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Adsorption and inactivation of bacteriophage fr on iron oxide coated sand

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Abstract

(english version)

Waterborne viruses create a significant public health risk, accounting for around 50% of waterborne diseases in the US between 1971-1994. Viruses can be removed during flow through porous media (e.g. soil, constructed wetlands), though the main removal mechanism is adsorption rather than inactivation. Metal oxide coated sands are often used to enhance adsorption. The goal of this study was (1) to characterize the adsorption and inactivation of bacteriophage fr onto synthesized iron oxide coated sands, and (2) to examine how adsorption and inactivation are affected by changes in environmental conditions (pH, ionic strength, humic acid concentration). Batch experiments were run to test: (1) virus adsorption and inactivation on IOCS compared to uncoated sand, (2) adsorption of IOCS after changing environmental conditions both (a) at the time of virus adsorption and (b) after viruses were pre-adsorbed onto IOCS under control conditions (pH 7, ionic strength 20 mM), and (3) adsorption of IOCS aged with buffer and humic acid solution.

The iron oxide coating greatly improved the adsorption capacity of the sand, though no virus inactivation was observed within the time scale of our experiment. Therefore, release of infective viruses was observed.

The predominant environmental factor was pH: viruses did not adsorb when incubated with IOCS at pH 9, and pre-adsorbed viruses were released when raising the pH from 7 to 10. Increasing the ionic strength did not impact adsorption onto IOCS, though it did increase adsorption onto uncoated sand. Humic acid decreased virus adsorption at concentrations greater than 30 mg HA/l, and 50 mg HA/l released a large fraction of pre-adsorbed viruses.

IOCS aged in buffer solution and with humic acids caused little and no reduction in the adsorption capacity, respectively.

These results demonstrate that systems designed to remove viruses by adsorption onto IOCS may not completely eliminate the threat of infective viruses. Due to the potential re-release of infective viruses by changes in environmental conditions, designing a filtration system to simply remove viruses by adsorption without causing inactivation may not eliminate the public health threat.

Furthermore, these batch experiments provide important knowledge for the future implementation of continuous flow experiments.

Abstract

(version française)

Aux Etats-Unis, entre 1971 et 1994, les virus ont été à l'origine d'environ 50% des maladies transmissibles par ingestion d'eaux contaminées. Le transport à travers un milieu poreux (ex. sol, marais artificiel) est un moyen efficace de diminuer la concentration de virus dans les eaux. Cette diminution est principalement due à l'adsorption des virus au milieu, plutôt qu'à leur inactivation. Dans l'optique d'améliorer l'adsorption des virus, le sable recouvert d'oxide de métaux est fréquemment utilisé. Les objectifs de cet étude étaient de (1) caractériser l'adsorption et l'inactivation de fr bacteriophages sur du sable recouvert d'oxide de fer (IOCS) synthétisé en laboratoire, et (2) d'examiner comment l'adsorption et l'inactivation sont influencées par les changements de conditions environmentales (pH, force ionique et concentration d'acide humique). Les expériences en mode " batch " ont été réalisées afin de tester : (1) l'adsorption et l'inactivation de virus sur le IOCS en comparaison avec du sable non recouvert d'oxide de fer, (2) l'adsorption du IOCS en changeant les conditions environmentales, (a) pendant l'adsorption des virus et (b) après que les virus aient été adsorbés au IOCS dans des conditions de control (pH 7, force ionique 20 mM), et (3) l'adsorption du IOCS altéré par contact avec une solution tampon et une solution contenant de l'acide humique.

La couverture d'oxide de fer améliore grandement la capacité d'adsorption du sable, cependant nous n'avons observé aucune inactivation à l'échelle de temps de notre expérience. Par conséquent, on a observé le relâchement de virus infectieux dans la solution.

Le facteur environmental prédominant était le pH : en effet les virus n'étaient pas adsorbés lorsqu'ils ont été mis en contact avec le IOCS à pH 9, et les virus déjà adsorbés ont été relâchés lorsque le pH a été augmenté de 7 à 10. L'augmentation de la force ionique n'a eu aucun impact sur l'adsorption au IOCS, mais a augmenté l'adsorption au sable non-recouvert d'oxide de fer. L'acide humique(HA) a diminué l'adsorption des virus à partir d'une concentration de 30 mg HA /l et à 50 mg HA/l une large partie des virus déjà adsorbés ont été relâchés.

L'altération du IOCS par la solution tampon et par l'acide humique a causé, respectivement, une faible diminution et aucune réduction de la capacité d'adsorption.

Ces résultats ont démontré qu'un système conçu pour épurer des virus par adsorption sur du IOCS ne pouvait pas complètement éliminer le danger de retrouver des virus infectieux dans l'eau après traitement. En effet, à cause de la possibilité de relâchement de virus infectieux lors de changements de conditions environnementales, la conception d'un système de filtration de virus par adsorption sans causer d'inactivation de ceux-ci, ne permet pas d'éliminer complètement le danger dans un cadre de santé publique.

Par ailleurs, ces expériences " batch " nous fournissent de bonnes connaissances pour de futures expériences en mode " écoulements continus ".

Contents

Chapter 1

Introduction

1.1 Context

There were 650 outbreaks of waterborne disease in the U.S. between 1974 and 1999 leading to 569,754 cases of illness. Of these outbreaks, 58% were associated with groundwater sources and 33% were associated with surface water sources. Eigth percent of all reported outbreaks were due to enteric viruses (hepatitis A virus, Norwalk virus and rotaviruses). It is suspected that many waterborne disease for which no etiological agent was indentified (about 47 % of all reported outbreaks) may be caused by viruses [1].
Therefore, in order to assess and to prevent the risk from contaminated groundwater, it is useful to understand the transport behaviour of viruses in the soil and in alternative wastewater treatment installations such as constructed wetlands or sand filter.

In order to examine the fate of viruses through porous media, bacteriophages (i.e., viruses that infect bacteria) are frequently used as surrogates for human viruses because they are not pathogenic to human, easier to prepare in large quantities and relatively easy to manipulate [30].

Thus, many studies have been performed with different phages, different porous media, and in different environmental conditions.
Two important environmental conditions that have been well studied are pH [17] and ionic strength [25][40]. More recently, the influence of organic matter [39] and other environmental parameters have been studied including the effect of anoxic conditions [35], anions (e.g. phosphate, nitrate, sulfate) [38][36], and microbial growth [37].
Most of the previous experiments were done in saturated water conditions, but transport in unsaturated flow conditions has also been recently studied [34][4].
The choice of phages that can be used as surrogates is relatively large and it can be time-consuming to work with several phages. Therefore, the selection of representative phages with high degrees of similarity to human viruses and adsorption characteristics are required. To ensure that the surrogate phages are not overestimating removal by

adsorption, researchers often select poorly-adsorbing phages as conservative indicators. For example, in adsorption studies performed with quartz sand, phages MS2 and PRD1 [30][11] are often studied. Indeed, MS2 and PRD1 have isoelectric points that are near the isoelectric point of the quartz sand [30]. However, other studies [6] have compared numerous phages together in order to establish the relationship between phage characteristics (e.g. isoelectric point, size, hydrophobic/-philic nature) and their behaviour in porous media.
As previously stated, most of the studies were done with quartz sand but increasingly more studies are done with metal oxide (e.g. iron oxyhydroxides, aluminium oxide) coated quartz sand [18][40][7]. The benefits of using coated sands include the presence of favorable sites for phage attachment, and the coating methods allow for the production of defined amounts of various metal oxide species. Besides uncoated and coated quartz sand, natural soils [32] [23] have also been studied, however, it is more difficult to determine the effect of the characteristics of the highly complex soil matrix.
While most studies have been performed in the laboratory, field scale experiments have also been done [32] [28].

Two main theories provide a theoretical and qualitative framework for adsorption: the Derjaguin-Landau-Verwey-Overbeek theory (DLVO), which is based on potential energy changes resulting from electrostatic interactions[27], and another unnamed theory based on hydrophobic interactions [8].

Some studies quantify phage adsorption by calculating the mass recovery [40], others calculate the sticking efficiency (that take into account the grain size diameter and the porewater flow velocity) [7], but the most current way to characterize phage sorption is now kinetic modeling with attachment/detachment to different kinds of attachment sites, and inactivation in the bulk liquid and on the solid surface [31][3]. Indeed, the development of modelling software has provided efficient tools to develop complex models that can be used to analyse and characterize data measured after the passage of phages through a soil.

1.2 Goal of the project

It was reported that inactivation rates in the solution or onto the surface are ten to hundred times much slower than detachment rates to the unfavorable fraction of attachment sites of a porous medium [29]. Thus attenuation of the phage concentration in water during soil passage occurs mainly because of adsorption behaviour (attachment/detachment) rather than because of inactivation.

Therefore, this work is focused on the effect of environmental changes on the release of infective viruses pre-adsorbed on synthesized iron oxide coated sand, because environmental changes appear often in natural conditions.
Due to the duration of the master thesis (four months), this work investigate the main

environmental condition changes (pH, ionic strength and natural organic matter content of the water) by batch experiments, that were easier to implement than continuous flow experiments.

This work was made keeping in mind that (1) it would introduce further continuous flow experiments that will lead to observe adsorption kinetic behaviour and, (2) that our IOCS would be used in the context of wastewater treatment as post-treatment for a conventional wastewater treatment plant or as additive element within a constructed wetland or sand filter.

Chapter 2

Theory

2.1 Fate of viruses in porous media

Viruses consist of two main components: a nucleic acid genome (RNA or DNA) surrounded by a protein shell called the capsid. Some viruses, called naked viruses, have just a capsid containing the viral genome while others, called enveloped viruses, have an extra membrane composed of a lipid bilayer with proteins around the capsid [20].
The amino acids in this protein coat (capsid or envelope) contain weakly acidic and basic groups, like carboxyl and amino groups, that determine the amphoteric[1] nature of the virus particle [30].
When such particles are travelling through a porous media, which consists of solid inorganic (e.g sand, clay) and/or organic (e.g organic material, polymer) particles, a number of interactions and reactions may take place. Depending on the surrounding environmental conditions like pH, ionic strength, temperature of the solution, presence of hosts cells and reactive chemical species, the virus could be inactivated in the solution or on the solid particles, adsorbed or desorbed to/from the solid particles, and also could replicate if the host cells are present.

