring mass of the residue. Total suspended solid (TSS) is the portion of TS retained by a filter (usually 0.45 \( \mu m \) but ranging till 2.0 \( \mu m \)) measured after being dried (Metcalf and eddy, 2004). Both total solids and turbidity are lumped parameters that do not provide any information on particle size or
distribution of the individual particles in the water, which can be obtained by particle size analyzers. Even though TSS is a universal standard to evaluate performance of conventional wastewater treatment plants, correlation of turbidity with the weight or particle number concentration of suspended matter is difficult because the size, shape, and refractive index of the particles affect the light-scattering properties of the suspension. There is no relationship between turbidity and TSS in untreated wastewater but a reasonable relationship may exist in filtered secondary effluent from activated sludge and is plant specific (Metcalf and Eddy, 2004).

4. WHY/HOW DO MICROORGANISMS ATTACH TO PARTICLES?

4.1. Interactions

Suspended bacteria may exist as free-living cells or attached to particles in an aggregation or floc. Researchers found that attached cells could be more active, as active or less active than the free-living cells (Cammen and Walker, 1982). Particles can be a surface that promotes growth of microorganisms, can be a source of particulate nutrient in low dissolved nutrient water source (Paerl, 1975) and bacteria attached to particles may enhance remineralization of particulate carbon (Kirchman, 1983). Particle aggregates are heterogenous and composed of the dissolved, colloidal and particulate material and vary in size and composition in a process that involves chemical or physical destabilization and could occur due to polymers or electrolytes (Droppo et al. 2005).

An infinite variety of interactions can occur between the particles, microorganisms and dissolved constituents in water that may involve processes such as sorption, redox and acid-base reactions. This occurs as many of the chemical and microbiological contaminants partition between the particles and the dissolved phase. Two examples are described herein: (1) The adsorption coefficient for polysaccharide constituents of biofilms onto kaolin clay increases with effluent ionic strength, calcium ion concentration, and reduced pH. An adsorbed polysaccharide macromolecule may simultaneously attach to several particles to form a larger aggregate with a smaller charge density (Kamani et al. 1992). Consequently, changes in effluent quality can affect the size of aggregates and their components; (2) Inorganic particles can adsorb dissolved organic macromolecules, which
result in negative surface charge (Muschelienm et al. 1989). Dissolved organic matter (DOM) when absorbing UV light may yield transient photo-oxydants (Schwarzenbach et al. 2002) that can cause transformation reactions to occur.

In natural environments, microorganisms can attach to particles by means such as adhesive stalk formation, capsular secretion (fibrous matrix at cell surface), surface appendages (flagella, fimbrils and pili), webbing and slimy materials and by sorption of bacterial cells to particles without aid of cellular appendages or secretions (Pearl, 1975). Natural interactions between microorganisms and particles were investigated in diverse systems as freshwater pond (Kirchman, 1983), mesotrophic lake (Simon, 1985), sea water (Jones and Jannasch, 1959), freshwater and marine ecosystem (Pearl, 1974 and 1975), river-flood plain systems (Luef et al. 2007), marine sediments (Veji and Albright, 1985), salt marsh sediments (Lucas et al. 2003), estuarine waters (Bent and Gouldier, 1981; Fries et al. 2006), coastal lagoons (LaMontagne and Holden, 2003).

Non-natural interactions between microorganisms and particles can occur during treatment, such as during coagulation and flocculation or iron/manganese oxidation during water treatment (Cairns et al. 1993; Petri et al. 2000; Templeton et al. 2006) or during activated sludge floc formation in wastewater treatment (Parker and Darby, 1995; Emerick et al. 1999, 2000; Luge et al. 1996, 2001a, 2001b; Jolis et al. 2001). UV disinfection may be negatively affected by aggregation of particles that may occur naturally or during flocculation or with wastewater effluents if these flocs are not adequately removed.

4.2. Surface charge

The classical DLVO Model (Derjaguin-Landau-Verwey-Overbeek) of the electrical double layer describe coagulation as a collision between two colloids leading to aggregation depending on the attractive van der Waals forces (VDW) and the repulsive electrostatic forces that result from the negative electric charge of most colloids (Buffie and Leppard, 1995; Buffie and van Leeuwen, 1993). Sum forces can be either repulsive or attractive depending on media, chemical structure and surface potential. Other non-classical DLVO forces exist such as hydrogen bonding, hydrophobic interactions and steric interactions play a role in environmental systems
(Grasso et al. 2002).

Particles acquire a surface charge for various reasons; the most common reason is that the surface has chemical groups that can ionize in the presence of water to leave a residual charge on the surface, which can be positive or negative. Thus the chemical nature of the particle may influence floc charge (Morgan et al. 1990). Most natural particles as well as bacterial surfaces, when in aqueous solution, acquire a negative charge that is balanced by positively charged ions very close to the surface. A diffuse layer, which depends on the ionic strength of the solution, then extends further from the surface to balance the charges. Changes in pH also influence the electrostatic forces as reactions between protons and the charged surface functional groups change the net surface potential. Thus the ionic strength and the pH can influence the range and magnitude of electrostatic forces. Numerous researchers have indicated electrostatic interactions to influence bacterial adhesion to surfaces (Mozes et al. 1986; Husmark and Ronner 1990).

Zeta potential is an indicator of this charge and can be used to predict and control the stability of colloidal suspensions (Adin, 1999a), is a function of electrophoretic mobility, diffuse layer thickness, dielectric properties and viscosity of the medium, and is the potential at the at the plane of shear (Lawler and Benjamin, 2004), essentially describing the magnitude of the repulsion or attraction between particles. The colloid titration technique is common and relatively reproducible in determining the floc charge (Morgan et al. 1990; Droppo and Ongley, 1992). With this method, the actual net surface charge of a suspension containing both negative and positive surface groups may be estimated. Mikkelsen (2003) studied the application and limitations of this method for surface charge determination of sludge from wastewater. Rice et al. 1996 measured the zeta potential of Bacillus subtilis spores spiked into filtered lake water and reported values ranging between -16 to -20 mV at pH values from 5 to 10.5. Drozd and Schwartzbrod, (1996) found that the zeta potential for Cryptosporidium oocysts decreased slowly as pH increased starting at -35 mV for alkaline pH and reaching the isoelectric point for optimum adhesion at acidic pH. The isoelectric point for optimum attachment or clumping due to charge reversal would be reached at acidic pH values which are not typical of natural waters. In environmental pH surface charge may not a dominant factor affecting the aggregation of microorganisms.

In the DLVO models small colloids (<0.1μm) disappear quickly by
aggregating in the size range of 0.1-1 μm, which are stable for long periods. Particles larger than approximately 10 μm disappear by sedimentation or can be retained by filtration. The net result is that inorganic particles in the size range of 0.1-1 μm are the most stable colloidal matter in the absence of organic matter. These models assume that individual colloids do not change with time; however, in the presence of microorganisms this assumption is not fulfilled. Microorganisms produce macromolecular material that leads to aggregation between microorganisms and colloids (Buffie and Leppard, 1995). Therefore, in aquatic systems, the contributions to the structure of a particle/solution interface are from the electrical double layer (EDL) and also from the macromolecular absorbed layer (MAL). The ratio between the thickness of MAL/EDL at neutral to alkaline pH and low ionic strength as in surface water supply may be smaller than 1 because the EDL layer is not compressed, therefore even though the negative charge on the particle may be due to adsorbed layer of NOM the interactions will be primarily electrostatic (Buffie and van Leeuwen, 1993). However Droppo et al. (1992) stated that in freshwater systems the electrochemical forces are less important than the biological processes in the formation of flocs, while in marine environment with high salt content floc formation is highly dependent on electrochemical flocculation.

Zita and Hermansson (1994) showed that at ambient ionic strength in the effluent wastewater interactions between the floc components can be explained by the DLVO theory. However the DLVO theory cannot explain all interactions within the flocs. It is probable that the polymer matrix in the central parts of the floc mediate a close contact between cells, a situation that is not well described by the DLVO theory. Moreover, when the ionic strength was increased, a different kind of mechanism seemed to affect the floc structure, when according to the DLVO theory, the interparticle distance in the suspension is fairly constant at these high salt concentrations and should not affect the adhesion. Thus DLVO interactions cannot explain in all cases mechanisms of bacterial attachment and studies have shown that other forces, such as hydrophobic interactions, may be important in microbial floc formation.

4.3. Hydrophobicity

Doyle (2000) cited hydrophobicity as the mechanism causing a non-polar
species to aggregate in water thus decreasing the interfacial area of the aggregate. Hydrophobic colloid suspended in water results in a discontinuity in the hydrogen-bonded structure of water due to the presence of this nonpolar surface such that water molecules adjacent to the surface become oriented to maximize the number hydrogen bonds (Grasso et al. 2002). The outer surface of bacterial cells can have a hydrophobic surface that can be responsible for interactions such as attachment of bacteria to hosts, attachment of bacteria to surfaces, and growth on hydrocarbons (Rosenberg et al. 1980).

It was previously shown that different species possess different hydrophobic characteristics (Rosenberg et al. 1980; Doyle et al. 1984; Koshikawa et al. 1989) and hydrophobicity plays a major role in attachment or adhesion of microorganisms to surfaces, with the most hydrophobic ones having greater affinity for hydrophobic surfaces (Faille et al. 2002; Ronner et al. 1990). Ronner et al. (1990) attributed adhesion to hydrophobic proteins that contribute to overcoming electrostatic repulsion. Some areas on the surface of the particle contain hydrophobic components covalently bound to cell walls or to the outer or cytoplasmic membrane (Doyle, 2000). Hydrophobicity and weak van der Waals forces may explain better than electrostatic double-layer interactions the infiltration of Cryptosporidium parvum oocysts through unconditioned sand filters (Adin et al. 1999b). In addition, studies have shown that cells that are in stationary phase of growth are more hydrophobic than cells in the logarithmic phase and might result from minimization of adhesive structures during and immediately after the division period (Allison et al. 1990). Thus the average physiological age is important in determining the hydrophobicity, and consequently surface attachment. Hydrophobicity of bacterial cells can be simply and easily measured by the microbial adhesion to hydrocarbon (MAT^{H}) method (Rosenberg et al. 1980), cell surface and flocs can be measured by the contact angle measurement (van Oss et al. 1988, 1999), polystyrene microsphere assay (Hazen and Hazen, 1987) and by salt aggregation test (SAT) (Lindahl et al. 1981).

Colloids or macromolecules are very small particles in the size range of 1 nm to 1 μm, and can be classified as "hydrophilic" or "hydrophobic" which refers to materials that are soluble or insoluble in water respectively (Gregory, 2006). Hydrophilic colloids are macromolecules such as proteins in the size range of 1-10 nm and macromolecules of natural organic matter
such as humics. Hydrophobic colloids are dispersed as very small particles such as clays and oxides. Environmental colloids that are hydrophobic in nature have a tendency to aggregate in water to reduce the area of contact with water but they may remain dispersed due to repulsive forces. The characterization of colloids is problematic as hydrophilic natural organic matter (NOM) can adsorb to inorganic particles that may obtain hydrophilic properties. Therefore, in the aquatic environment most particles have similar surface properties, such as zeta potential, characteristic of organic coating (Gregory, 2006).

To summarize, the nature of the particle-solution interfacial chemistry such as hydrophobicity and zeta potential of particles (and microorganisms) will also influence aggregation of particles with microorganisms and are linked to the formation and the structure of a floc (Bura et al. 1998).

4.4. Extra-cellular polymeric substances

Activated sludge flocs are composed of microorganisms (mainly bacteria), extra-cellular polymeric substances (EPS), inorganic particles (such as calcium phosphate and iron oxides) and divalent cations (Urbain et al. 1993). The EPS are microbial products or secretions that are located on or outside cell surfaces that aggregate cells into flocs or adhere to surfaces, provide resistance to surrounding toxins or biocides, accumulate enzymes and nutrients for cell use, and facilitate communication between cells (Wingender et al. 1999). The EPS are a complex mixture of organic substances: proteins, carbohydrates, acid polysaccharides, lipids, DNA, and humic acid substances that surround cells and create a matrix of microbial flocs and films (Liao et al. 2001). Protein was found to be the dominant component in EPS composition of activated sludge flocs (Bura et al. 1998; Comte et al. 2006). The production of EPS by bacteria involves a significant investment of carbon and energy.

In general molecules with conjugated bonds (such as those present in the EPS matrix) are good UV absorbers below 300 nm. These wavelength spectra inflict irreversible damage on cellular DNA as well as on other molecules, such as: proteins, enzymes, coenzymes, hormones and electron carriers (Kalivaart, 2004). The absorbance spectra of proteins show a maximum peak at 280 nm, where the peptide bond in proteins display a significant absorbance below 240 nm due to the large number of these bonds.
The disinfection of floc associated pathogens (with MP lamps) can be effected by the UV absorbance of EPS present in the floc matrix (Farnood, 2005). Analysis of EPS extracted from cultures of *Klebsiella* sp. showed that EPS strongly absorbs UV light, however the reduction of UV intensity within the floc can vary according to the distribution of EPS, whether it is accumulating around a single target microorganism or coating a thin film on the floc surface (Luh, (2003) cited by Farnood (2005)).

The components of the aggregates or flocs are inorganic components, biota and bioorganic components as EPS and organic decay, bound and free water and the floc pores (Droppo, 2001), and EPS is the major component of the activated sludge floc matrix. The origin of EPS is complex thus different approaches exist that defines EPS. Therefore experimentally EPS is defined by the method used to separate or extract EPS. EPS separated by centrifugation can be classified as follows; (a) bound or attached EPS (bound closely to cell surfaces as peripheral capsules) and located in the pellet after centrifugation and (b) soluble or colloidal EPS which is loosely attached to the cell or totally unattached and able to move freely between sludge flocs and surrounding liquor as a less organized slime and found in the supernatant after centrifugation (Foxon and Darby 1997; Thornton 2002; Comte et al. 2006). In another study, Rosenberger and Kraume (2002) suggested that EPS in activated sludge occurs as a capsule surrounding the bacterial cell wall which enhances flocculation ("extractable EPS") and in solution in the supernatant as slime polymers ("suspended EPS"). Certain species of bacteria are not capable of forming EPS (or capsular EPS which surrounds many bacteria cells) while others do so under competitive nutritional environments where it provides bacteria a selective advantage.

Tsuneda et al. (2003) studied the role of EPS on bacterial adhesion to poor and rich EPS strains and concluded that if the EPS amount is small, cell adhesion onto solid surfaces is inhibited by electrostatic interaction (polymereic interaction is weak and van der Waals force is almost independent of bacterial species), and if it is large, cell adhesion is enhanced by polymeric interaction (high-molecular-mass polysaccharides as polyelectrolytes). In addition, they found no correlation between hydrophobicity and cell adhesiveness. While others found that EPS possess both hydrophobic and hydrophilic properties (Jorand et al. 1998), and a strong correlation exist between the hydrophobicity of cells and their degree of attachment to activated sludge flocs (Zita and Hermansson, 1997). Moreover, Liao et al.
(2001) found a strong inverse correlation between the surface charge and hydrophobicity of sludge, and suggested that ionizable groups present on sludge surfaces, increase the polar interactions of EPS with water molecules.

Thus EPS play a significant role in the formation and function of microbial aggregates; however it is not clear whether hydrophobic interactions, electrostatic interaction or polymeric interaction or their combination may be important in microbial floc formation, and the role of EPS in adhesion and aggregation is case specific.

TEP is another type of particles that recently gained acknowledgement in fresh water systems. EPS can be found in cell coating, in soluble or colloidal EPS and also as transparent exopolymer particles (TEP) (Thornton 2002). TEP are a form of EPS that were first identified in diatom cultures and natural seawater using polysaccharide specific staining techniques (Allredge et al. 1993). TEP are discrete particles (rather than dissolved molecules or coatings on other particles) and are detected as transparent sheets and films or strings ranging from 3 to 100 μm in the longest dimension and occurring in the coastal waters. These TEP particles promote aggregation by increasing the overall stickiness of suspended particles (Dam and Drapeau, 1995). The number of large aggregates in water was found to be related to the TEP concentration in water rather than to the total or dissolved organic carbon (Li et al. 2008). Other studies suggested that TEP in source water can be related to biofilm formation on surfaces of membranes and that conventional pre-treatment with sand filtration can lower the TEP concentration, however most of the literature on TEP is on natural environments and not in the water treatment industry (Berman, 2005). Very little is known about the relationship between the fractions or pools of EPS (EPS cell coatings, soluble EPS and TEP) and how this affects aggregation, how EPS is transferred between pools, and the role or organisms and the environment in these processes (Thornton 2002).

5. IMPACT OF PARTICLES ON UV DISINFECTION OF MICROORGANISMS

5.1. Overview

Particulate matter in water interferes with transmission of UV light and in particular important when UV disinfection is applied (Crittenden et al. 2005).
Mechanisms that are important when evaluating the impact of particles on UV disinfection are shielding and enmeshment. Figure 6 illustrates the impact of particle “shielding” on UV disinfection, while Figure 7 illustrates the impact of particles enmeshed associated with microorganisms on UV disinfection, where microbial inactivation depends on accessible pathways to UV light.

Particles can “shield” target organisms from UV light by refraction, reflection, absorption and scattering. Definition for absorbance and scattering and the impact of particles on UV absorbance and scattering is detailed in section 5.2.1 and 5.2.2. Refraction is defined as the change in the direction of light propagation as it passes through the interface between one medium and another. Refraction may change the angle that UV light strikes a target pathogen. While, reflection is the change in the direction of light propagation when it is deflected by the interface between two media.

Interactions between dissolved organic matter and particles present in water and the impact of this interaction on UV disinfection is detailed in section 5.2.1.2 and the potential impact of iron on UV absorbance and disinfection is detailed in section 5.2.1.3. In water systems, shielding and enmeshment are less important in low turbidity waters where filtration is used, but is more important for unfiltered water supplies and process upset that release particles to water as detailed in section 5.3. Post UV disinfectant effect on biofouling in water distribution systems is clarified in section 5.3.6. Particles can also protect microorganisms by enmeshment that relates to particles that are associated with microorganisms and this phenomenon has been mostly studied with wastewater effluents as further detailed in section 5.4. Kinetics of UV disinfection of microorganisms associated with particles is further explained in section 5.4.2.

![Diagram](image_url)

**Fig. 6:** The impact of particle “shielding” on UV disinfection by absorption, scattering, refraction and reflection.
Fig. 7: The impact of particles enmeshed/associated with microorganisms on UV disinfection, where microbial inactivation depends on accessible pathways to UV light.

5.2. Impact of particles on UV absorbance and scattering

5.2.1. Absorbance

5.2.1.1. Overview

Absorption in water is caused when a light beam illuminates water at a specific wavelength that results in radiation interacting with atoms or molecules to raise their energy level, thus light is absorbed, and energy is lost from the beam. In other words, absorbance is characterized by the decrease in the amount of incident light as it passes through a water sample over a specified distance or path length. UV absorbance of a substance varies with the wavelength (λ) of the light. When UV light is absorbed in the water matrix, it is no longer available to disinfect microorganisms, thus water with high absorbance may not be efficient for UV disinfection. For example, iron compounds in water absorb UV, lower water transmittance thus increase the UV dose required.

The use of UV irradiation for water disinfection purposes relies on accurate measurement of light absorption in water. The standard method used to measure light absorption of a water sample relies on transmittance of light captured by a detector that is placed in front of the sample, using a spectrophotometer. However, when light beam illuminates a suspension of particles in water, the intensity of transmitted beam may be reduced as a result of both scattering and absorption of light (Gregory, 2006). When light scattering occurs, there is no loss of energy from the beam, and energy is
radiated in all directions at the same wavelength of incident irradiation. Thus scattered light can still disinfect water, whereas absorbed light is no longer available for disinfection. Qualls et al. (1983) suggested that with turbid effluent samples the absorbance can be separated to soluble absorbance, absorbance due to particles and scattering due to particles. In most applications, the standard spectrophotometer measures bulk water absorbance which includes both soluble and particulate contributions (for water with non-light scattering particles). Loge et al. (1999) described a technique for directly measuring UV absorbance and internal scattering characteristics within wastewater solids by using a fiber optic microelectrode apparatus. It was determined that bulk liquid absorbance provides insufficient information regarding the ability of UV to penetrate the organisms within wastewater particles.

Natural waters contain various substances that can affect its optical properties such as mineral particles, particulate organic matter, and microorganisms. Because of unique absorbance and scattering properties, particles interfere with the measurement of UV absorbance, determination of fluence rate, and the mathematical modeling of UV disinfection systems. Various studies on UV absorbance of water related to UV disinfection included the impact of particles on absorbance (Du and Rabani, 2004; Linden and Darby, 1998; Cabrera et al. 1996; Babin and Stramski, 2002; Loge et al. 1996).

A number of substances in water influence UV absorbance directly such as iron, nitrate and natural organic matter (NOM). For example, nitrite (NO\textsubscript{2}\textsuperscript{-}) formation during polychromatic UV photolysis of nitrate was studied as a function of pH and NOM concentration, and it was demonstrated that NOM had an impact on nitrite formation (Sharpless and Linden, 2001). NOM however can impact UV disinfection processes indirectly. Indirect photolysis can occur when there is a transformation due to energy transfer from other excited species such as NOM or by reaction with radicals such as hydroxyl and peroxy radicals (Schwarzenbach et al. 2002). For example, NOM in surface waters plays an important role in sunlight induced photochemical processes (photosensitizer), not only as a radical scavenger but also as a precursor for reactive oxygen species (ROS) (Doll and Frimmel, 2004).

5.2.1.2. Impact of natural organic matter on UV absorbance

Aquatic NOM is typically comprised of biogenic, polyelectrolytic organic
molecules and polymers, with mass concentration ranging from 0.5 to 100 mg/L of organic carbon. Aquatic NOM has been operationally classified as humic substances (HS) and non-humic substances based on the separation by XAD resin. Humics are absorbed on the resin, while non-humics are not retained (Frimmel, 1998). Humic substances (HS) are colored, refractory NOM and can be classified into three fractions based on their water solubility. The fraction that is insoluble in water at pH < 2 is defined as humic acid (HA), fulvic acid (FA) is the fraction that is soluble in water under all pH conditions. Humin, the third fraction, is not water soluble at any pH (Aiken, 1985). The contribution of HS to the Total Organic Carbon (TOC) as defined by specific isolation procedures can be found in the range between 50% to 80%, primarily of FA (Wilkinson et al. 1997). The characterization with respect to elemental composition, molecular weight and functional groups provide information that HS possess high molecular weight and have diverse functionality. HS can absorb light in the ultraviolet and visible range mostly due to its unsaturated structures (Frimmel, 1998). In average surface water the absorbance of FA was found to be 120 (L/mgC/cm) and for HA was 240 (L/mgC/cm) (Aiken, 1985).

All contributions to organic carbon in water are referred to by the term TOC which is comprised of Particulate Organic Carbon (POC) and Dissolved Organic Carbon (DOC) (Frimmel, 1998). The DOC fraction is determined as the carbon concentration of the water passing through 0.45 μm pore size filters, while the carbon retained on the filter constitutes the fraction of particulate organic carbon (POC) that is a small fraction out of TOC in most aquatic systems. Total organic matter (TOM) also includes other elements such as oxygen, hydrogen, nitrogen and phosphorus in addition to C and is, together with dissolved organic matter (DOM) and particulate organic matter (POM), analogous to TOC, DOC and POC. DOC in water is mostly from NOM.

NOM is ubiquitous in wastewaters and it can absorb to suspended particles and change its surface properties (Dai and Hozalski, 2002). The chemical nature of natural organic matter and its abundance will determine whether colloids and aggregates in water will be stabilized or destabilized by NOM. Numerous studies showed that the majority of particles in natural waters are in the form of aggregates and not in dispersed suspension, and addition of organic matter may lead to aggregate stabilization which depends upon the composition of organic matter and aggregate size (Walker and Bob,
Negative surface charge of inorganic particles formed by absorption of DOM can retard the rapid physical aggregation (collision, surface charge attraction) and may promote longer timescale biological aggregation (bacterial attachment and growth) (Muschenheim et al. 1989).