2.2 Adsorption/desorption behaviour

2.2.1 General principles

Viruses are smaller than prokariotic cells, ranging in size from 20-300 nm [20]. In this size range, virus particles are often considered colloids. In porous media like the sand used in this study, the size of the pores are bigger than size of the viruses. Thus, the capture of viruses by straining, i.e. where particles are larger than the pores, does not take place. However, unlike soluble substances which can move freely with soil water movements, there are various restrictions on the movement of virus particles, mainly via sorption onto components of the soil [21]. Sorption sites in soil occur both in the static soil matrix and on the mobile soil particles [21].

[1] Amphoteric molecules can either react like base or like acid.

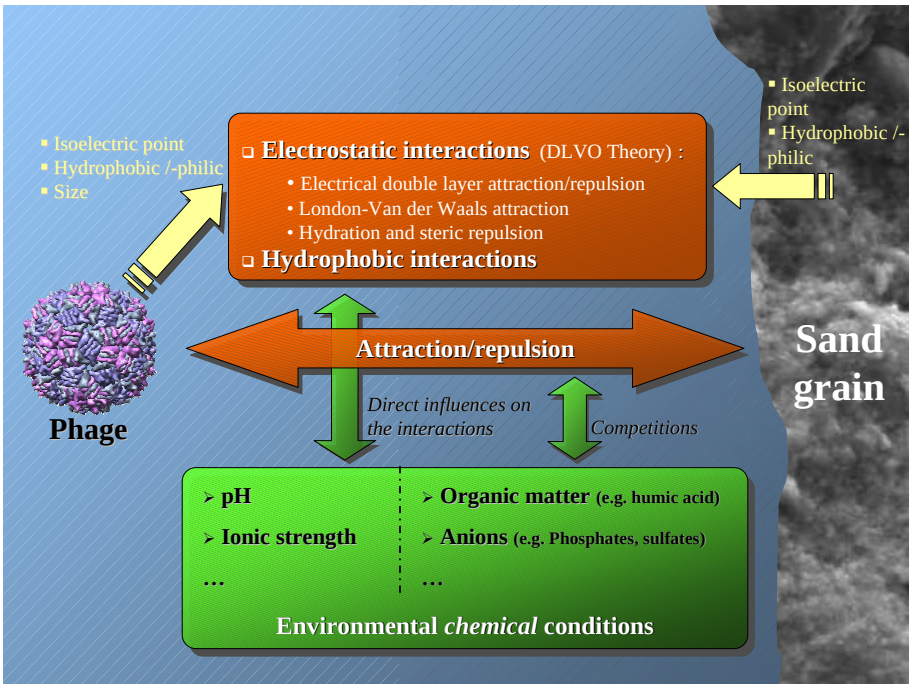

Figure 2.1: **Overview of the interactions of a virus with a solid surface in case of saturated conditions.**

At a particle scale, adsorption and desorption are governed by attractive and repulsive forces that exist between colloids (e.g. viruses) and the porous media [30]. These intersurface forces include electrical double layer repulsion or attraction, London-van der Waals attraction, and poorly characterized short-range forces such as hydration[2] and steric repulsion [3]. The net effect of these attractive and repulsive forces on the interactions between surfaces is described by the DLVO theory (see Section 2.2.2) [27]. Hydrophobic interactions (that are not described by the DLVO theory) could also have an influence on attraction/repulsion (see section 2.2.3).
All these attractive/repulsive forces depend mainly on electrostatic and structural properties of the surfaces of the viruses and the porous media.
Firstly, these properties depend on the virus and the porous media type. For the virus that means mainly the isoelectric point[4] (pI) and the hydrophobic/hydrophilic nature

[2]Reaction in which a hydroxyl group (OH^-) and a hydrogen cation (H^+) are added to atoms that are bonded together.

[3]Effect observed when molecules are brought too close together. Indeed, because a molecule occupies a certain amount of space, the associated cost in energy due to overlapping electron clouds may affect the molecule's preferred conformation.

[4]pH at which the surface exhibits a neutral net electrical charge or pH at which the colloidal particle remains stationary in an electrical field. Sometimes, people use the concept of point of zero charge

of the coat proteins, and for the porous media that means mainly the pI. Secondly, predetermined by their own characteristics, the electrostatic properties of the viruses and the porous media are *influenced directly* by environmental chemical and physical conditions. Environmental chemical influences arise mainly from pH and ionic strength of the bulk solutions. Indeed, the pH determines the net charge depending on the pI. The ionic strength affects mainly the double layer compression/expansion and thus attraction/repulsion.
Environmental chemical conditions, like the presence of organic matter, affect also attraction/repulsion by *competing* with viruses for the attachment sites. Environmental physical conditions, like the flow velocity or the viscosity of the fluid, can affect the thickness of double layer and then the attraction/repulsion.

2.2.2 DLVO theory

The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory provides a conceptual framework to have a better understanding of interactions between virus particles and solid surfaces and the role played by the environmental conditions (e.g. pH, ionic strength). However, we have to keep in mind that this model has several shortcomings in predicting attachment and detachment behaviour. It has generally been shown that under unfavorable conditions for attachment, which means when virus particles and solid surfaces have the same net surface charge, DLVO theory underestimates attachment by many orders of magnitude. Furthermore, this theory was formulated for smooth bodies with ideal geometries and uniforms properties, while, in practise, real particles are irregular and heterogeneous in composition, structure and charge [30].

The DLVO theory describes the attractive and repulsive forces between colloid and grain surfaces in terms of potential energy as a function of the separation distance between them. The potential energy profile is constructed by summing the electrical double layer attractive/repulsive potential energy, London-van der Waals attractive potential energy, and short-range repulsive potential energies over the separation distance between the colloids and grains [27]. This model does not take into account hydrophobic interactions.

The *electrical double layer* potential energy (ϕ^{DL}) arises from the overlap of diffuse clouds of ions (double layer) that accumulate near charged surfaces to balance the surface charge. If the interacting surfaces are liked-charged, the electrical double layer potential energy will be repulsive (Figure 2.2.A and 2.2.B). If the surfaces are oppositely charged, the double layer potential energy will be attractive (Figure 2.2.C) [27].
All formulations of the double layer potential energy are sensitive to changes in solution chemistry. Indeed, the double layer potential energy is influenced by variations in

(pH_{pzc}) to described the point at which the surface exhibits a neutral net electrical charge. The pH_{pzc} refers to the absence of any type of surface charge, while the isoelectric point refers to a state of net neutral surface charge. In this work we will talk about isoelectric point to describe the point at which the surface exhibits a neutral net electrical charge.

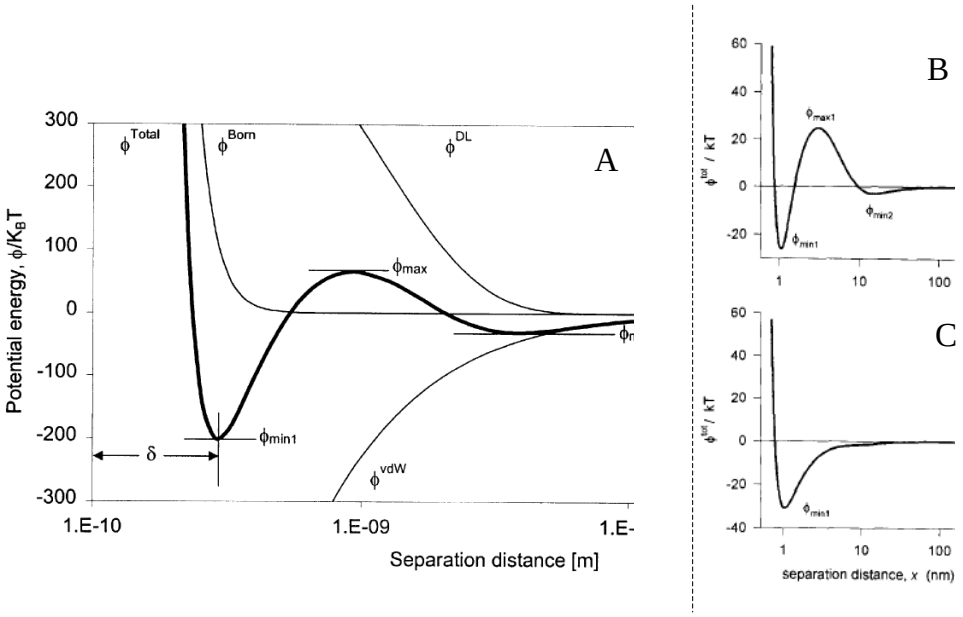

Figure 2.2: **(A) Potential energy between two surfaces (virus - porous media) as a function of the separation distances. The total potential energy (ϕ^{Total}) is the sum of the double layer potential energy (ϕ^{DL}), the van der Waals potential energy (ϕ^{vdW}), and the Born potential energy (ϕ^{Born}). Furthermore, there is a minimum separation distance that is illustrated by the fact the the total potential energy curve seems to increase infinitely at a very low separation distance. A negative potential energy value means low energy requirements, then conditions are favorable or attractive. A positive potential energy value means high energy requirements, then conditions are unfavorable or repulsive.**
(A and B) Case of liked-charged surfaces. The DL potential energy is positive or unfavorable. In this case, the total potential energy curve is characterized by an attractive well at a very small separation distance, the primary minimum (ϕ_{min1}), a repulsive energy barrier (ϕ_{max}), and a shallow attractive well at a larger separation distance (ϕ_{min2}).
(C) Case of oppositely charged surfaces. The DL potential energy is negative or favorable. In this case, the total potential energy curve is only characterized by the primary minimum (ϕ_{min1}), and there is no energy barrier.[17][30]

both (1) the surface potentials of the colloid and the collector, which depend mainly on their charge and structural properties (pI) and the pH of the solution, and (2) the ionic strength of the solution [27].
In general, surface potential is zero near the isoelectric point (pI), positively charged at

pH values below the pI and negatively charged at pH values greater than the pI. Surface potentials cannot be measured directly, but they can be calculated using various models of the oxide-water interface or approximated by zeta potentials derived from measurements of electrophoretic mobility [27].
The ionic strength of the solution controls the extent to which double layers extend from the surface into the bulk solution. At high ionic strength, the surface charge can be balanced by a small ("thin") double layer because the ion concentration near the surface is high; conversely, low ionic strength will produce large ("thick") double layers. At high ionic strength, the double layers of approaching surfaces will overlap only at small separation distances and the double layer repulsion between the surfaces is reduced. At low ionic strength, the double layer repulsion is increased [27].

The *attractive London-van der Waals* interactions (ϕ^{vdW}) are long-range forces caused by instantaneous dipole-dipole interactions between surfaces. The magnitude of the attraction depends on the density and porlarizibility of the interacting surface and the medium [27]. Attracive van der Waals forces are independent of changes in solution chemistry like pH or ionic strength.

The *short-range repulsive* forces that are often attributed to some form of hydration or steric repulsion are not well understood currently. Despite the lack of understanding, these forces most likely play an important role in colloid mobilization because they define a primary minimum of finite depth. Without these short-range repulsive forces, the probability of colloid escape from the infinitely deep primary minimum (ϕ_{min1}) would be zero [27]. The effect of short-range repulsion has been included in DLVO profiles in two ways: (1) designation of a minimum separation distance corresponding to the shear plane distance and the thickness of the layers of water hydration between surfaces; and (2) calculation of the Born potential energy (ϕ^{Born}) [27]. Changes in solution chemistry may affect short-range repulsion forces [27].

Thus, the combination of these three potential energy gives a profile of the total energy potential as function of the separation distance.
When the surfaces of the virus and the porous media are *like-charged* (Figure 2.2.A and B), the double layer potential energy becomes more and more positive with the decrease of the separation distance. The total potential energy curve is characterized by an attractive well at a very small separation distance, the primary minimum (ϕ_{min1}), a repulsive energy barrier (ϕ_{max}), and a shallow attractive well at a larger separation distance (ϕ_{min2}). The formation of this secondary minimum results when the magnitude of the van der Waals attraction exceeds the magnitude of the double layer repulsion; this phenomenon occurs at a given separation distance due to the fact that the van der Waals attraction decays linearly with distance whereas the double layer repulsion decays exponentially [27].
When the surfaces of the virus and the porous media are *oppositely charged* (Figure 2.2.C) the double layer potential energy, as the van der Waals potential energy, be-

comes more and more negative with the decrease of the separation distance. Thus, the total potential energy curve is only characterized by the primary minimum (ϕ_{min1}) and there is no energy barrier. The primary minimum in the case of like-charged particles is usually less deep than the primary minimum in case of oppositely charged particles [17].

2.2.3 Hydrophobic interactions

In addition to the electrostatic interactions explained in DLVO theory, hydrophobic interactions also contribute to the adsorption/desorption behaviour of viruses. In fact, the coat proteins of virus contain spans of amino acids that are hydrophobic. Depending on the way these proteins are folded, such hydrophobic parts may either be on the inside or the outside of the virus coat [30]. Furthermore, some viruses have an envelope that is made of proteins and lipids [20]. Thus, viruses have different hydrophobic behaviours depending on the composition and structures of their outermost layers.
Hydrophobic "attraction" refers to the preference of non-ionic surfaces to associate with each other rather than with water. A favorable entropy change resulting from the disorganization of hydrogen-bonding water molecules arranged around hydrophobic surfaces drives this "attraction" rather than any significant attraction between non-ionic surfaces [11].

Hydrophobic behaviour of some bacteriophages (MS2, ϕX174) and some animal viruses was characterized by adsorption on a solid with hydrophobic groups (octyl-Sepharose) that seems to adsorb viruses mainly by hydrophobic interactions. They found, by doing a ranking of the tested viruses, that MS2 was the most hydrophobic and ϕX174 the least hydrophobic [33]. These results were confirmed by the ranking of another study that used nitrocellulose membranes as the hydrophobic surface [19]. A continuous-flow column experiment showed that the removal of MS2 by attachment to silica beads was dependent on the amount of hydrophobic surface present on the beads [2].
These studies show that hydrophobic interactions can play a role in attachment/detachment of the virus; however the relative importance of these interactions compared to electrostatic interactions was not quantified.
According to other authors [17], the amendment of a silica surface by addition of hydrocarbon chains will decrease the negative surface charge of the silica surface in addition to providing a potential hydrophobic "harbor" for virus attachement. Thus, increased virus attachment may be more reasonably attributed to the decrease in double layer repulsion rather than hydrophobic expulsion of the virus from the solution [17]. Therefore, the electrostatic concept, through the DLVO theory, seems to explain virus attachment in the case of like-charged virus/porous media particles more "reasonably" than the hydrophobic interactions concept.

2.2.4 Irreversibility and reversibility of attachment

The sorption of viruses on surfaces is governed by attachment and detachment. As the DLVO theory shows (see section 2.2.2), there are situations that are favorable to sorption (oppositely charged particles) and others that are unfavorable to sorption (liked-charged particles). They depend mainly on virus type, solid type and environmental conditions.

Sorption is generally considered to be irreversible for oppositely-charge particles and reversible for liked-charged particles [11].
Actually, irreversible attachment does not really exist. In fact, in the case of irreversible attachment, the rate of attachment (k_{att}) is much greater than the detachement rate (k_{det}). Even if detachment rate is very small, it takes place. Indeed, in a study modelling the transport of bacteriophage through dune sand[31], they found a ratio k_{det}/k_{att} between 0.0003-0.02 for the favorable sorption sites.
From the point of view of the DLVO profiles, attachment in favorable conditions is assumed to occur in the deep primary minimum [3](see Figure 2.2.C).
In unfavorable conditions, attachment is assumed to occur in the shallow secondary minimum because of the energy barrier between the secondary and the primary minimum. It is also hypothesized that the viruses temporarily reside in the diffusion boundary layer around porous media grains [17].

In many natural porous media, because of their heterogeneity, favorable and unfavorable sites for attachment are present at the same time.
In typical natural conditions, during transport of groundwater (pH 6-8) where most viruses are negatively charged, a part of sorption sites are positively charged (e.g., iron and aluminum oxides, edges of clay minerals) and favorable for attachment, while other sites are negatively charged (e.g. quartz) and unfavorable for attachment [11][3]. With fluctuations or changes of the chemical (pH, ionic strength, etc.) and physical (flow velocities,etc.) environmental conditions, the favorable/unfavorable disposition of the sorption sites can change.

Finally, it is important to note that only favorable sites are considered to remove viruses from water, until their maximal adsorption capacity is reached.

2.3 Inactivation

We usually distinguish between the inactivation of free and attached viruses.

Inactivation of *free* viruses, i.e. those present in the bulk solution unattached to surfaces, depends on both time and the physicochemical conditions. Adverse conditions (e.g. UV light, active oxygen species) and higher temperatures can accelerate damage to specific viral components that are required for infection, most notably degradation of the viral genome and conformational changes in the host recognition site (protein) [11].

Inactivation of *surface-attached* viruses can either be enhanced or reduced.

In the case of inactivation *slowed* by attachment, viruses appear to be protected from inactivation by attachment to geological media with high organic matter and clay-sized particle content [11]. This reduced inactivation may be the result of protection from proteolytic enzymes or other virus-inactivating substances, increased stability of the virus capsid when attached, blocking from ultraviolet radiation.[30]

In the case of inactivation *accelerated* by attachment, enhanced degradation seems to be due to attachment to geological media like iron and aluminum oxides and other materials that bind viruses strongly [11]. This strong electrostatic attraction appears to be sufficient to disrupt virus structure and cause inactivation.
However, the nature of the direct role of attachment in inactivation is not clear. Viruses interact with mineral surfaces through their protein capsids. It is well-known that mineral surfaces can have a denaturing effect on proteins by dehydration and disruption of interactions within peptide chains. The distortions and unfolding of protein structures may be the primary cause of virus inactivation. Such interactions could result in virus degradation and separate release of protein capsid components and nucleic acids [28].

2.4 Modelling of adsorption/desorption/inactivation behaviour during transport through porous media

Modelling of the transport is essential to have a quantitative understanding of virus behaviour. Indeed, measured data fitted by a mathematical model allow us to quantify several parameters that are defined during the construction of the model. Thus, it is possible to quantify and identify processes, as far as they are well defined, that govern virus behaviour during passage through porous media.

Advection, dispersion, sorption and inactivation are the main processes affecting the transport behaviour of a virus through porous media.

- *Advection* and *dispersion* are hydrodynamic parameters that can be measured directly.

  Advection (average interstitial water velocity) through a porous medium can be expressed by Darcy's law. Darcy's law 1D expression:

  $$Q = \frac{\kappa A}{\mu} \frac{(P_b - P_a)}{L} \tag{2.1}$$

  where Q is the discharge through the porous media $[m^3/s]$; A is the cross-sectional area to flow $[m^2]$; κ is the permeability of the porous media $[m^2]$; (P_b - P_a) is the pressure drop between the inflow and the outflow region [[kg/m·s]; μ is the

dynamic viscosity of the fluid[kg/m·s]; and L is the length where the pressure drop is taking place over. Expressed in term of head drop and hydraulic conductivity constant:

$$Q = KA\frac{\Delta h}{L} \tag{2.2}$$

where K is the hydraulic conductivity constant [m/s]; Δh is the head drop between the inflow region and the outflow region [m]. From Q and A, we can find the average interstitial water velocity v [m/s].

Dispersion, similar to the diffusion coefficient in turbulent flow conditions, can be determined by measuring the breakthrough curve of a tracer (e.g. salt). By fitting the measured data (breakthrough of the tracer) with the advection-dispersion equation, it is then possible to determine the dispersion coefficient [34]. Basically, 1D expression of the advection-dispersion equation is expressed like:

$$\frac{\delta C}{\delta t} = D\frac{\delta^2 C}{\delta x^2} - \nu\frac{\delta C}{\delta x} \tag{2.3}$$

where C is the concentration of the transported species in our case viruses [pfu/m^3] or salt [mg/m^3]; D is the dispersion coefficient [m^2/s]; v is the pore flow velocity [m/s]; t is time [s] and x is the distance [m].