Generally organic matter can be removed by coagulation with inorganic or synthetic organic coagulants, flocculation and sedimentation prior to the disinfection process. Numerous researchers studied the effect of NOM or DOC on coagulation (Tseng et al. 2000; Krasner and Amy, 1995; Bell Ajy, 2000). Alum demand in water containing high concentration of NOM is usually higher and indicates the necessity for pH control by alkalinity addition to target pH (Gregory and Carlson, 2002; Tseng et al. 2000). The process of using higher doses of coagulants to remove TOC is termed enhanced coagulation and is a regulatory requirement to control precursors of disinfection by products (Edzwald and Tobiason, 1999; Volk et al. 2000). Still organic matter remains in surface waters and wastewater effluents after treatment and may exert a photon demand either in the dissolved or particulate form when exposed to UV light.

A question remains whether UVC light itself can impact NOM characteristics. Studies showed that UV light at conventional doses for disinfection did not change the nature of natural organic matter and specifically of humic substances in water, and only at extremely high doses of 25600 mJ/cm² a change in the organic content (hydrophobicity, aromaticity and biodegradability) was observed that had also an impact on enhanced microbial regrowth (Camper et al. 2001). Another study also determined that UV treatment at typical UV doses for water disinfection does not increase in general the biodegradable fraction of NOM; however the impact on potential regrowth should be evaluated specifically with regard to the water characteristics (Shaw et al. 2000).

The coagulation model of Smoluchovskvky does not consider the possible interactions of inorganic colloids with organic macromolecules. When the organic macromolecules are much smaller than the inorganic colloid they may adsorb as a thin layer at the colloid surface and modify the interaction energy barrier (Buffe and Leppard, 1995). Sorption of organic matter on colloids or larger particles may impact UV disinfection. In support of this possibility, studies showed that soil-derived humic acid possibly coated the surface of viruses and bacteria, protecting them from UV disinfection (Templeton et al. 2006; Cantwell et al. 2008).
5.2.1.3. Potential impact of iron on UV absorbance and disinfection

Iron can occur naturally in groundwater, or iron may be added in water and wastewater treatment as a ferric coagulant, thus forming UV-absorbing ferric floc particles. When ferric ions are added to water sequential reactions occur as the ferric ion hydrates to form a variety of soluble species as well as insoluble ferric hydroxide precipitate (Crittenden et al. 2005). Moreover, UV radiation can induce photochemical alterations of metal-containing particles or organic/inorganic moieties of particles. Fe(II) and Fe(III) complexes are known to exhibit photolysis upon exposure to UV light (Schwarzenbach et al. 2002). These reactions may impact bacteria that are embedded within or attached to the particle surface.

Cairns et al. (1993) observed a correlation between higher ratios of Fe/SS (iron to suspended solids) to poorer UV disinfection of effluent fecal coliforms. Higher counts of surviving bacteria at higher Fe/SS ratios could be due to: (i) increased number of smaller particles to be disinfected (iron impacts size and number flocs in coagulation); (ii) UV absorbance by the iron itself; (iii) compressed packing of the iron-rich particles, or (iv) a combination of these factors. In addition to the impact of iron on the particles, iron can impact the free microorganisms by facilitating association with particles. Petri et al. (2000) suggested that lower MS2 virus counts than expected were observed upon performing a UV bioassay possibly due to high dissolved iron concentration in raw groundwater in a reduced state that oxidized upon exposure to air by sampling and co-precipitated with MS2. Thus increased resistance could be due to shielding of virus with iron containing particles as previously suggested by Cairns et al. (1993). UV inactivation of MS2 was higher with addition of a chelating chemical (such as coffee or EDTA) to the water that kept the iron dissolved and prevented co-precipitation of the virus MS2.

A study by Templeton et al. (2006) showed that phages associated with iron oxide particles in groundwater are shielded from UV light even at a relatively low turbidity level of 2.7 NTU, when water was experimentally aerated for 2 hrs. Iron precipitation is a result of oxidation of reduced dissolved iron in groundwater. However, the impact on non aerated iron-containing groundwater and also the impact of iron on lower turbidity values within the regulated limits should be examined. Another study by Templeton et al. (2005) showed little protection of viruses even at very high turbidities.
with kaolin clay particles (70–100 NTU), possibly due to the natural difference in UV absorbance of clay and iron particles (Bitton et al. 1972; Cairns et al. 1993).

Moreover iron impacts fouling on the quartz sleeves of MP UV reactors that reduce light transmission and disinfection efficacy. Petri et al. (2000) observed rapid fouling on with high iron levels of 1.3 mg/L iron and hardness of 500 mg/L as CaCO₃ in raw groundwater.

5.2.2. Scattering

5.2.2.1. Overview

When particles are present in solution, each particle scatters light in unique directions and patterns. As a result, the mutual influence of scattering from particles can lead to enhancement or cancellation of the integrated scattering effect. Scattering is influenced by the particle size relative to the light wavelength, shape of particles, chemical composition, concentration (Bohren and Huffman, 1983), relative refractive index between particles and suspending medium. For example, large diameter particles such as 5 μm are dominated by forward scattering whereas with small diameter particles (i.e., 0.05 μm) the scattering is equally distributed in all directions (Huber and Frost, 1998).

The fraction of light absorbed is generally obtained as the fraction of light transmitted subtracted from unity. However with particles that scatter light, the fraction of light absorbed (by the particles) equals the sum of the fraction of light transmitted and the fraction of light reflected all subtracted from unity (Hanssen, 2001; Tassan and Allali, 2002). For highly concentrated particle samples, multiple scattering and inter-particle interferences are of concern and in the case of highly charged particles or particles with refractive indices close to 1 the inter-particle interferences can become significant (Schnablegger and Glatter, 1995). Above 89% transmittance multiple scattering effects are insignificant (Nelson et al. 1993).

When particles are present in water the transmitted beam shows energy loss (even though scattering does not involve net energy loss), because light is scattered in all directions so less light would reach a detector placed opposite to light source (Figure 8). Thus, the drawback of using the standard spectrophotometer relates to light scattering at angles outside the reception angle of the detector, therefore most of the light does not reach the detector.
(Tassan and Allali, 2002; Du and Rabani, 2004). Furthermore, with the standard spectrophotometer it is not possible to separate the incident beam from the light scattered in the forward direction, as the detector responds to a superposition of the incident and forward scattered fields (Bohren and Huffman, 1983), while the scattered light in other directions (besides forward direction) will be measured as absorbed light. Therefore measurement of UV absorbance for a suspension with large scattering can result in significant error in absorbance measurements (Qualls et al. 1983; Tassan and Ferrari, 2003), or in UV fluence (Linden and Derby, 1998; Christensen and Linden, 2003, Mamane-Gravetz and Linden, 2004).

Fig. 8: Effect of particles on light scattering in all directions.

5.2.2.2. Measurement of light scattering -- Integrating sphere

True UV absorbance, including all transmitted and scattered radiation, can be measured using integrating sphere (IS) spectrophotometers (Scheible, 1987; Nelson and Prezelin, 1993; Linden and Derby, 1998; Tassan and Ferrari, 2003; Christensen and Linden, 2003; Mamane and Linden, 2006b; Mamane et al. 2006), which are optical devices that integrate the radiant flux (Castiglioni and Albertini, 2000) of most reflected and transmitted radiation simultaneously, and presents an integrated signal or radiances field to the detector. The inside of the sphere is coated with a white thermoplastic material named Spectralon® that reflects over the entire UV-Vis wavelength range (Storm and Springsteen, 1998). In practical terms, positioning the
turbid water sample in the center of the sphere by a center mount holder, allows absorbance measurements of turbid samples in one measurement (Storm et al. 1998, Mamane and Linden, 2006b). A sample in which particles and absorbing media exist together is defined as a heterogeneous sample, whereas a sample devoid of particles is defined as a homogeneous sample. The absorbance coefficient (\( \alpha \)) of a homogeneous sample is obtained by direct absorbance measurements as follows (Eq. 4):

\[
\alpha (cm^{-1}) = \frac{A_D \cdot 2.303}{b}
\]  

(4)

where \( A_D \) is the direct absorbance measured by a standard spectrophotometer and \( b \) is the path length of the cuvette or depth of water.

The extinction coefficient (\( \beta \)) is defined as the sum of the absorbance and scattering effects, as obtained by standard spectrophotometric measurements of heterogeneous samples (Eq. 5).

\[
\beta (cm^{-1}) = \frac{A_D \cdot 2.303}{b}
\]  

(5)

The absorbance coefficient is the extinction coefficient corrected for light scattering. The absorbance coefficient of a heterogeneous sample is obtained by IS absorbance measurements, \( A_{IS} \), as follows (Eq. 6):

\[
\mu (cm^{-1}) = \frac{A_{IS} \cdot 2.303}{b}
\]  

(6)

The scattering albedo (\( \omega \)), the fraction of light scattered away, is defined in percentage as (Eq. 7):

\[
\omega(\%) = \left(\frac{\beta - \mu}{\beta}\right) \times 100
\]  

(7)

As previously mentioned, the average germicidal UV irradiance in water is a function of several factors including the irradiance (mW/cm\(^2\)) incident to the water, absorbance, and the pathlength of light in the water (Bolton and Linden, 2003). The UV fluence (mJ/cm\(^2\)) is obtained by multiplication of the average germicidal irradiance by exposure time. Therefore there is a direct
correlation between UV absorbance and the fluence a microorganism is exposed to. Consequently, not using integrating sphere spectroscopy to account for scattering in absorbance measurements will result in overdosing the UV system. For example, absorbance measurements using standard spectrophotometry were falsely higher compared to integrating sphere spectroscopy measurements for water suspensions with turbidity values above 3 NTU, which resulted in significant overdosing of LP and MP bench scale samples (Christensen and Linden, 2003).

Results of a study by Mamane et al. (2006) showed that the penetration of germicidal UVC irradiance in water was dependent on the scattering properties of particles in a water matrix including particle size, particle chemical composition and water absorbance. In addition, using an integrating sphere for absorbance measurements resulted in correct fluence rate determination and subsequent microbial inactivation (Mamane and Linden, 2006b). The same microorganism associated with particles that scatter light differently, will likely be more inactivated within the aggregate that is more scattering, therefore the magnitude of particle scattering is important in UV disinfection studies. Natural particles from wastewater effluent exhibited less scattering at 254 nm (approximate 20-40% scattering) compared to inorganic alumina (90-100% scattering) and Na-Montmorillonite clay particles (50% scattering). However scattering of wastewater effluent at wavelengths above approximately 240 nm was not evident in the direct measurement. The UV fluence rate in a scattering and absorbing media is not simply an average of the fluence rate of the individual scattering and absorbing components (Mamane et al. 2006). Figure 9 illustrates the extinction coefficient of clay suspension as a function of wavelength. Inorganic particles like clay scatter light at all wavelengths tested. Medium pressure (MP) lamps contain wavelengths over a wide spectrum in the UVC and UVB ranges; therefore this complexity unique to MP lamps enhances the need for understanding the spectral scattering characteristics of particles, especially for disinfection of microorganisms.
Fig. 9: Direct (D) and IS spectral absorbance measurements and scattering albedo of 250 mg/l Na-rich montmorillonite clay suspended in deionized water (rev Mamane et al. 2006).

5.2.2.3 Measurement of light scattering – actinometry

Research studies previously used spherical actinometers in UV reactors to measure fluence rate distribution of light from different directions from multiple lamp sources or reflected light, with use of monochromatic or polychromatic lamps. A spherical quartz vessel containing a chemical actinometer can measure UV fluence in air or in aqueous systems by placing the actinometer solution inside the sphere, placing the sphere in the UV irradiated suspension, and measuring the photochemical reaction occurring inside the sphere (Rahn et al. 1999; Stefan et al. 2001; Jin and Linden, 2002). Spherical actinometry can be also utilized to quantify the effect of particle scattering in UV reactors, as the actinometer can record every photon that comes from different directions due to a scattering suspension (Mamane et al. 2006). According to the scheme in Figure 10, an actinometer is inserted inside the sphere which absorbs UV photons directly from the UV source and indirectly from particles that scatter UV light and this forms a photochemical reaction which is measured with a spectrophotometer.

\[ 8 \Gamma + IO_3^- + 3 H_2O + h\nu \rightarrow 3 I_3^- + 6 OH^- \]  

(8)
Fig. 10: Iodide/iodate actinometer absorbance with spherical actinometry at 352 nm used to quantify the effect of light scattering by particles as illustrated by the arrows.