- *Sorption* of viruses on solid surfaces can be described as equilibrium and/or kinetic processes. In batch experiments, soprtion can be described as an equilibrium process if sufficient time has passed to achieve steady-state conditions. In continuous flow experiments, however, it was often shown that virus behaviour could not be well explained by equilibrium processes [31]. Thus, kinetic sorption is the better way to understand virus behaviour during transport through a porous media.

  In the case of *saturated conditions* (full saturation of water content), over the past two decades, the modelling of virus attachment and release kinetics has evolved from a simple equilibrium distribution approach, to single-site irreversible and reversible kinetic models [32], to a dual-site approach with kinetic attachment and release for a site of favorable attachment and a site of unfavorable attachment [31][29] and, finally, adding to this dual-site approach, the effect of blocking, i.e., a decrease in the attachment efficiency of the geologic medium as its surface is filled with attached viruses [11].

  Often, semi-log plots of breakthrough curves from tests with block-input of viruses exhibit a typical skewness: a rather slowly rising limb and a smooth transition from a declining limb to a very long tail. Dual-site approach was developed because the one-site kinetic models fail to fit the rising and declining limbs together with the tail satisfactorily (see Figure 2.3) [31].

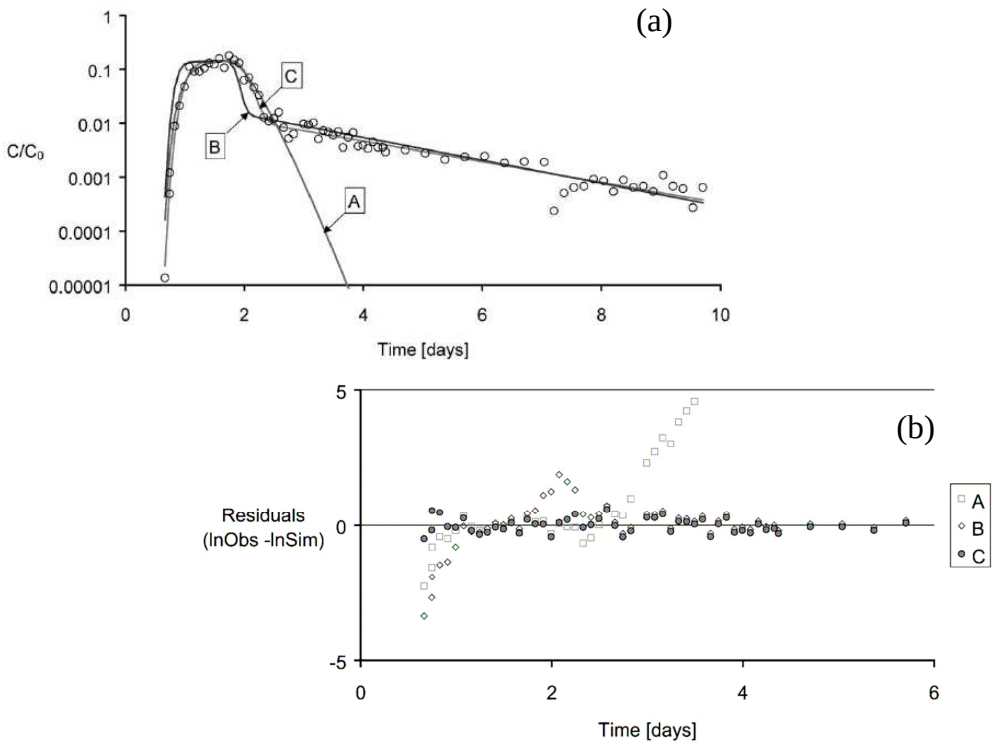

Figure 2.3: **(a) Measured breakthrough curve (open circles) of MS2 phage in a column (after around 2 days, they stopped adding virus) fitted with a one-site kinetic model (A and B) and a two-site kinetic model (C). (b) Residual values (observed minus fitted values) that show us the failing of the one-site kinetic model (A and B). The straight line at 0 corresponds to the fitting curve.**

The governing equations for an advection-dispersion model, including sorption to two types of kinetic sites and inactivation of free and attached viruses, in the case of uniform one-dimensional flow, are as follows [31]:

$$\frac{\delta C}{\delta t} + \frac{\rho_B}{n}\frac{\delta S_1}{\delta t} + \frac{\rho_B}{n}\frac{\delta S_2}{\delta t} = D\frac{\delta^2 C}{\delta x^2} - \nu\frac{\delta C}{\delta x} - \mu_l C - \mu_{s1}\frac{\rho_B}{n}S_1 - \mu_{s2}\frac{\rho_B}{n}S_2 \quad (2.4)$$

$$\frac{\rho_B}{n}\frac{\delta S_1}{\delta t} = k_{att1}C - k_{det1}\frac{\rho_B}{n}S_1 - \mu_{s1}\frac{\rho_B}{n}S_1 \quad (2.5)$$

$$\frac{\rho_B}{n}\frac{\delta S_2}{\delta t} = k_{att2}C - k_{det2}\frac{\rho_B}{n}S_2 - \mu_{s2}\frac{\rho_B}{n}S_2 \quad (2.6)$$

where C is the concentration of free viruses [pfu/m^3]; S is concentration of attached viruses [pfu/kg]; t is time [day]; x is distance [m]; aL is dispersivity [m]; v is average interstitial water velocity [m/day]; ρ_B is dry bulk density [kg/m^3]; n is porosity [-]; k_{att} and k_{det} are attachment and detachment rate coefficients, respectively [day^{-1}]; μ_l and μ_s are the inactivation rate coefficients of free and attached

viruses, respectively [day^{-1}]. Subscripts 1 and 2 refer to the two different kinetic sites.

After the measurement of the average interstitial water velocity, the bulk density, the porosity and the dispersivity, it is possible to determine all the others kinetic parameters of the equations by fitting the equations (2.4), (2.5) and (2.6) to the virus breakthrough data with an appropriate modelling software [34].

Recently, studies that were made under *unsaturated conditions* (water content) introduced the interactions of viruses with the air-water interface in addition to the solid-water interface [34][4]. Indeed, removal of viruses was enhanced in unsaturated conditions compared to saturated conditions. Thus, modelling of virus removal in unsaturated flow conditions should consider these additional interactions.

- *Inactivation* was considered in these models. Most of the time, models separate inactivation in the bulk and inactivation onto the surface (as in equation (2.4), (2.5) and (2.6)).

2.5 Measurement of adsorption/desorption/inactivation behaviour: batch and continuous flow experiment

In *batch* experiments, a suspension of viruses is agitated with a quantity of the solid material of interest in a container. After a certain time, almost steady-state situation is reached. Thus, batch experiments are mainly used to study equilibrium adsorption and to construct sorption isotherms [30].
Batch experiments were used to try to determine kinetic sorption parameters. In fact, in batch suspensions of viruses and soil, sorption equilibrium is not reached instantaneously. The kinetic behaviour that is operative before apparent equilibrium is reached can be described by the same kinetic parameters that we have for continuous flow experiments (see section 2.4).
Most of the results for the kinetic parameters calculated from batch experiments show a ratio k_{att}/k_{det} that is greater than in the case of continuous flow experiments. In fact, batch experiments are like well-mixed systems that increase the number of accessible attachment sites compared to a continuous flow experiment [30].
Furthermore, it is argued that results from batch experiments have been neither consistent nor reproducible, largely due to the fact that there is no standard protocol. That is to say, different sizes and types of containers and different methods of agitation are used [30].

Therefore, *continuous flow* experiments (often column experiments) are the only way to quantify the sorption behaviour of viruses during transport through a porous media.

Nevertheless, *batch* tests can be used to investigate the effects of various environmental factors (e.g., pH, organic matter,ionic strength), to compare adsorptive behavior of different viruses in combination with different solid materials under a given set of experimental conditions and to have a good appreciation of the maximal adsorption capacity [30]. Moreover, batch experiments are easier to implement. Thus, it is a good way to do preliminary experiments to get a first idea of the virus sorption and inactivation behaviour in contact with a certain porous medium.

Chapter 3

Material and methods

3.1 Material

3.1.1 Porous media: Iron oxide coated sand (IOCS)

Sea sand was purchased from Merck (Darmstadt, Germany) with a mean diameter of 0.1-0.315 mm. To coat the sand, we first acid washed it to remove metals from the sand (Appendix A) and then coated it as described by Foppen & al. [7](Appendix B). Thus, we obtained a coated sand with a high iron content (see Table 3.1).

Then, we characterized our IOCS by determining the iron content, surface area and iron:oxygen ratio of the iron oxide coating to aid in identifying the iron-oxide type (see Table 3.2)
The coating procedure of Foppen & al. [7] was used to synthesize goethite, but the Fe:O ratio of our iron oxide coating (see Table 3.1) was between 1:1 and 2:3. Based on the color of our IOCS that was ochre (see Figure 3.1), our iron oxide coating is probably mainly made of hematite (see Table 3.2).
The knowledge of the characteristics of our IOCS are of great importance and interest. Indeed, the number of adsorption sites is strongly dependent on the amount of iron coated. In addition, the electrostatic behaviour of our iron coating as function of the

Table 3.1: **Characteristics of the IOCS used for the batch experiments.**

Characteristics	
Iron content [mg Fe/g of sand] [a]	5.6 ± 0.7
Surface area [m^2/g of sand] [b]	1.8
Fe:O [c]	1:1 until 2:3 → FeO and Fe_2O_3

[a]Sand digestion according to McGrath and al.[22] and analysis with ICP-AES.
[b]NOVA BET surface area and pore size analyser (QUANTACHROME, Florida, USA).
[c]Scanning electron microscope FEI XL30S FEG (The Netherlands, Endogen) was used with X-ray energy dispersive spectroscopy (EDS, software from INCA, Oxford).

Table 3.2: **Some iron oxyhydroxide species by decreasing order of Fe:O ratio [5].**

Iron oxyhydroxides species	*Fe:O ratio*	*Main colour*	*Isoelectric point (pI)*[a]
Wüstite (FeO)	1:1	black	
Magnetite (Fe_3O_4)	3:4	black	
Hematite (Fe_2O_3)	2:3	red	8.0 - 9.0
Goethite (FeOOH)	1:2	yellow-brown	5.9 - 6.7

[a][24]

pH depends a lot of the iron oxyhydroxides species.

Figure 3.1: **Iron oxide coated sand (left) and uncoated acid washed sand(right).**

The scanning electron microscope provided images of the iron coating on the sand (see Figure 3.2). Generally, we can note that the coating area is relative low compared to the total area of the sand grain.

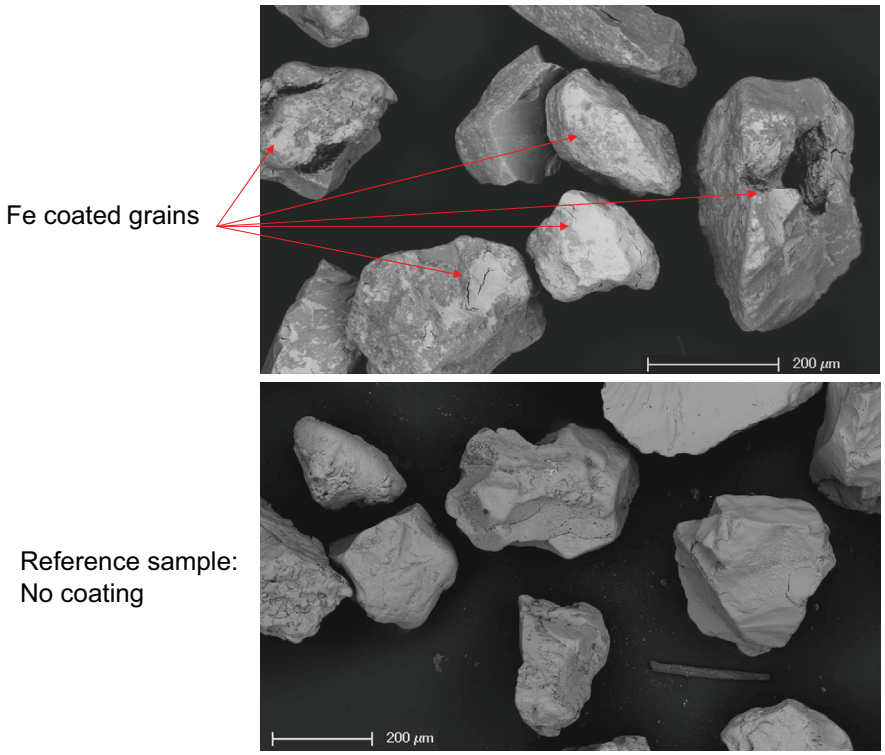

Figure 3.2: **View of the coating on the IOCS(above)(in white: iron oxide coating area) and the uncoated sand (below).**

3.1.2 Phages

We used enterobacteria phage fr for our experiments. Fr phage has more or less the same size as two other phages MS2 and ϕX174 (see Table 3.3), that are commonly used in sorption and inactivation studies. Its isoelectric point was measured around 9.0, but, as our batch experiments show (see section 4.2), this value seems to be too high.

Table 3.3: **Size and isoelectric point of the most commonly used phages compared to fr phage.**

Phages	*Equivalent diameter size [nm]*	*Isoelectric point*
fr	26 [a]	9.0 [b]
MS2	24 [c]	3.9[c]
PRD1	63[c]	4.2[c]
ϕX174	27[c]	6.6[c]

[a][42]
[b][12]
[c][6]

3.1.3 Experimental material

Table 3.4: **Material used during the experiment.**

Experimental material	
Sand	Uncoated sand
	Iron oxide coated sand (IOCS)
Standard buffer solution	Tris-HCL 50 mM + NaCl 20 mM
Environmental parameters tested	*pH*: adjusted with NaOH and HCl
	Ionic strength (IS)[a]: adjusted with NaCl
	Humic acid concentration: Humic acid (Sigma Aldrich, Buchs, CH)
Desorption step	made by adding beef extract solution (pH 8): 20 ml deionized water +2.4 g beef extract + 0.3 g glycine

[a]The Tris-HCl buffer was not included in the ionic strength calculation. Thus, the ionic strength was dependent only on the NaCl concentration. Therefore, when we are talking about a buffer solution with IS of X mM, it is Tris-HCl 50 mM + NaCl X mM.

3.2 Methods

3.2.1 General experimental protocol

All our experiments were made at ambient temperature (20-25°C).
First, a stock of known virus concentration was diluted with buffer solution adjusted at the different environmental conditions to obtain a given concentration.
Then, we added 1 ml of this virus solution to 0.5 g of sand in 1.5 ml microcentrifuge tubes. This ratio virus solution:sand was optimized in preliminary experiments.
After that tubes were mixed on a rotating plate during 1 hr slowly to avoid inactivation of phages by sand friction and sand alteration. In preliminary experiments, we saw a stabilization of the virus concentration in the supernatant after 30 min. Thus, we assumed that after 1 hr equilibrium was reached.
Next, 500 ml of the supernatant was taken and diluted serially (by 10x or 100x dilutions) to obtain between 10 and 300 plaque forming units [pfu] per 100 microliter sample. The standard buffer solution (Tris-HCl 50 mM + NaCl 20 mM) was always used during the dilution step. With this first step we measured the non-attached fraction of the phages (*unbound fraction*).
Infective phages was quantified using the double-layer agar technique [14] and infective virus concentrations were measured in plaque-forming units per ml (pfu/ml).
To measure the amount of attached phages (*bound fraction*) or to measure the effect of changes in the solution chemistry (environmental conditions) after an adsorption step, we replaced the 0.5 ml of removed supernatant with, respectively, 0.5 ml of beef extract (pH 8; 20 ml deionized water + 2.4 g beef extract + 0.3 g glycine) or a solution with

new chemistry.
Controls without sand to check the initial phage concentration was kept during all the experiment and plated at the same time as the other samples.
In order to have a statistical treatment of our data, we always tried to triplicate our measurments. Thus, the mean and the standard deviation were calculated and ploted.

3.2.2 Detailed protocols

Adsorption behaviour onto uncoated sand and IOCS with changes in phage concentration. We worked with Fr, MS2 and ϕX174 phages with standard buffer solution (pH 7, IS 20 mM). The adsorption on uncoated sand and IOCS was tested by mixing different initial phage concentrations. Only measurements of the unbound fraction of the phages were made.

Inactivation behaviour of iron oxide coated sand. We worked with Fr phages with standard buffer solution (pH 7, IS 20 mM). The bound and unbound fraction of the phages to IOCS was tested by mixing 10^9 and 10^{10} pfu/ml of initial phage concentrations. Measurements of the unbound fraction of the phages were made after a first mixing. Then, we added beef extract in order to measure the bounf fraction of the phages.

Adsorption behaviour of iron oxide coated sand with changes in environmental parameters. We tested the influence of changes in environmental conditions (A) at the same time as virus adsorption, or (B) after the viruses have been adsorbed onto the media (see Figure 3.3).
pH. We worked with Fr and MS2 phages with standard buffer solution (pH 7, IS 20 mM). We adjusted the pH of the buffer with NaOH and HCl. (A) The adsorption on uncoated sand and IOCS was tested by mixing 10^8 pfu/ml of initial phage concentrations with buffers at different pH. (B) For the desorption from the IOCS, some drops of NaOH was added to the sample in order to increase the pH to 9 and 10. The amount of NaOH to add to reach the right pH was determined in a preliminary experiment. Nevertheless, the pH obtain was not precisely measured.
Ionic strength. We worked with Fr phages with standard buffer solution (pH 7, IS 20 mM). We adjusted the ionic strength of the buffer with NaCl. The Tris-HCl buffer was not included in the ionic strength calculation. (A) The adsorption on uncoated sand and IOCS was tested by mixing 10^8 pfu/ml of initial phage concentrations with buffers at 20, 100 and 300 mM ionic strength. (B) For the desorption from the IOCS, 500 ml of the supernatant was removed and we added 500 ml of a solution with a NaCl concentration that was two times more than the wanted concentration (Dilution factor of 2. We had 500 ml of a solution with 200 or 600 mM ionic strength to 500 ml of a solution of 20 mM in order to obtain around 100 mM and 300 mM ionic strength).
Humic acid concentration. We worked with Fr phages with standard buffer solution (pH 7, IS 20 mM). Humic acid (HA) content was created with Aldrich humic acid. (A) The adsorption on uncoated sand and IOCS was tested by mixing 10^8 pfu/ml of initial

phage concentrations with buffers at 1, 10 and 100 mg HA/l. (B) For the desorption from the IOCS, 500 ml of the supernatant was removed and we added 500 ml of a solution with a HA concentration that was two times more than the wanted concentration (Dilution factor of 2. We had 500 ml of a solution with 20, 60, 100, 200 and 500 mg HA/l to 500 ml of a solution of 0 mg/l in order to obtain around 10, 30, 50, 100 and 250 mg HA/l).