Nanoparticles (NPs) may impact UV disinfection efficacy by absorbing UV light, by scattering UV light and as a technology to improve disinfection by UV by interaction with microorganisms in water. The distribution of light intensity in a UV reactor due to scattering particles in the size between nano to submicron particles was investigated previously using a spherical chemical actinometry method (Mamane et al. 2006). Previous results showed that aluminum oxide submicron particles suspended in DI water (size of 400 nm) increased fluence rate distribution in the annular bench scale UV reactor up to three folds compared to DI water alone as illustrated in Figure 11. These results imply that adding aluminum oxide nanoparticles to a UV reactor can potentially be used as a tool for improved disinfection.

For full scale UV system validation testing, a typical practice is to measure the effect of changing solution transmittance on UV fluence in a reactor by adding absorbing material such as coffee, humics and other chemicals into a reactor feed water (Wright, 2000; Stefan et al. 2001). These solutions added to the UV reactors are void of particles however in reality especially when using UV to disinfect secondary wastewater effluents; these solutions do not truly represent particles in effluent waters.
Fig. 11: 3D surface plots of fluence rate in the annular UVC reactor at various X, Y locations for conditions of: (top) reactor filled with deionized water, (bottom) reactor filled with alumina particles (rev Mamane et al. 2006).
5.3. Disinfection of water systems by UV

5.3.1. Overview

In water treatment, the coagulation process involves the addition of a chemical coagulant used for particle destabilization, thus transforming a stable suspension into an unstable suspension capable of aggregation. The flocculation process is used to increase the rate of particle collision and produce particles, by means of aggregation that can be subsequently removed by gravity sedimentation, floatation and filtration prior to UV disinfection. In conventional water treatment plants UV disinfection is applied usually after filtration or to unfiltered waters with low turbidity.

Particle count/characteristics and UV transmittance (UVT) of the water can be controlled by unit processes and chemical addition upstream of UV reactors thereby optimizing the design and costs of the UV reactor. For example, coagulation, flocculation, and sedimentation remove solubles and particulate material, filtration removes particles and activated carbon absorption also reduces soluble organics.

5.3.2. Unfiltered systems

Under the US surface water treatment rule (SWTR), finalized in 1989, all drinking water systems using surface water must filter and disinfect their waters to protect the public from exposure to Giardia lamblia cysts and viruses, unless they meet EPA filter avoidance criteria. The SWTR specified conditions which a water system could avoid filtration (40 CFR 141.71), which include parameters such as good water quality that is measured by coliforms and turbidity, watershed control and absence of waterborne outbreaks (USEPA, 1989). The Interim Enhanced SWTR, finalized in 1998, extended the previous rule to include control of Cryptosporidium, control of filter performance and address disinfection by products (USEPA, 1998). At that year, the EPA extended watershed control requirements for unfiltered waters, serving more then 10,000 people, to include Cryptosporidium control wherever Giardia is mentioned. The Long Term 1 Enhanced SWTR, finalized in 2002, required even more stringent filtration requirements for public water systems serving small water plants (USEPA, 2002). In 2003, the EPA proposed the Long Term 2 Enhanced SWTR that will assign water plants a treatment category that will determine extent of additional treatment to meet disinfection goals, based on Cryptosporidium concentration (USEPA, 2003a). In addition, the EPA developed a guidance manual for public water
systems using UV light to meet drinking water disinfection standards for filtered and unfiltered systems (USEPA, 2006).

UV disinfection is very effective for inactivating Cryptosporidium, without forming residual disinfection byproducts produced with chlorine disinfection. This information was very important for unfiltered supplies, because filtration was one of the only barriers available for removal of Cryptosporidium. Using UV for disinfection provides a barrier for Cryptosporidium especially for unfiltered water sources. New York’s water supply system is one of the largest of its kind in the USA that meets the EPA conditions for unfiltered water supplies (Pires, 2004). Cantwell et al. (2008) found that for selected river water with turbidity values of 5.4 NTU, more than 92% of natural occurring particles were below 11 µm, and particles larger than 11 µm are able to protect indigenous coliforms from LP UV at a dose of 40 mJ/cm². These finding have implications on disinfecting unfiltered surface waters, as larger particles (above 11 µm in this study) may potentially harbor and protect pathogens from disinfection.

5.3.3. Filtered systems

In water treatment plants microorganisms can aggregate with particles by coagulation and flocculation process through addition of hydrolozing chemicals (as alum or iron salts) aided with synthetic organic polymers that results in the formation of precipitates followed by gravity separation and/or filtration to remove particles, pathogens and NOM from water. The goal is to remove particulate matter and portions of the NOM in water. For example, Livsted and Cripps (1999) suggest filtration of seawater aquacultural purposes before UV disinfection to avoid transmission of fish pathogenic bacteria from sea to land based aquaculture such as salmonid hatcheries. A 50 µm filter followed by a UV dose of 22 mJ/cm² can achieve more than 5 logs reductions indicating that after filtration the majority of the bacterial counts were single cells or cells attached to small fragments.

Particles, however, can pass filtration media during filter ripening, regular filtration operation and at the end of filter cycle and effect the subsequent UV disinfection. For example, disturbances in operation during filtration of flocculated aggregates in water may result in a filter breakthrough event and release of flocculated particles that will negatively affect UV disinfection. During depth filtration suspended particles are removed by attachment to the filter media or to particles that were previously retained and serve as
additional collector sites that result in improved removal efficiency (Darby et al. 1992). Removal of particles by granular filtration is a simultaneous process of particle attachment to the collector, detachment and reattachment of those detached particles in deeper layers of the filter or detachment. This occurs since the structure of accumulated deposits on the filter are not equally strong, and under the hydrodynamic forces caused by the flow this structure may be partially destroyed and may disintegrate previously fixed flocs (Mintz, 1966). Mamane et al. (2008) observed spikes, when plotting percent removal of particles by granular filtration as a function of equivalent circular diameter (ECD), that relate to sudden lower removal efficiencies in particle removal. This may imply that larger particles above 10 μm may be leaving the filter compared to particles that entered the filter, suggesting particle detachment. However, particles with lower circularity were removed more efficiently compared to the highly circular particles. These results also suggest that the spikes in the size based removal may be due to the more circular particles for similar size range that are removed less efficiently and not necessary due to detachment of previously retained particles as suggested by other researchers. Particles in the size range above approximately 10 μm, may potentially harbor pathogens and pass filters under routine operation. Moreover, Templeton et al. (2007) studied the removal of particle-associated bacteriophages by dual-media filtration at different filter cycle stages and impacts on subsequent UV disinfection. UV disinfection of the filter effluent was hindered during ‘off-spec’ filter periods, especially at the end of a filter cycle.

5.3.4. Influence of turbidity – case studies

Generally, the impact of particles on ultraviolet (UV) disinfection has typically been studied by adding clays or natural particles to increase water turbidity and seeding microorganisms into sample water and contacting them via stirring, essentially measuring the effect of particle absorbance and scattering on UV performance. Varying water turbidity up to a limit that still meets the regulatory requirements for unfiltered effluents did not have an impact on the UV dose-response curves of microorganisms (Malley et al. 2001; Batch et al. 2004). Even at higher turbidity values, some studies showed no impact on UV disinfection. For example, Passantino et al. (2004) concluded that clay turbidity up to 12 NTU, also did not affect the performance of UV disinfection of seeded MS2. Amoah et al. (2005) also
found that UV inactivation of dispersed *C. parvum* and *Giardia cysts* was not affected by adding natural turbidity up to a value of 10 NTU. A summary of studies on the impact of turbidity on UV disinfection of seeded microorganisms is presented in Table 1.
Table 1  
Impact of turbidity on UV disinfection of seeded microorganisms

<table>
<thead>
<tr>
<th>Water type</th>
<th>Seeded organisms type</th>
<th>Particle type for enhanced turbidity</th>
<th>Lamp type</th>
<th>Turbidity value, NTU</th>
<th>Absorbance measurement</th>
<th>Impact on UV dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline buffer</td>
<td>Klebsiella aerogenes</td>
<td>Hectorite, Dickite, Kaolinite, Halloysite</td>
<td>LP</td>
<td>0.3% (w/v)</td>
<td>Standard</td>
<td>No</td>
<td>Bitton et al., 1972</td>
</tr>
<tr>
<td>Saline buffer</td>
<td>Klebsiella aerogenes</td>
<td>Nontronite and K, Ca, Zn - montmorillonite</td>
<td>LP</td>
<td>0.3% (w/v)</td>
<td>Standard</td>
<td>Yes (dose not specified)</td>
<td>Bitton et al., 1972</td>
</tr>
<tr>
<td>DI water</td>
<td>MS2</td>
<td>Montmorillonite and algae</td>
<td>MP</td>
<td>5.7 to 12</td>
<td>Standard</td>
<td>No up to 140 mJ/cm²</td>
<td>Passantino et al. (2004)</td>
</tr>
<tr>
<td>GW, lake, river after treatment</td>
<td>MS2</td>
<td>Ambient turbidity</td>
<td>LP, MP</td>
<td>0.3</td>
<td>Standard</td>
<td>No up to 100 mJ/cm²</td>
<td>Batch et al., (2004)</td>
</tr>
<tr>
<td>Lake untreated</td>
<td>G. muris</td>
<td>Natural lake particles</td>
<td>MP</td>
<td>7.5 to 20</td>
<td>Standard</td>
<td>0.4 log decrease at 5 and 40 mJ/cm²</td>
<td>Amoah et al., (2005)</td>
</tr>
</tbody>
</table>

Impact of Particles on UV Disinfection Of Water and Wastewater
<table>
<thead>
<tr>
<th>Lake untreated</th>
<th>C. parvum</th>
<th>Natural lake particles</th>
<th>MP</th>
<th>0.3 to 20</th>
<th>Standard</th>
<th>0.8 log decrease at 5 and 40 mJ/cm²</th>
<th>Amaoh et al., (2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffered water</td>
<td>MS2 and T4</td>
<td>Kaolin clay</td>
<td>LP</td>
<td>200 mg/L</td>
<td>IS</td>
<td>No</td>
<td>Templeton et al., 2005</td>
</tr>
<tr>
<td>Simulated drinking water</td>
<td><em>Bacillus subtilis</em> spores</td>
<td>Montmorillonite</td>
<td>LP</td>
<td>0 to 5</td>
<td>Standard</td>
<td>0.5 log decrease at 60 mJ/cm²</td>
<td>Mamane and Linden, 2006b</td>
</tr>
<tr>
<td>Simulated drinking water</td>
<td><em>Bacillus subtilis</em> spores</td>
<td>Montmorillonite</td>
<td>LP</td>
<td>0 to 5</td>
<td>IS</td>
<td>No</td>
<td>Mamane and Linden, 2006b</td>
</tr>
<tr>
<td>GW</td>
<td>MS2 and T4</td>
<td>Oxidation to induce iron particles</td>
<td>LP</td>
<td>2.7 NTU</td>
<td>IS</td>
<td>Yes</td>
<td>Templeton et al., 2006</td>
</tr>
<tr>
<td>River water</td>
<td>E. coli</td>
<td>Natural particles</td>
<td>LP</td>
<td>12-32</td>
<td>Standard</td>
<td>No</td>
<td>Liu et al., 2007</td>
</tr>
</tbody>
</table>

Note: groundwater (GW), integrating sphere (IS)
A distinction should be made between the effect of water turbidity on inactivation of microorganisms which relates mainly to the scattering effect of disperse inorganic particles on UV irradiance, to the impact of particle-microbe association on UV (USEPA, 2003b). Studies showed that for the same UV fluence, microorganisms aggregated with montmorillonite clay particles using alum were more resistant to UV disinfection compared to microorganisms co-suspended with particles (Mamane and Linden, 2006b). Malley (2000) investigated the effect of flocs collected from water and wastewater processes on MS2 disinfection by mixing those particles with the virus and concluded that different particles can effectively shield MS2 differently from UV inactivation. For example at a turbidity value of 4 NTU, an additional 50 mL/cm² is needed to achieve 2 log inactivation of MS2 with settled alum floc and wastewater solids compared to filtered drinking water. Templeton et al. (2005) found that MS2 and T4 virus aggregated within a floc prior to UV exposure resulted in reduction of viral inactivation compared to disperse particulate-free virus, however in several cases reduction was not observed compared to a non coagulated mix of particles with viruses. Wu et al. (2005) found that even small goethite particles can protect E. coli and Pseudomonas putida from UV inactivation when allowing for 24 hr attachment of cells to particles. For example, at a UV dose of 15 mL/cm² and 50 NTU, the log value of E. coli was 2 logs less than at 0 NTU (no goethite particles). Liu et al. (2007) found that floc particles formed by coagulation and flocculation led to lower (more than 1-log) inactivation of E. coli at 10-40 mL/cm² compared to non particle associated E. coli at similar turbidity values. Cantwell et al. (2008) found that a limit of 2.5 log was achieved for inactivation of indigenous E. coli in unfiltered raw surface water, compared to more than 3.4 log inactivation with filtered waters.