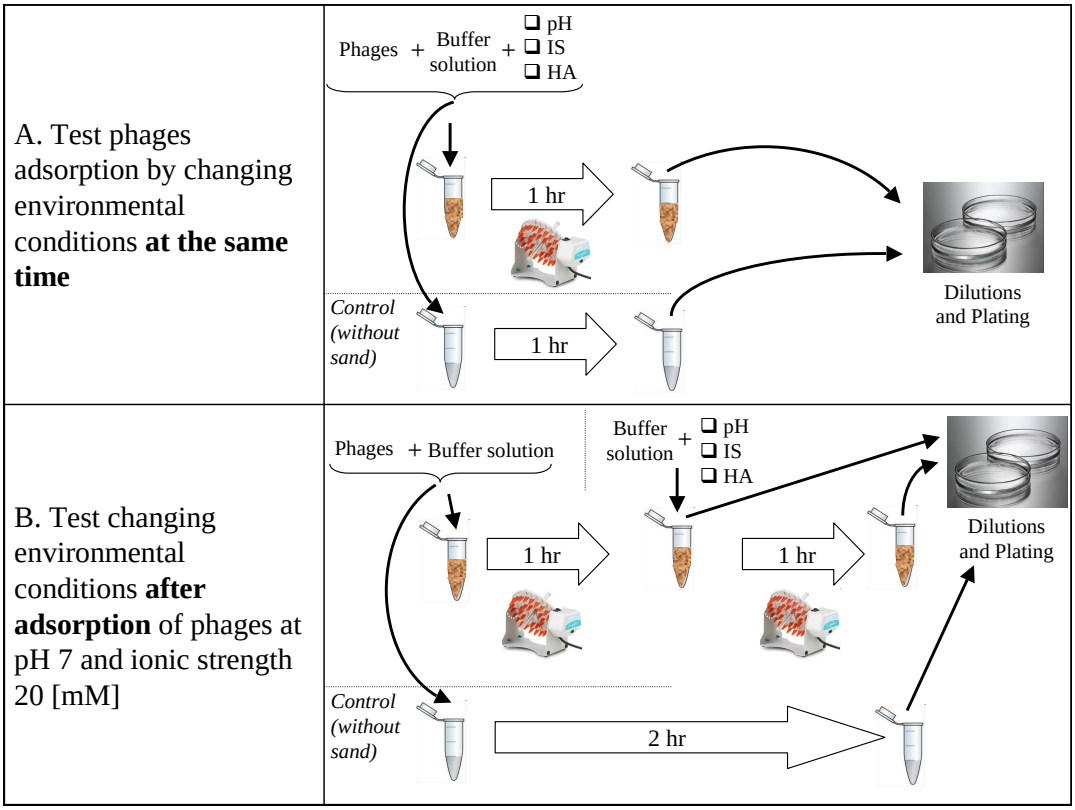

Figure 3.3: **Summary of the protocol difference between: (A) testing virus adsorption by changing environmental conditions at the same time. (B) Test changing environmental conditions after adsorption of viruses at pH 7 and ionic strength 20 mM.**

Adsorption behaviour of aged iron oxide coated sand. The protocol for testing the aged IOCS was the following (see also Figure 3.4): the aging phase consisted of putting the IOCS in contact with solution without shaking it. Each day during 5-6 days the samples were washed several times in order to reduce the concentration of potential dissolved iron and, thus, simulate the renewal of the solution that take place in a continuous flow situation.
Aging by standard buffer solution (pH 7 and IS 20 mM) and by standard buffer solution with 1 and 10 mg HA/l were tested with a range of initial fr phages concentrations.

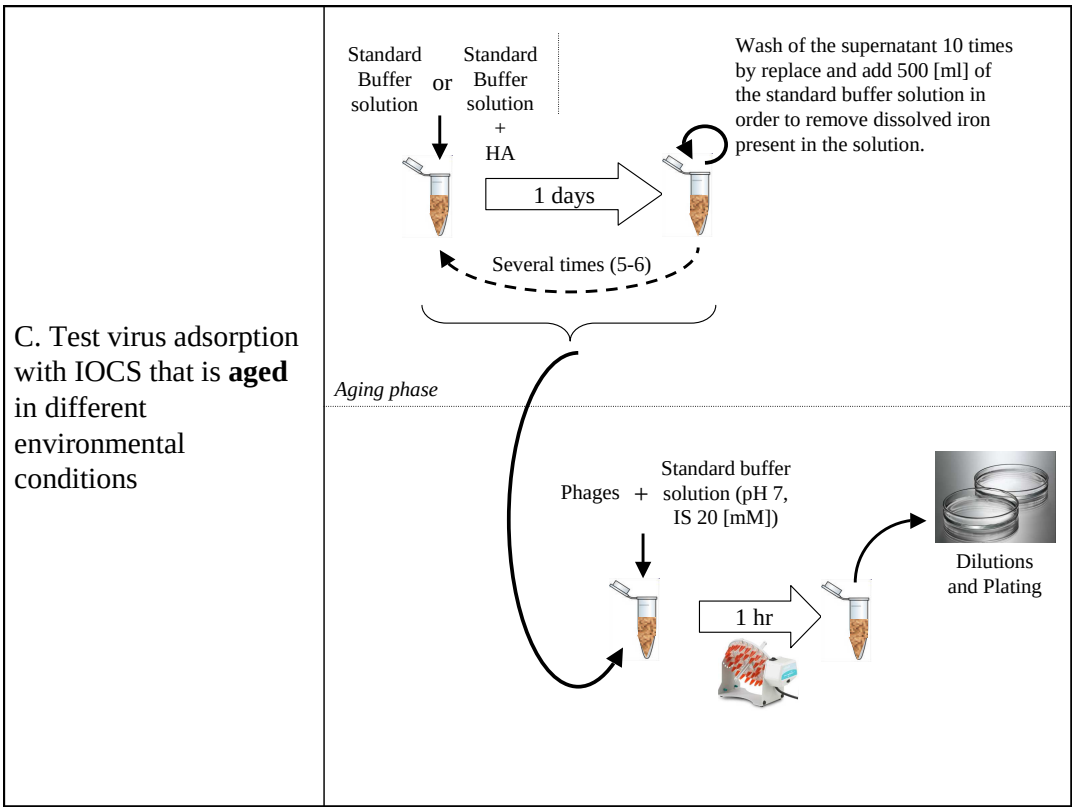

Figure 3.4: **Summary of the protocol for testing aged IOCS.**

Chapter 4

Batch experiment

4.1 Aggregation within the time scale of the experiments

Aggregation of phages can take place in solutions. In fact, in the same way that viruses can be adsorbed to solid surfaces, viruses can also be adsorbed to other viruses depending on electrostatic and hydrophobic interactions. These factors are again dependent on the virus type and environmental conditions (e.g. pH, ionic strength, and the concentration of monovalent and multivalent ions).

Langlet et al. [15] showed by dynamic light scattering (DLS) that viruses, even those with similar isoelectric points, did not have the same aggregation behaviour. Indeed, over a broad range of pH values (1.5-7.5), the aggregation of MS2 phages was not observed for pH higher than the isoelectric point (pI 3.1-3.9 depending on the ionic strength) and large ionic strengths, while aggregation of SP (pI 2.1-2.3) and GA (pI 2.1-2.6) viruses took place over the whole range of pH and ionic strength conditions examined.

Therefore, a measurement of the size distribution of our fr phages in standard buffer solution (Tris-HCl 50 mM + NaCl 20 mM) at different pH (4, 7 and 9) after several hours, was required for the interpretation of the batch experiment's results. For all tested pH values (see Figure 4.1), the size distribution of the phages showed a peak slightly scattered around 20 nm which is approximately the size of our phages (see section 3.1.2). Thus, we concluded that our test phage is not aggregated in our standard buffer solution within pH values between 4 and 9.

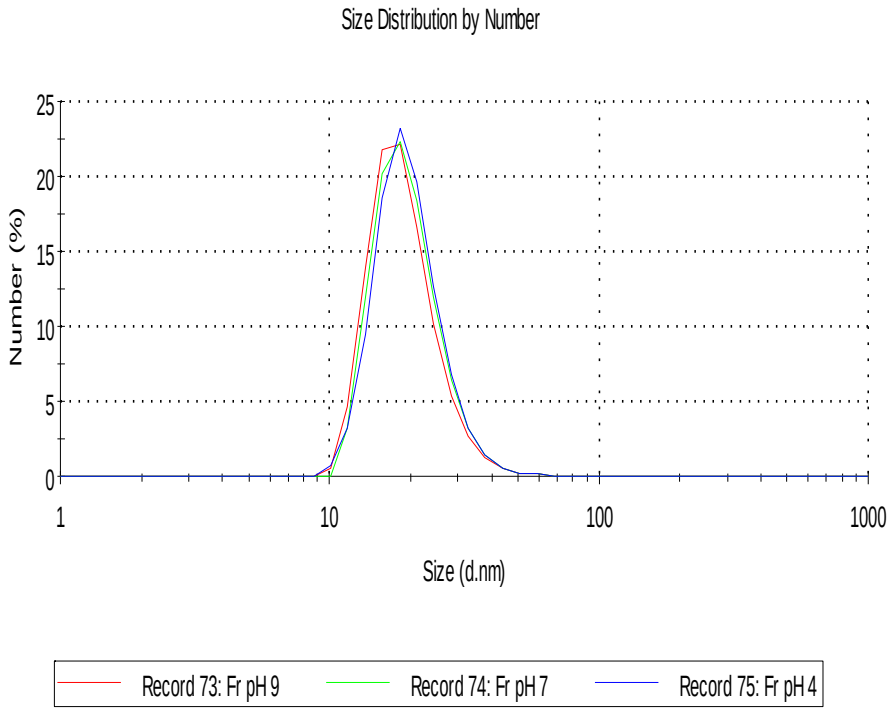

Figure 4.1: **Size distribution of fr phages as function of pH after around 3 hours in standard buffer solution, as measured with DLS.(Fr initial concentration = 10^{11} pfu/ml)**

4.2 Adsorption behaviour onto uncoated sand and iron oxide coated sand (IOCS) with changes in phage concentration

a. Results

For fr phage, we tested adsorption with uncoated sand and IOCS. Firstly, the reduction of the unbound phage concentration with uncoated sand is often less than 1 log, i.e., between 40 - 80 % of the initial phage concentrations remain free in the supernatant solution (see Figure 4.2).
Secondly, the adsorption of fr phages on IOCS is much greater. Indeed, we can always see between 3-4 Logs reduction of the phage concentration in the supernatant. In other words, compared to the uncoated sand, IOCS adsorbed 100 to 1000 thousand times more phages (see Figure 4.2).

Adsorption onto uncoated sand was also tested for two other phages.

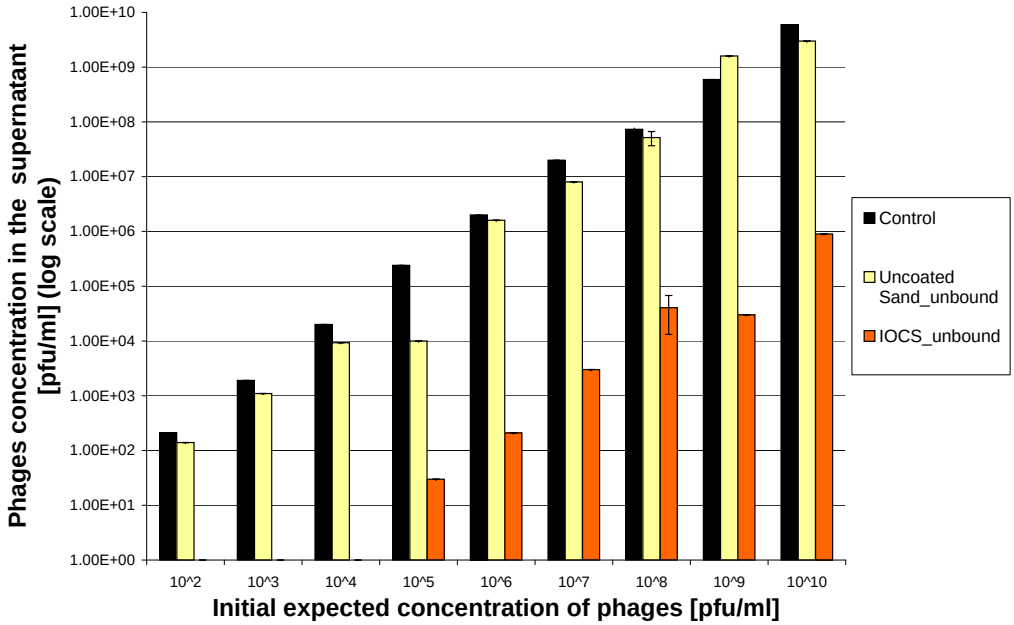

Figure 4.2: **Log plot of the fr phage concentration in controls (without sand) and after mixing with uncoated sand and iron oxide coated sand (IOCS) at different initial phage concentrations, in standard buffer (pH 7 and IS 20 mM).**

Generally, we saw the same order of reduction of the unbound phage concentration, i.e. less than half of the concentration. However, the mean removal efficiency shows a slight difference between the phages themselves. Indeed, MS2 had slightly higher adsorption than ϕX174 that in turn had higher adsorption than fr (see Figure 4.3). But, these differences were negligible compared to the difference in fr adsorption onto uncoated sand and IOCS (see Figure 4.2).

From the data on fr phage adsorption onto IOCS, it should be possible to have an idea of the adsorption capacity of the sand by plotting the adsorption density (phages concentration/g of sand) versus phage concentrations in the supernatant at equilibrium and then fitting the data to an isotherm model (Langmuir, Freundlich, etc.) (see Figure 4.4). Unfortunately, there was no way to fit a relevant curve with these data. The removal efficiency for the different initial concentrations showed no detectable decreasing trend with increasing initial concentrations (see Table 4.1).

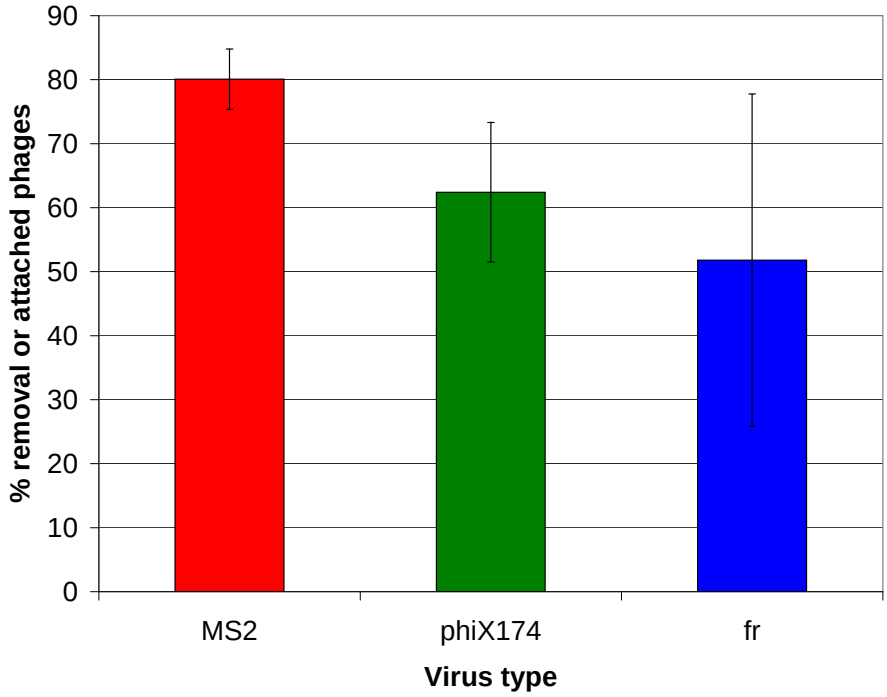

Figure 4.3: **Mean of removal efficiency* for the different phages with uncoated sand from a data set of 5 different initial concentration 10^4-10^9 (we did not consider the results of 10^9 pfu/ml, because they were completly different form the others). The error bars represent 95% confident intervals. * % removal = 100*(initial concentration - concentration in the supernatant)/initial concentration.**

b. Discussions

Adsorption onto the uncoated sand. The combination of the low degree of adsorption of fr phages onto uncoated sand with the high degree of adsorption onto IOCS does not agree with the pI value of fr found in the literature [12] (see also section 3.1.2). In fact, if fr had an isoelectric point around 9, it would have a positive net surface charge at pH 7, while uncoated sand (pI around 3) would have a negative net surface charge (see Figure 4.5). Thus, the phage and the sand would be oppositely charged and, according to the DLVO theory (see section 2.2.2), there would be a high level of adsorption.
Therefore, we could advance that the pI of fr is lower than 7. This conclusion is supported by the fact that MS2 (pI 3.9) and FX174 (pI 6.6), which are negatively charged at pH 7, are reducing in the same order of concentration in contact with uncoated sand.

Actually, it is important to mention that like-charged particles could also attach to

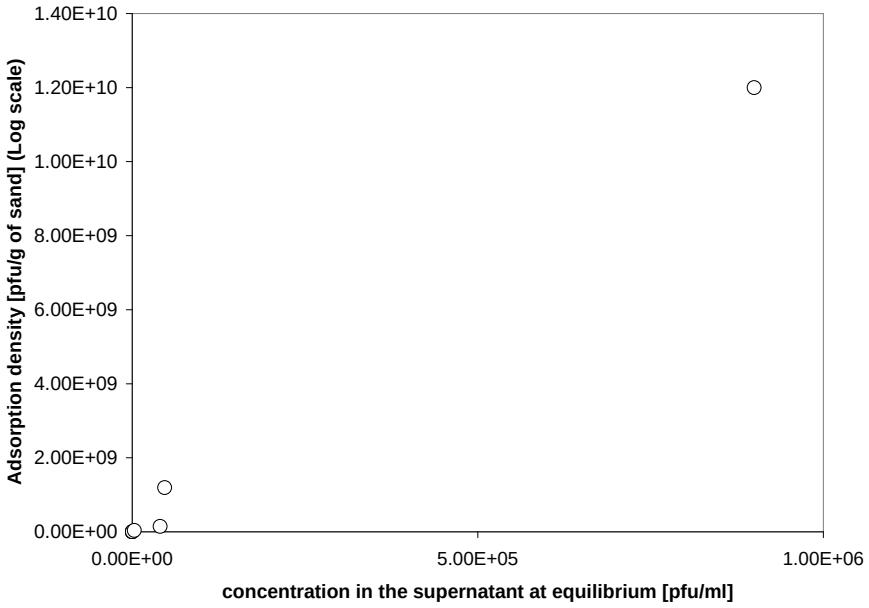

Figure 4.4: **Plot of the adsorption density vs. concentration of fr phages in the supernatant at equilibrium with iron oxide coated sand.**

a certain degree even in the presence of electrostatic repulsion, as determined by the DLVO theory (see section 2.2.2). Loveland et al. [17] found a near complete attachment of PRD1 phages (calculated pI around 3.5) to uncoated sand (calculated pI around 2.5) until pH around 5.5 while for IOCS (calculated pI around 5.1), a near complete attachment was observed until pH around 7. Assuming there was (1) no inactivation (see section 4.3), (2) insignificant adsoprtion to the eppendorf tube, and (3) no aggregation between the phages (see section 4.1), and according to the results of Loveland et al. [17], we can affirm a certain amount of fr phages are attached at pH 7 despite both having net negative surface charges (see Figure 4.3).

Adsorption onto the IOCS. Adsorption of fr phage onto IOCS is much greater than

Table 4.1: **Removal efficiency for the different initial concentrations tested of fr phages with IOCS.**

Initial phages concentrations [pfu/ml]	2.00E+04	2.40E+05	2.00E+06	2.00E+07
% removal[a] (mean)	100.000	99.988	99.990	99.985
Initial phages concentrations [pfu/ml]	7.34E+07	5.97E+08	6.00E+09	
% removal (mean)	99.945	99.992	99.985	

[a]% removal = 100*(initial concentration - concentration in the supernatant)/initial concentration.

adsorption on uncoated sand. This could be easily explained by the opposite charges of the phages and the IOCS. Indeed, assuming that the iron oxide coating is made mainly of hematite (pI 8.0 - 9.0) (see section 3.1.1) and phage pIs lower than 7 (see above), then at pH 7 the IOCS is positively charged and the phages are negatively charged (see Figure 4.5). Loveland et al. [17] and Lukasik et al. [18] found the same difference in attachment behaviour at pH 7 between uncoated sand and IOCS for PRD1 and MS2, respectively.
We could not fit a relevant adsorption isotherm due to the logarithmic increase of the equilibrium concentration (see Figure 4.4) and also to the fact that the removal did not show a decreasing trend with increasing virus concentrations (see Table 4.1). Furthermore, adsorption isotherms are based on the assumption that free particles in the liquid phase are in equilibrium with the particles attached to the solid phase. For the IOCS, however, we are in the case of favorable attachment sites, i.e., the adsorption to the iron oxide coated surfaces is likely to be irreversible (see section 2.2.4). Thus, one of the main assumptions of the current adsorption isotherm model is not satisfied and could explain the difficulties in fitting our data to a model.
Given our results, the maximum adsorption capacity is probably larger than 10^{10} [pfu/g of IOCS]. Moore et al. [23] found surface saturation of uncoated Ottawa sand for poliovirus type 2 in a synthetic freshwater medium at pH 7 at 2.5×10^{9} [pfu/g of sand].
Thus, we can conclude that the iron oxide coating, even when present on only a small fraction of the sand grain surface (see Figure 3.2), provided a large number of favorable attachment sites.