To conclude, the influence of turbidity up to values of approximately 10 NTU on the pattern of UV inactivation was insignificant for seeded viruses, bacteria and even parasites in water. Particles however do show a real protective effect when aggregated or attached to microorganisms, or when dissolved organic matter is coating the microorganism (Templeton et al. 2005). Particle may show a false protective effect when absorbance measurements did not account for light scattering particles (Mamane and Linden, 2006b).
5.3.5. Sources of particle-associated microorganisms in water

Stormwater runoff from municipal separate storm sewer systems can readily raise the bacterial concentration in the monitored receiving water. Borst and Selvakumar (2003) found that all microorganisms except *E. coli* showed a significant increase in measured concentration after blending runoff samples at 22,000 rpm, indicating that particle-associated organisms exist in stormwater runoff. In untreated stormwater runoff, Schillinger and Gannon (1985) reported that about 10% of the fecal coliform cells were attached to suspended particles with a fraction larger than 52 μm, about 3% were attached to fraction between 30-52 μm, another 2% were attached to fraction between 10-30 μm, 1-2% were attached to fraction between 5-10 μm, and about 80% remained in the dispersed state (below 5 μm). For comparison, about 70% of *Pseudomonas aeruginosa* remained in the dispersed state, thus bacterial species show a markedly different behavior in their tendency to attach to particles.

Granular activated carbon (GAC) is used widely in water for absorbing a wide range of organic compounds which are nutrients for indigenous bacterial attachment. Analysis of particles released from granular activated carbon filter beds showed that 40% of the samples contained bacterially colonized GAC particles, and over 17% of these samples contained were colonized with coliform bacteria, 28% of which tested positive as fecal coliforms (Camper *et al.* 1986). This may be an important mechanism by which microorganisms penetrate disinfection barriers and enter finished drinking water supplies. Some of the organisms attached to carbon particles have been identified as possible pathogens (Camper *et al.* 1985b). Results showed that the accumulation of carbon fines was dependent only on the amount of water filtered and not on the time period before or after filter backwash (Camper *et al.* 1986). Camper *et al.* (1985a) performed bacteria extraction from suspended GAC particles by blending the effluent of water treatment plants with the chemical extractants. Using scanning electron microscopy (SEM), Stewart *et al.* (1990) found that only 8% of the particles had no detectable colonized bacterial cells, while 77% of the GAC particles in the effluent of a water treatment plant were colonized with less than 50 bacteria and 8% were colonized with several hundred to several thousand bacteria. Thus, the release of only a few particles could introduce significant numbers of organisms into the distribution system.

Another source of introducing particle-associated microorganisms into
water sources is through contamination of wastewater effluents. Madema et al. (1998) induced attachment of cysts and oocysts by mixing with secondary effluent and found that approximately 35% of both cysts and oocysts were almost instantly attached and as much as approximately 70% attachment was attained after 24 h. Since discharges from activated sludge treatment systems of sewage are an important source of surface water contamination with *Giardia* and *Cryptosporidium*, this rapid attachment to particles have implications on the survival of (oo) cysts and their removal during drinking water treatment by filtration and disinfection processes.

In wastewater the particles associated with coliforms were above 10 μm and 12% of the total particles associated with bacteria were in the range of 10-80 μm (Emerick et al. 2000). The minimum particle size that below it the microorganism are not shielded in effluents may not be relevant to water because in water treatment plants prior to disinfection, water is usually filtered, if not the turbidity is low and the extent of particle aggregation is low. Since the fraction of the fluid incorporated within the floc structure increases with size of the floc (Buffie and Leeuwen, 1993) then it is reasonable to assume that surface water flocs are denser then wastewater flocs and therefore the path of light that reaches the microbe will be reduced at smaller size flocs. In addition, chemical flocs in water may be denser compared to biological flocs in wastewater, thus if they reach UV disinfection unit they may shield UV light from the embedded microorganisms to a greater extent even though the size of the floc maybe smaller than in effluents. Taking in to consideration that water is a dilute system with very low coliform counts (range of 20-300/100 ml) then the coliforms that can be protected by UV are small. Nevertheless, water regulations are more stringent compared to effluents. Bacteria attached to particles in lakes and freshwater samples were counted on 3μm filters, while unattached bacteria were counted on 0.2μm filters (Simons, 1985; Kirchman, 1983).

**5.3.6. Post UV disinfectant effect on biofouling in water distribution systems**

Microorganisms associated with particles may disintegrate over time to receiving waters and result in bacterial re-growth. The extent to which microorganisms become associated with the inner surfaces of pipes or with suspended particulate matter within the water column could significantly
enhance their survival and regrowth potential in distribution lines and reservoirs. In drinking water distribution systems, bacterial growth mainly occurs on the internal surface of the pipes irrespective of the type of material (cementitious, metallic or plastic) (Momba and Makala, 2004). In examining a distribution system pipe surface at a specific sampling location and time, Ridgway and Olson (1981, 1982) found that 17% of the particles recovered contained attached microorganisms with 10-100 bacteria per particle, which means that 1-mL of sample could contain anywhere from 1,500 to 15,000 particle-associated bacteria, mostly in association with particles greater than 10 μm. Herson et al. (1987) showed that a drinking water distribution system made of cast iron-lined pipe released particles associated with Enterobacter cloacae as observed by using SEM images.

The unwanted deposition of microorganisms on all kind of surfaces, which in turn interferes with technical, economical and hygienic requirements, is termed biofouling (Flemming, 2002). There is an overwhelming evidence that biofilms grown on surfaces can develop significant tolerance against most water disinfectants compared to suspended planktonic cells (Stewart et al. 2000; Spoering and Lewis, 2001; Lewis, 2001; Donlan and Costerton, 2002; Schwartz et al. 2003; Kierék-Pearson and Karatan, 2005, Kim et al. 2006; Buckingham-Meyer et al. 2007; Arciola et al. 2008). But microorganisms in biofilms differ from their planktonic counterparts also regarding their physiology and metabolism, and can include various types of pathogenic and opportunistic microbes (Engel et al. 1980; Arnon et al. 1997; Mackey et al. 1998; Momba et al. 2000; Schwartz et al. 2003; Moreau-Marquis et al. 2008; Arciola et al. 2008).

Studies showed that biofilm formation occurred even in the presence of free residual chlorine concentrations higher than 2.5 mg/l (Momba and Binda, 2002), and also ozonation had no effect on biofilm formation on polyvinyl chloride (PVC), polyethylene (PV) and stainless steel pipe surfaces (Zacheus et al. 2000). Also, biofilms induce a disinfectant demand and consequently promote disinfectant decay in distribution systems (Flemming, 2002). Chlorination is most frequently applied to drinking water, however the risk of elevated levels of harmful disinfection by-products of chlorine in drinking water have given rise to consider alternatives for biofilm control. Oxidative disinfectants may enhance the formation of easily biodegradable organic substances (BDOC) which can in turn be utilized by microorganisms and so promote biofilm formation in water distribution systems (Glaze, 1987;
The effects of UV disinfection in water distribution systems have been demonstrated by Lund and Hongve, 1994; Lund and Ormerod, 1995; Momba et al. 1998; Schwartz et al. 2003; Pozos et al. 2004; Langmark et al. 2005; Rand et al. 2007; Dykstra et al. 2007 and Murphy et al. 2008. Pozos et al. (2004) found that UV did not impact heterotrophic bacteria in a simulated distribution system and that the resistance of microbial population to UV irradiation in the planktonic form did not help in predicting microbial community in the biofilms. Lund and Ormerod, (1995) found that UV irradiated water showed less biofilm production however attributed this to formation of hydrogen peroxide and oxygen radicals during irradiation of humic water at dose of 42 mJ/cm². They also found that ozone increased biofilm production while free chlorine residual of 0.05 mg/l was found to control biofilm formation in the distribution net. Some studies also suggested that an improved synergistic effect of UV and other disinfectants for mitigating biofilms. For example, Murphy et al. (2008) studied the combination of chlorine based disinfectants (free chlorine, chlorine dioxide and chloramines) with UV and concluded that combination of UV and chlorine based disinfectants showed better control of biofilm in water distribution systems compared to chlorine based disinfectants alone. Mofidi et al. (2002) found that heterotrophic bacteria treated with a high UV dose of 140 mJ/cm² were able to regrow, emphasizing the importance of applying residual disinfectants to UV treatment.

5.4. Impact of UV on disinfection of wastewater effluent

5.4.1. Overview

Domestic wastewater is mainly comprised of water (99.9%) together with relatively small concentrations of suspended and dissolved solids. The bulk of the organic matter consists of microbial cellular constituents and their degradation products. In a typical wastewater treatment plants, the activated sludge process promotes particle interactions in water with other particles and microorganisms to form aggregates or flocs, which are further separated by sedimentation.

Aggregation and clumping in wastewater effluents have been shown to interfere with chemical disinfection of bacteria (Stewart and Olson, 1996). Particles can protect embedded microbes in various types of disinfectants
because penetration of a chemical disinfectant (i.e. chlorine, chlorine dioxide, ozone) into particles can be limited (Qualls et al. 1983). With chlorine disinfection, Dietrich et al. (2003) and Berman et al. (1988) found that the inactivation of coliforms associated with sewage effluent was decreased because of the effect of the solids. Sobsey et al. (1991) showed that hepatitis A virus (HAV) associated with cells required ten-fold the dose of free chlorine to achieve a 4 log_{10} inactivation compared to dispersed viruses. The effect of particle association with microorganisms on UV disinfection efficacy was studied with wastewater effluents (Parker and Darby, 1995; Getr and Nicell, 1996; Loge et al. 1996, 1999, 2001a, 2001b; Emerick et al. 1999, 2000; Jolis et al. 2001; Madge and Jensen, 2006). The effect of wastewater particles on protecting microorganisms associated with particles is demonstrated in Figure 12, which illustrates the LP UV dose response curve of Salmonella typhimurium suspended in PBS and in unfiltered wastewater effluent, with high particulate load, mixed to allow aggregation.

Fig. 12: UV dose response curve of Salmonella typhimurium suspended in PBS and in unfiltered wastewater (WW) effluents, from Durham NC (Mamane and Linden, not published).

Aggregation of microbes with particles can result in reducing the effectiveness of UV disinfection by decreasing light through an aggregate due to absorption and scattering (Qualls et al. 1983; Loge et al. 1999). Loge
et al. (1999) found that UV light attenuation within wastewater aggregates is mainly due to absorbance of the particles and not due to scattering. The organisms can be located on the liquid surface interface with an unobstructed pathway or either on the interface or within the aggregate without a direct light pathway. In general, inactivation of residual indicators or pathogens depends on accessible pathways for chlorine or UV to diffuse or penetrate the aggregates (Emerick et al. 1999; Dietrich et al. 2003). Density of the floc is important to determine the floc strength, since compact structures have more inter-particle interactions, stronger flocs and impact pathways for UV light to penetrate the particle and reach an embedded microorganism. For example, Loge et al. (1999) considers chemically flocculated particles (as with AS with chemical P removal) to be denser than biological flocs produced by aeration tanks, therefore they shield UV light or possibly limit chlorine diffusion into the aggregate to a greater extent. However when polymeric flocculants are added to the chemicals used to induce chemically flocculated flocs, large and low-density flocs are formed (Gregory, 1997). Even so, biological flocs formed in wastewater have extremely high UV absorbance and may harbor coliform organisms and shield them from UV light (Emerick et al. 1999, Loge et al. 1999). The resistance of microorganisms in an aggregate is typically related to the apparent resistance of microorganisms by protection through physical association with particulates as previously discussed; however actual resistance of microorganisms may occur during certain growth conditions of the microbe (Berg et al. 1982) or due to starvation. Physiological growth state of a microbe will also affect its aggregation with particles and its actual resistance to UV, however this issue will not be discussed in this review.

Emerick et al. (1999) found that the survival of coliform bacteria in the effluents from different wastewater treatment plants such as conventional activated sludge (AS), AS with biological nitrogen removal and trickling filters appeared to have the same typical plot. However the percent of particles associated with coliform bacteria and the percent of residual coliforms was found to be different with the various pre-treatments. They determined that no residual coliform bacteria were present in particles with an average diameter below 10μm, after applying a high UV dose of 100 mJ/cm². In addition, number of coliforms associated with particles correlated to residual coliform at high UV dose; however this correlation was not tested for other microorganisms. Nelson (2000) examined the disinfection of
wastewater stabilization ponds and found that, despite the high TSS, the pond effluent was easily disinfected with UV as the majority of the coliforms were not associated with particles and strengthens the previous findings by Emerick et al. (1999). Loge et al. (1999) suggested that liquid absorbance less than 0.4 cm⁻¹ is recommended for UV systems to be viable; however this parameter does not define the ability of UV to reach target organisms inside an aggregate.

The level of pretreatment prior to the disinfection stage will effect the particle concentration and PSD prior to disinfection. Furthermore, regulations might consider to ensure the reliability of disinfection processes through implementing advanced tertiary treatments such as granular or membrane filtration to reduce the particle quantity or size in effluent or changes in wastewater treatment processes that will reduce the association of pathogens with particles. For example, disinfection was significantly improved in a sand filtered effluent compared to unfiltered secondary effluent (Qualls et al. 1983). They observed that unfiltered secondary effluent was limited to 3-4 log reduction, while filtered effluents (removed particles larger than 70 μm) increased log reduction in about 0.5 to 0.8 logs. Jolis et al. (1999) found that UV following microfiltration reliably met coliform standards for wastewater reclamation in California for coliforms with a UV dose of 45 mJ/cm² and for MS2 virus with a dose of 88 mJ/cm², while Jolis et al. in 2001, found a dose of 80 mJ/cm² is required when in-line filtration is applied.

Turbidity measurements do not provide information with regard to the extent of association of microbes with particles; however suspended solid concentration can be correlated to some extent with aggregation and is site specific. Various studies did not find a consistent relationship between TSS and UV disinfection performance (Qualls et al. 1983; Madge and Jensen, 2006).

5.4.2 Kinetics of UV disinfection of wastewater effluent

5.4.2.1 log-linear inactivation

In general, a typical dose-response curve for various microorganisms is illustrated in Figure 13. This curve shows a linear relationship representing free-swimming microorganisms (in this case MS2) that follow the Chick-Watson kinetic model.
Equation (9) describes the inactivation of disperse bacteria in completely mixed batch reactor or in ideal plug flow with no axial dispersion that receive the same intensity of UV light. The one hit model assumes that a single harmful event (hit) is sufficient to inactivate a biological unit. The dose-response curve is computed as log reduction (log10 N0/N D) as a function of UV dose and shows first order kinetics as a function of UV dose (Eq. 10).

\[ N_D = N_0 e^{-kt} = N_0 e^{-kD} \]  

(9)

\[ \log\left(\frac{N_0}{N_D}\right) = klt \]  

(10)

where

- \( N_0 \) = total number of surviving disperse bacteria (non-aggregated) at time t
- \( N_0 \) = total number of disperse bacteria before UV application (at time t=0)
- \( K \) = inactivation rate coefficient, cm³/mW·s or cm³/ml
- \( t \) = average intensity of UV light in bulk solution, mW/cm²
- \( t \) = exposure time in seconds, s
- \( D \) = UV dose or fluence, ml/cm²

5.4.2.2. Tailing phenomenon

In some cases, with increasing UV dose, a reduced inactivation termed
tailing is observed. Tailing implies a residual of microorganisms present in the water systems even at very high UV fluences, which could result in a public health concern. Various hypotheses have been postulated for tailing phenomenon. Interpretation of the tailing phenomenon can be classified by two schools of thought, one involves the vitalistic conception where individuals in a population cultured from a pure colony are not identical with respect to disinfection; the other involves the mechanistic conception where general similarity of resistance exists between individuals in a population but other factors are affecting the inactivation (Cerf, 1977). The mechanistic theory is generally accepted. Assuming all individuals in a population are similar, tailing could occur due to the following (Cerf, 1977):

A. Normal feature related to the resistance mechanism, where modification of cell resistance occurs during treatment.
B. Artifact due to inactivation of mixed populations with different resistance or same species with resistant and sensitive genetic variants.
C. Heterogeneity of treatment due to local variations in disinfection concentrations, pH and medium.
D. Artifact due to aggregation of cell suspension that occurs during treatment.
E. Artifact due to errors in method used for enumeration of low concentration of survivors. Less than $10^2$ cells per ml will require numerous repetitions to use the data in confidence.
F. Microbe-particle association (Loge et al. 2001a).

In addition to the reasons stated above, Blatchley et al. (2001) observed that in an un-irradiated culture most of the E. coli cells were discrete or in cell pairs while in the UV irradiated culture a fraction of the cells were found in clumps containing 50-100 bacteria; possibly these UV induced clumps observed resulted in tailing.

A typical plot for the log inactivation of coliforms as a function of UV dose in a secondary wastewater effluent is illustrated in Figure 14. A residual microbial concentration is observed at very high UV dose that is shielded from UV light. In various treatment processes a residual coliform concentration ranging between 2-120 MPN/100 ml was observed after exposure to a UV dose of 100 mJ/cm² (Emerick et al. 1999). A transition region was also observed between the linear and tailing zone for coliforms on the interface of particles without a direct pathway of light that reduces
inactivation efficacy (Loge et al. 2001a). UV light penetrates to wastewater flocs due to the porosity of the particles that creates pathways for light penetration and not due to light transmittance as UV light is unable to penetrate even a few microns within the floc due to the high absorbance of wastewater particles that is sufficient to block UV light (Loge et al. 1999).

Fig. 14: Typical responses of coliform bacteria to UV light in wastewater secondary effluent (data taken from Loge et al., 2001a).

In a study by Mamane and Linden (2006a) spores were induced to aggregate with clay particles in simulated drinking water. It was found that spores in the aggregates are protected from UV light across a UV fluence increase, with a steady decrease in the percent of spores within an aggregate that are protected from UV as the UV fluence increases. Tailing was not observed in this study and this decrease is expected if the aggregates contain zones that allow penetration of UV light over prolonged UV exposure with increase in fluence (Emerick et al. 2000). In a denser aggregate, UV light may not penetrate the aggregate, and an increase in UV fluence can result in tailing. As previously discussed, different distribution curves for the UV intensity within the aggregates may exist and depend on light-accessible pathways in the various aggregates and the positioning of microbes in and on the aggregate (Emerick et al. 2000).

In wastewater secondary effluent the residual population of coliform bacteria seems to resist disinfection is typically apparent at doses greater than
30 mJ/cm² (Loge et al. 2001a). However this value may not be relevant for other microorganism. Gehr et al. (2003) determined that UV inactivation curve for spiked microorganisms (fecal coliforms (FC), Clostridium perfringens and MS2) in wastewater effluent after mixing for several hours. A linear decline was observed with FC count at fluences up to 20 mJ/cm² followed by tailing at fluences above 20 mJ/cm², however the curves for C. perfringens and MS2 did not show the 2 stage behavior as C. perfringens showed a steady decline with increasing fluence, with an approximate 1-log reduction for an increment of 30 mJ/cm² and for MS-2, a 1-log reduction was achieved for each 10 mJ/cm². Gehr and Nicell (1996) evaluated disinfection of wastewater effluent indigenous FC with MP UV reactor and the transition to tailing zone occurred at approximately 25 mJ/cm². In a study by Cairns et al. (1993) the transition to the tailing zone for indigenous FC with a MP UV reactor occurred at 30 mJ/cm². Beltran and Jimenez (2008) studied the inactivation efficiencies of indigenous FC, fecal enterococi (FE) and Salmonella Typhi and spiked Acanthamoeba spp. Tailing was observed at UV light doses over 30 mJ/cm² for FC, FE and Salmonella Typhi and even at lower doses (above 15 mJ/cm²), however a dose of 30 mJ/cm² for the protozoon Acanthamoeba spp. did not result in any reduction in UV inactivation. Bohrerova and Linden (2006) found that mycobacterium inactivation was compromised with aggregates larger than 41 μm, and that they tend to naturally aggregate and result in tailing at a UV fluence above 20 mJ/cm² when spiked in wastewater effluent.

Tailing in wastewater effluent has been observed to occur both in experiments with collimated beam apparatus using indigenous or spiked bacteria (Parker and Darby, 1995; Emerick et al. 1999, 2000; Loge et al. 2001a; Gehr et al. 2003; Bohrerova and Linden, 2006; Madge and Jensen, 2006; Beltran and Jimenez, 2008) and with flow through UV reactors where a portion of the UV delivered may extend to the tailing region of the dose-response behavior of a target organism (Qualls et al. 1983; Schelte, 1987; Cairns et al. 1993; Ho et al. 1998; Jolis et al. 1999, 2001; Loge et al. 1996; Gehr and Nicell, 1996).

Pennell et al. (2008) hypothesized that resistant subpopulations of cells termed persister cells that exist in any normal cell population may be a contributing factor to tailing behavior in cells exposed to UV radiation. This phenotypic persistence is a non-inherited resistance process of cells to UV radiation. A study by Mamane-Gravetz and Linden, (2003) showed that the
presence of tailing in the fluence response curve may be a characteristic also of the surface properties of the microorganisms. Differences in hydrophobicity, PSD and volume distribution between spores support the hypothesis that increased hydrophobicity is accountable for enhancing spore self-clumping and as a result, the survival of clumped spores exposed to UV irradiation and the level of log reduction achievable before tailing. It was concluded that is important to assess whether aggregation or cell clumping is an inherent property of isolated spore surface, if it is related to the water quality or a combination of both. To conclude, tailing could be a result of physical factors (particle-microorganism and microorganism-microorganism interactions) and cell population heterogeneity with regard to UV sensitivity (Pennell et al. 2008).

5.4.2.3. Tailing models

The kinetics of UV inactivation of microorganisms in water is often more complex than a simple log-linear inactivation. Alternative models for microbial inactivation that accounts for shouldered survival curves are the multi-target model or the multi-hit model also known as the series event model (Zimmer, 1961). With the multi-target model all targets in an organism must receive a hit prior to full inactivation with a finite number of targets, while the multi-hit model assumes that an organism must receive multiple hits prior to inactivation. Severin et al. (1983) described mathematically and tested experimentally the UV inactivation of pure cultures by the multi-target kinetics and series-event kinetics. It was concluded that both theories mathematically describe inactivation, however it is more logical to accept the series-event model for inactivation of discrete microorganisms due to the biochemical reaction of thymine dimer formation, while the multi target model better describes inactivation of a clump of microorganisms where each organisms act as a single target in a clump of many targets. Deviation from first order kinetics, in practice, could also include reduced disinfection performance due to uneven dispersion in UV plug flow reactors that result in distribution of residence time in the reactor.

A simple method to account for tailing of dose response curves is to consider a microbial population that consists of two subgroups that are each inactivated by the one hit model, where one is sensitive and the other is resistant (Jagger, 1977). The double exponential model includes tailing but not shoulder, thus another development is the modified two-population model.
where the sensitive subpopulation follows the multi-target model and the resistant subpopulation follows the one hit model (Jagger, 1977). Cantwell et al. (2008) used the double exponential model to allow quantitative assessment of tailing curves for indigenous coliforms in unfiltered surface water.

Cabaj and Sommer, (2000) developed an empirical model similar to the model presented by Jagger (1977) to describe the survival function of microorganisms with shoulder and tailing using four parameters \(K_1, K_2, \delta_1, a\). Equation 11 was developed for a case of a mixture of two microorganisms with different sensitivities to UV irradiation; one more sensitive with a shoulder \(d\) and the other less sensitive. A shoulder refers to the section of the curve in which survival is higher than the expected linear response at low UV fluences. The survival function was adapted to describe the inactivation of cultured isolated spores that theoretically thought as a mixture of spores with different sensitivities to UV within the same suspension. As illustrated in Figure 15, each spore isolate exhibited two different fluence based kinetic zones where the linear zone corresponded hypothetically to the sensitive variant and the tailing zone corresponds to the less sensitive variant (Mamane-Gravetz and Linden, 2005). This equation was experimentally validated with spores (Mamane-Gravetz and Linden, 2005; Bohrerova et al. 2006b).

![Figure 15](image.png)

**Fig. 15:** UV fluence-response curve of *Bacillus* spore isolate (rev Mamane and Linden, 2005).
\[
\frac{N_D}{N_0} = 1 - (1 - 10^{-k_1 \cdot H_0})^{10^d} + a \cdot 10^{-k_2 \cdot H_0}
\]

(11)

\[
a = \frac{N_{0,2}}{N_{0,1}}
\]

(12)

where

- \(k_1\) = Absolute value of fluence based inactivation rate constant of the linear zone in the fluence-response curve plotted as \(\log(N_D/N_0)\) vs. \(H_0\) (cm\(^2\)/ml).
- \(k_2\) = Absolute value of fluence based inactivation rate constant of the tailing zone in the fluence-response curve plotted as \(\log(N_D/N_0)\) vs. \(H_0\) (cm\(^2\)/ml).
- \(d\) = intercept with y axis (\(\log(N_D/N_0)\)) of the linear zone in the fluence response curve.
- \(N_0\) = initial concentration of sensitive \((N_{0,1})\) and less sensitive \((N_{0,2})\) microorganism.
- \(\log(a)\) = intercept with y axis (\(\log(N_D/N_0)\)) of the tailing zone in the fluence response curve \((N_{0,2} \ll N_{0,1})\).
- \(H_0\) = the fluence at 253.7 nm (mJ/cm\(^2\)) (same as \(D\) is eq. 9).
- \(N_D/N_0\) = survival rate.

It is complex to predict the tailing phenomenon mathematically (Chiu et al. 1999). In an attempt to mathematically describe how particles affect UV inactivation, Scheible, (1987) modified the first order kinetics of the dose-response curve by adding to the dose-response equation (Eq. 9) a term for the bacterial density that is associated with particles (Eq. 13). \(N_p\) represents the bacterial density associated with particles where UV radiation is unable to penetrate the aggregate (colonies/100 ml), while \(N_0\) represents the sum of initial non-aggregated density. In this case it was assumed that \(N_0\) may include also bacteria associated with particles however it was assumed that in effluents \(N_0\) is mostly of dispersed bacteria. This model is different than the models previously described in its approach, as the tailing portion is not considered as the resistant subpopulation but is considered as a population that is physically associated or occluded within the floc.
\[ N_D = N_0 e^{-k_D t} + N_p \]  

(13)

A model to account for the interaction of light with free microorganisms that incorporates particle size distribution, total number of microbial counts associated with particles and transmittance of particles to UV was proposed by Cairns et al. (1993). The model assumes that the number of particle-associated counts is distributed proportionally over the PSD, and the intensity of light at the inner core of the particle is assumed to be the intensity at the surface of the particle corrected for UV absorbance of the particle. In addition, this model considered a reference point of 9 μm that differentiates between the free and attached microorganisms. The model can be used for predicting the impact on dose with varying water quality parameters such as PSD and absorbance. Application of this model requires knowledge on the size distribution of the particle-associated flocs which are not generally known, although they can be calibrated with empirical data. Another model that will be further described in detail is the model presented by Emerick et al. (2000). A summary of the kinetic models for UV inactivation and their equations is detailed in Farooq (2005).

Scheible (1987) developed a correlation between the log of the number of particle associated coliform residual bacteria after exposure to UV radiation (at high doses) and the log of total suspended solids (TSS in units of mg/ml) (eq. 14). It was assumed that at high doses any residual coliforms after exposure would be attributed to the coliforms associated with suspended solids. Scheible (1987) found that residual effluent coliforms are significantly influenced by TSS as the empirical coefficients c and m were 0.26 and 1.96 respectively. Beltran and Jimenez (2008) varied effluent TSS by adding samples from the mixed liquor to obtain the empirical coefficients c and m. Results showed that inactivation of free fecal coliforms were not dependent of the TSS value, while in the tailing region inactivation rates decreased with increase in TSS values between 15-100 mg/l with c and m values of 0.06 and 2.42 respectively.

\[ N_p = c(TSS)^m \]  

(14)

where

c represents the intercept of the regression line and m represents the slope of the regression line. The empirical coefficients c and m are specific to each type of effluent.
Loge et al. (1996) developed an empirical UV model for the tailing region to predict coliform inactivation in unfiltered secondary wastewater effluent. This empirical model correlated between the effluent coliform density after exposure to UV light to parameters such as TSS, unfiltered UVT at 253.7 nm, influent coliform concentration prior to UV exposure and the applied UV dose. Because this model is empirical and based on parameters that are related to but not necessarily a direct measure of dose reaching target organisms, it should be calibrated at a particular WWTP prior to full scale design with a recommended minimum $R^2$ of 0.7. In addition, the number of influent coliforms was statistically insignificant in this model.

Sakamoto and Zimmer (1997) concluded that UV light can be easily penetrated through particles below 10 µm while particles above 40 µm in size are difficult to penetrate even at high UV doses. Ho et al. (1998), concluded, that correlation between residual total coliforms at high UV dose greater than 90 mJ/cm² (presumably associated with particles) and TSS or total PSD of large particles above 40 µm was not successful especially with water with high variability, and that UV models do not provide predictions of UV disinfection and a pilot study is preferred. In addition, it was reported that there is no correlation between TSS and the number of particles containing coliforms and that the degree in which coliforms are embedded in particles determines level of inactivation achieved. Beltran and Jimenez (2008) found that the inactivation efficiencies of indigenous FC, FE and Salmonella Typhi in three effluents from activated sludge (AS), rotating biological reactors (RBC) and trickling filters (TF), with different volumes of particles larger than 40 µm (2, 15 and 7% respectively), did not effect UV inactivation efficacy. It should be noted that in terms of number of particles larger than 40 µm (and not volume) there was no difference in all effluents.

Qualls et al. (1983) noted that filtration of effluent with 8 µm filters inactivated 99% of the coliforms up to 4.3 logs survival when exposed to LP UV lamps. Consequently the protected coliforms with the unfiltered effluent samples are a minority that leveled off at 2-3 logs of survival at doses greater than 12 mJ/cm². Using the data reported by Qualls et al. (1983), Emerick et al. (1999) confirmed that irradiating 10 µm filtered wastewater at high UV dose of 150 mJ/cm² resulted in a decrease in residual coliforms near detection limit of 2 coliforms/100 ml. Thus 10 µm was a critical size or lower limit capable of completely shielding microorganisms. In addition, Emerick et al. (1999) found that although different WWTP treatment processes result in
different percentage of influent particles associated with at least one embedded coliform and different total PSD, the same percent (above 99%) of particles with associated coliform bacteria were inactivated by high UV dose. Thus the number of particles with associated coliforms in the influent (measured for particles above 10 μm) and not the total PSD is directly related to the residual coliforms in the effluent of UV system.

Emerick et al. (2000) proposed an explicit mathematical approach using fundamental parameters to describe the inactivation of coliforms associated with particles, which received a reduced UV intensity compared to the bulk liquid. It was assumed that in the enumeration of coliform bacteria at least one coliform was embedded in the particle with the multiple tube fermentation (MTF) technique, and that the numbers of particles associated with coliforms are known as well as the particular UV intensity for that associated coliform. The critical coliform is the coliform that is mostly shielded from high UV dose. In addition it was assumed that the probability of inactivating the critical coliform is independent of the size of the particle containing the organism. The inactivation model for the total number of particles containing at least one surviving coliform at time t (surviving after exposure to high UV dose), \( N_p(t) \) is (Eq. 15):

\[
N_p(t) = N_p(0) \int_0^1 e^{-k\omega t} d\omega
\]

(15)

where

\( \omega \) = fraction of the average UV intensity that reaches the critical coliform, \( \omega \) can approach 1 for a bacteria that is on the surface of the particle and approach 0 for a bacteria that is totally shielded from the UV light.

\( N_p(0) \) = initial number of particles containing at least one embedded coliform at time \( t=0 \) (for particles larger than 10 μm).

Summation of all particles and integration resulted in (eq. 16):

\[
N(t) = N_D(0)e^{-kt} + N_p(t) = N_D(0)e^{-kt} + \frac{N_p(0)}{kt}(1-e^{-kt})
\]

(16)

To verify the assumption that particle size does not affect the survival of residual UV coliform exposed to high UV doses using LP lamps, Emerick et
al. (2000) serially filtered wastewater to three size classes (10-11, 10-20 and 10-40 μm) and enumerated the surviving coliforms at each size class. The conclusion above was supported as the confidence intervals of the surviving coliforms at the different degree of filtration after high UV dose overlapped. To remove or eliminate tailing there are two possibilities: (a) physically remove particles (reduce Νp variability) by sand or disc filtration (Loge et al. 2001a) or (b) modify activated sludge process (Loge et al. 2001b). From this study it was concluded that: (a) residual coliforms are uniformly distributed throughout all particles containing coliforms and independent of particle size; (b) particles smaller than the minimum particle size do not contain regions that can shield microbes from UV light; (c) at size greater than the critical size (particles with associated bacteria above 10 μm), particle size is not important in determining shielding of coliforms from UV light. However, this conclusion was criticized by Madge and Jensen (2001), who stated that the hypothesis cannot be accepted as large uncertainties imply not to reject the alternative hypothesis rather than accept the hypothesis that residual coliforms are uniformly distributed independent of particle size and that UV disinfection is independent of particle size. In addition they criticized the assumption that the parameter ω is independent of particle size.

Wang et al. (2006) found that larger particle size fraction of effluent above 10μm consistently produced a lower efficiency in UV inactivation of FC than the smaller fractions between 5-10μm and 2-5μm, and the efficiency of three were 1.2, 2.8 and 4.1 log respectively; while the free FC was easiest to be disinfected, with efficiency of more than 5.3 log. Madge and Jensen (2006) fractionated wastewater effluent to separate particles into different size fractions of 5 μm and 20 μm from wastewater effluents representing a size that was considered to include small particles that do not shield bacteria and a size that was considered to shield bacteria and significantly impact UV disinfection. Whole sample and fractions were exposed to UV, and they hypothesized that the influence of particles depends both on the number of particle-associated bacteria and both on the size of the particles, but also other factors such as the nature of the particles and culture age. The resulting fractions (<5 μm, 5-20 μm, >20 μm) were exposed to LP UV light, where 55-65% were free floating or small fractions under 5 μm and 30-45% were in the fraction above 20 μm. Results showed that particle size is an important factor in determining disinfection rate as the fluence based rate constant for the linear portion varied for the different fractions and a trend was observed.
between deceasing rate with increase in percent of large fraction that also tends more to tail.

Pennell et al. (2008) developed a model to describe tailing behavior as a result of both phenotypic persistence and external shielding (PPES). This model was based on the assumption that two subpopulations exist, shielding is equally distributed across subpopulations, and persistence is not inherited and intrinsic. The total initial concentration for the microorganisms prior to UV disinfection not associated with particles is the sum of the concentrations for susceptible microorganism and persistent microorganisms. The overall expression rate for dispersed microorganisms includes two terms, where the susceptible subpopulation follows the series event model and the persistent subpopulation obeys the one-bit model. The model should account for external particle shielding in the case where particle association may also affect the dose-response curve. The kinetic model for UV disinfection that accounts for particle shielding ($N_p(t)$) was adapted from Emerick et al. (2000) (Eq. 15) with modification that includes the series-event model.

Biodosimetric measurements depend on the survival function of the microorganisms and on the shape of the fluence distribution in the UV reactor, as a wide distribution will result in reduction equivalent fluence (REF) measurements that depend on the microorganism tested (Sommer et al. 1999). MS2 is typically used as a challenge organism due to its first order kinetics, while the tailing and even the shoulder complicates the physical explanation of the REF phenomenon. With spores, tailing can affect REF values in this case where the UV fluence in the reactor is in the tailing region of the spores' fluence-response curve (Bohrerova et al. 2006b). However using non-linear equations (as presented in eq. 11) to calculate the REF at various water transmittance values confirms that the comparison of the first order rate constants between organisms with tailing versus organisms with linear inactivation is not sufficient for the determination of REF when considering the differences in sensitivity of the microorganisms (Bohrerova et al. 2006b). Consequently using a Lagrangian particle tracking approach (Ducoste et al. 2005) in conjunction with the non linear equation with allows REF calculation with reasonable agreement with all measured data (Bohrerova et al. 2006b).

5.4.2.4. Floc stability

Floc stability during sampling and obtaining the true count for the
microorganisms embedded within the floc samples are major issues to consider. Even the sampling of flocs can cause disaggregation of these fragile structures (Droppo and Ongley, 1992); therefore there is a possibility that aggregates can disaggregate to a certain extent under exposure to disinfectant due to mixing and under the particle size analysis. Control of disaggregation can be optimized by applying laboratory precautions. In addition, not using extraction techniques to release the particle-associated microorganisms can result in one colony on a plate that originates from one or more surviving microbes. However, the extent microorganisms are associated with particles can be determined by using chemical extractants combined with physical separation techniques such as blending or homogenizing (Camper et al. 1985a; Parker and Darby, 1995; Ormeç and Linden, 2003; Caron et al. 2007; Plancherel and Cowen, 2007). The difference in the microbial counts with and without these separation techniques will indicate the significance of particle-associated microbes in the water samples that were shielded from the UV light.

5.4.2.5. Reuse applications

Water reuse regulations tend to have very strict microbiological water quality requirements (e.g., very high virus reduction levels), which could make particle effects even more relevant, if falling restricts the maximum log reduction that is achievable. Physical and microbiological standards are used to assess the suitability of reclaimed water for the specific application. The stringent standards defined by the California Wastewater Reclamation Criteria (CWRC) specifies the level of treatment required for various applications of reclaimed water in California, with disinfected tertiary reclaimed water that meets the following criteria: (a) filtered effluent turbidity < 2 NTU (daily average); (b) final effluent total coliforms < 2.2 colony forming units (cfu)/100 ml (7-day median); (c) five-log (99.999%) virus reduction (for non-chlorine disinfection systems) demonstrated using MS2 phage, poliovirus, or any virus as resistant to disinfection as poliovirus. Jolis et al. (1996) compared in-line filtration to microfiltration (MF) followed both by UV disinfection to test their ability to meet the CWRC requirements. Both met the turbidity requirements; however, MF also produced effluent that was free of suspended solids and coliforms with 1.9 log removal of MS2 virus. This resulted in a minimum of 80 mJ/cm² dose needed to achieve 5-log virus removal with in-line filtration versus 45 mJ/cm² required with MF.
6. IMPACT OF PARTICLE-MICROBE ASSOCIATION ON CRITERIA FOR CHOOSING AN INDICATOR

Water and wastewater may contain a wide variety of bacteria that are opportunistic or overt pathogens of animals and humans. Many bacteria that are non-pathogenic to humans have their natural habitat in water, and some opportunistically pathogenic bacteria occur naturally in water as well (APHA, 2005).

The fecal coliform group is the traditional indicator of fecal pollution. However, other studies suggested enterococci as indicators for fecal pollution (Cabelli et al. 1983), or Clostridium perfringens (Fujioke and Shizamura, 1985). The indicator concept has been used for over 50 years and some of the basic assumptions behind choosing an ideal indicator are as follows: (1) it is present when pathogens are present, (2) it cannot grow in the environment, (3) it should be indigenous to intestinal tract, (4) it should not be removed at a greater rate than the pathogen in treatment processes, (5) it is inexpensive and easy to count, and (7) it is found in higher numbers than pathogens (Bitton, 1999; Griffin et al. 2001). With respect to the treatment plant, indicators should represent the pathogen’s removal through treatment processes, and thus become a tool to integrate source and effluent quality parameters with treatment plant operation. It is important to examine variability in raw water quality on the use of an indicator to assess the pathogen; and the effect of treatment on the use of the indicator through treatment performance (Neiminski et al. 2000). Therefore an indicator should ideally represent a tool for improving treatment process. In some circumstances it was found that the traditional fecal bacterium is not representative of pathogens. For example, Neiminski et al. (2000) could not find any bacterial indicator for Cryptosporidium removal by water treatment processes.

Pathogens post disinfection may be either non-particle associated or particle associated. However, the criteria for indicators are based on assumptions of disperse indicators and pathogens, and do not consider whether the assumption for ideal indicator also holds between indicators associated with particles and pathogens associated with particles. Cryptosporidium parvum oocysts are inactivated by relatively low UV doses (Clancy et al. 2000; Craik et al. 2001; Shin et al. 2001), however reduction in UV inactivation was observed with addition of naturally occurring particles.
above 10 NTU; probably due to interaction with particles beyond considering the adjustment of increased absorbance (Amonh et al. 2005). Other studies showed that natural particulate matter in water can protect indigenous aerobic spore forming bacteria from UV radiation and these organisms may serve as a natural model organisms for cryptosporidium associated with natural particles in water (Caron et al. 2007). Also natural occurring indigenous coliforms in unfiltered surface waters showed tailing in the UV dose-response curve (Cantwell et al. 2008). These results imply that natural particles at certain size and characteristics may results in increased resistance.

Moreover, indicators are counted through plate culture assays that identify specific species of colony forming units (CFUs) of bacteria in the water sample (Griffin et al. 2001). One colony can originate from a single wastewater particle even if that particle was associated with several indicators. This raises the question of whether the quality of the particle can influence not only the number of surviving indicators associated with that particle but also the number of surviving pathogens associated with that particle. Currently little is known about the association of pathogenic bacteria, viruses and protozoans to particles in wastewater (Linden et al. 2004).

UV inactivation of various pathogens and indicators seeded in wastewater effluents vary in their inactivation kinetics both in the first order and tailing zone (Hassen et al. 2000). Consequently, it may be helpful to find two indigenous indicators for meeting UV disinfection goals. A sensitive indicator for the first order kinetics such as E. coli and a resistant indicator for the tailing phase such as Clostridium perfringens which will correlate to inactivation of E. coli associated with particles. Comparing first order and tailing kinetics of different indicators to pathogens free and associated with particles, with various particle types, may enable correlating risk of indicator survival post - UV disinfection to pathogen survival and ultimately protect public health.

7. PRACTICAL IMPLICATIONS OF PARTICLES ON UV DISINFECTION FOR WATER UTILITIES

Water utilities should recognize that turbidity is not the only important parameter when evaluating the effect of particles on UV disinfection efficacy. Interactions between particles, microorganisms and UV light can affect
disinfection efficacy. Particles add turbidity to waters but also may associate with microorganisms in an aggregate. Turbidity and absorbance measurements of waters are not reliable indicators of aggregation because particle-associated microbes can exist with lower turbidity and absorbance values, compared to a system with similar concentration of particles co-suspended with microorganisms (Mamane and Linden, 2006a,b).

To evaluate aggregation, various parameters can be measured such as size, volume, feret number and circularity, depending on the method used for particle counting. Dividing particle counts into fraction of counts below and above a certain value (Figure 16), which differentiates between free organisms (sus) and aggregated particles (agg), and then into different size bands within the aggregate can define the extent to which microbes can be potentially protected in an aggregate. The most dominant fraction, with largest size band will likely have an effect on protection of microorganisms within aggregates. Natural particles aggregated with microorganisms will require an increased UV fluence compared to a dispersed system of particles co-suspended with particles. In this study, a decreased inactivation of microorganisms associated with particles with the particle size distribution of an aggregate containing larger size class or bands (Natural agg compared to Simulated agg), while no correlation of inactivation with dispese fraction (Natural sus compared to Simulated sus) (Mamane and Linden, 2006b). As a result, analysis of particle size distribution with a particle analyzer will provide insights into the extent of particle aggregated microbes in a water sample that may occur naturally or as a result of filter breakthrough.

![Figure 16](image_url)

**Fig. 16:** Particle counts for suspended and aggregated systems (reprinted from Mamane and Linden, 2006b).
Natural sus -- represents spores suspended with natural particles from natural raw water

Natural agg -- represents spores aggregated with natural particles with alum

Simulated sus - represents spores suspended with clay particles in simulated drinking water

Simulated agg - represents spores aggregated with clay particles in simulated drinking water

UV light penetration in water samples can increase several fold after coagulation and flocculation processes, due to removal of dissolved organic matter. Dissolved organic matter in natural waters appears to be the major absorbing component influencing light penetration in UV reactors. This has implications on the importance of placing UV reactors after processes that remove organic matter in surface waters. Utilities should evaluate the impact of UV light scattering by particles, in addition to particle shielding and aggregation with microorganisms. Light scattering of particles can result in over dosing the UV system, which raises energy costs to the water utilities. Falsely higher fluence can impact inactivation as follows: (a) a sensitive microbe associated with particles will likely result in increased inactivation compared to a resistant particle-associated microbe; (b) the same microbe associated with particles that scatter light differently, will likely be more inactivated within the aggregate that is more scattering.

The degree of particle scattering can be evaluated by measuring water absorbance with an integrating sphere spectroscopy unit attached to the spectrophotometer. Periodically, water samples at the inlet of the UV reactor can be analyzed to determine if scattering is an issue. In general, scattering is not an issue for waters with turbidity values below 3 NTU (Christensen and Linden, 2003). Inorganic mineral particles can scatter light to a greater extent compared to organic particles and particles associated with microorganisms can also scatter some of the UV light (Mamane et al. 2006). The “scattering ability” of a specific water sample can be evaluated by collecting a grab samples and measure absorbance with an integrating sphere concurrently with measuring turbidity and particle analysis. Such measurements, especially during or after disruption of a process in the treatment of water at the plant, will indicate the effect of light scattering of particles and aggregates for waters. Scheible (1987) suggested filtering a water sample to obtain a
reasonable true absorbance if an integrating sphere is not available.

The flow chart presented in Figure 17 indicates a step-by-step approach to evaluate the impact of particles in unfiltered waters or even effluents on UV disinfection efficacy. As previously summarized, UV disinfection of unfiltered waters will not be significantly effected with turbidity levels below 3 NTU unless there is a fraction of particles greater than a certain size that

![Flow Chart]

**Fig. 17: Flow chart for unfiltered waters**
may harbor microorganisms. Scattering of particles or aggregates in natural waters will probably not considerably affect the disinfection efficiencies. Thus, the state of the microbe whether dispersed or in an aggregate, may effect disinfection efficiencies, and can be monitored via particle sizing.

Difficulties arise as the particle concentration may vary over time in a matter of hours, days and over seasons. In addition, processes such as storm events, runoff, infiltration and inflow can increase the concentration of particles in the water. This review discussed thoroughly the impact of particles on UV disinfection; however the effectiveness of any disinfection technology may drop significantly as pathogens are associated or enmeshed within flocs.

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