Adsorption behaviour of the different phages with uncoated sand. Even if it is not so perceptible, the efficiency of attachment to uncoated sand for phages shows the following trend in decreasing order MS2 >ϕX174 >fr (see Figure 4.3).
As we saw previously, according to the DLVO theory, like-charged particles could attach until a certain level of electrostatic repulsion. So, even if phages and uncoated sand are negatively charge, we still observe some attachment.
However, we were expecting less attachment for phages with lower isoelectric points. Actually, this did not happen experimentally.
Dowd et al. [6] also found that MS2 attached more to aquifer sand at pH 7.5 than ϕX174.
The slightly higher attachment of MS2, compared to ϕX174, to uncoated quartz sand could be attributed to two different factors.
Firstly, the variation of the net surface charge of the phages with pH did not follow a linear pattern. In fact, the electrophoretic mobility curve which is used to measure the net surface charge as a function of pH, generally stabilized to a certain net surface charge after a certain pH value depending on the phage type. Thus, as described by Redman et al. [26] and Penrod et al. [25], two phages with pI values equal or greater to MS2, Norwalk (pI around 5) and λ (pI around 3.9) respectively, can be more negatively charged after a certain pH increase.
Secondly, MS2 seems to be more hydrophobic than ϕX174 (see section 2.2.3), thus some

hydrophobic interactions would promote the attachment of MS2.

pH	3	4	5	6	7	8	9	10
Quartz sand	+ pI	−	−	−	−	−	−	−
Hematite	+	+	+	+	+	+ pI		−
Goethite	+	+	+	pI	−	−	−	−
According to Herath and al. [22]	+	+	+	+	+	+	pI	−
fr Expected after our experiments.	+	+	+ pI	−	−	−	−	−
MS2	+	pI −	−	−	−	−	−	−
ΦX174	+	+	+	+	pI −	−	−	−

Figure 4.5: **Isoelectric point and expected surface net charge (qualitative value + or -) of quartz sand, iron oxides (hematite, goethite) and phages (fr, MS2, ϕX174) as function of the pH.**

4.3 Inactivation behaviour of iron oxide coated sand

a. Results

The addition of the bound and the unbound fraction roughly equalled the initial virus concentration (see Figure 4.6). Therefore, there was no inactivation due to the iron oxide coated sand within the time scale of our batch experiments.

b. Discussions

Inactivation in our experiments. Our results show that phage inactivation is neither accelerated nor slowed by mixing the phages with our porous media (IOCS) compared to free phages in the control solution. Thus, at the time scale (1-2 hr) and temperature

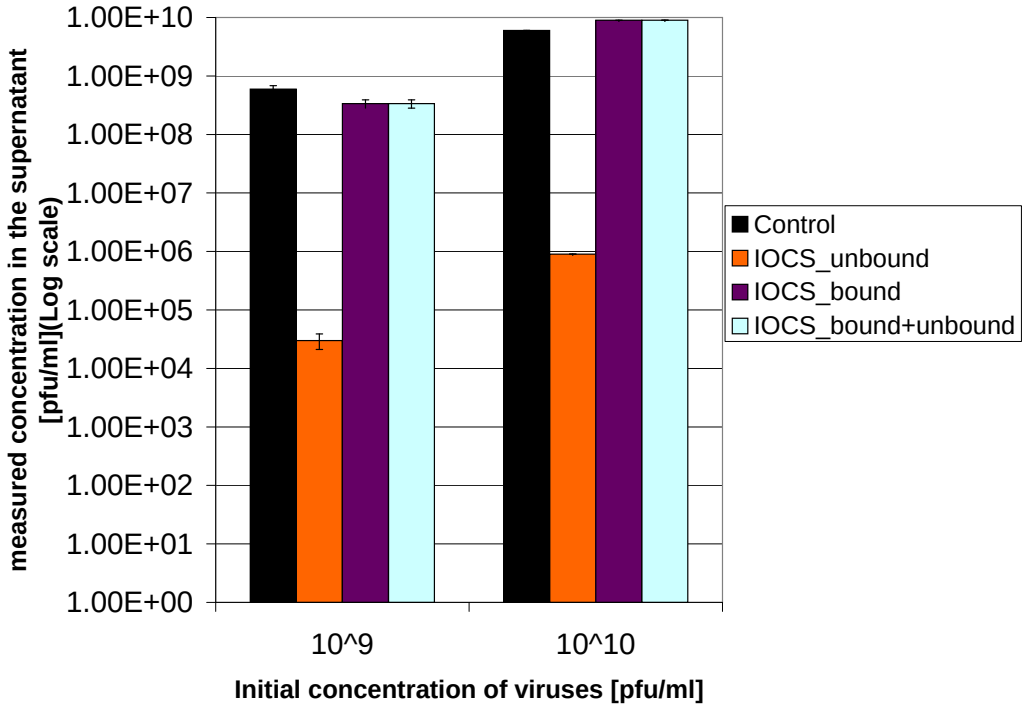

Figure 4.6: **Plot of fr phages concentration in the supernatant in controls (no sand) and after mixing with iron oxide coated sand (IOCS) at 10^9 and 10^{10} pfu/ml initial phages concentrations, in standard buffer (pH 7 and IS 20 mM). Non attached fraction (IOCS_unbound) measured after the mixing and attached fraction (IOCS_bound) measured after desorption with beef extract.**

conditions (ambient temperature 20-25°C) of our experiment, we can say that we do not have any enhanced or reduced inactivation due to our iron-oxide coated sand in a solution at pH 7 and 20 mM of ionic strength.
The uncoated sand was not tested because we first tested the IOCS and saw no inactivation. Therefore, we assume that there would be no inactivation at the time scale of our experiment in the case of phages attached to the uncoated sand

Inactivation in other studies. Ryan et al. [28] estimated that the solution inactivation rate was around 3 times faster than the combined surface inactivation/release rate. This study was conducted in field and column studies with contaminated sediment, mainly sand containing 3-4% iron and aluminium oxyhydroxides as porous media. The inactivation experiments (solution and surface inactivation) were conducted in raw and amended contaminated groundwater and contaminated sediment pH 6-7. The residence time of the phages was around 24 hr.
Zhuang et al. [38], in column studies with goethite coated sand, found some inactivation

of the phages MS2 and ϕX174 at pH 7. The residence time of the phages in porous media was only around 2 hr for the free phages. But, the inactivated fraction of the phages was measured as follows: around 20 pore volumes of phage solution were passed through the sand and immediately after that the sand was eluted with around 21 pore volumes of beef extract to examine the mass recovery. Thus, they measured the phages recovered during the elution and calculated the inactivation as the difference between the removal and elution fractions.
As illustrated by these two studies, the inactivation of the attached phages was probably mainly determined by the contact time between the phages and the porous media. Even if the porous media is thought to have an efficient attachment behaviour, the time during which the phages remain attach to it seems to be the most important parameter that determined the inactivation rate onto the porous media.

4.4 Adsorption behaviour of iron oxide coated sand with changes in environmental parameters.

4.4.1 Environmental parameters

Different environmental factors can influence the phage behaviour during its transport in solution through a porous media (see Figure 4.7).

Non exhaustive list of potential environmental factors influencing phage transport -in solution	
• pH	• Water content
• Ionic strength (IS) (from monovalent or multivalent ions)	•Temperature
• Natural organic matter (often simulated with humic acid in laboratory studies)	• Competitions with macromolecules (e.g. other phages, organic pollutants and anions like e.g phosphate, sulfate)
• Redox potential	• Microbial activity

Figure 4.7: **Main environmental factors**

In nature, all these factors interact together to create a complex system. In order to determine the influence of all these parameters together, it is often easier to first study each parameter individually. By testing each parameter alone, we would have a better

understanding of its influence and, thus, a better apprehension of systems with several parameters.
For this study, we did not have enough time to test all the environmental variables. So, we did our experimentation with several commonly tested environmental factors thought to be the major parameters affecting phage transport [30]. The pH, the ionic strength and the humic acid content of the solution were the parameters we tested.

4.4.2 *pH* influences on IOCS adsorption

a. Results

Results of phage adsorption when changing the pH at the same time. We tested pH values between 7 and 9, because these values are common values observed in nature. We worked with the same initial concentration for all pH values tested.
We saw no variation of the unbound fr phage concentration with the uncoated sand with variation of the pH. But, with the IOCS, the concentration in the supernatant increased when we increased the pH. Thus, at pH 9.5, most of the phages are unbound (see Figure 4.8).
In order to see if this increase of the unbound fraction was due to the iron oxide coating, we also tested MS2 for the same range of pH values (see Figure 4.9). We observed more or less the same behaviour: with increasing the pH, the unbound fraction increased. Thus, at pH 9, like in the experiment with fr, most of the MS2 phages were unbound.

Results of changing the pH after adsorption in a standard buffer. We saw a decrease of the concentration in the supernatant, when we increase the pH to around 9. By increasing the pH to around 10, we observed an increase of the unbound fraction of the phages (see Table 4.2 and Figure 4.10).

Table 4.2: **Fraction of the unbound phages remains in the solution as function of the pH increase.**

	pH 7	*pH 9*	*pH 10*
%unbound phages	0.079	0.006	4.221

b. Discussions

Effect of the pH with uncoated sand. As shown in the section 4.2, at pH 7, fr phages and uncoated sand are like-charged. Thus, by increasing pH we increased repulsion between two particles that have already repulsive interactions. But, we did not observe a significant increase in the unbound phages in the solution at pH 9.5 (see Figure 4.8). The results of Loveland et al. [17], for PRD1 phages (calculated pI around

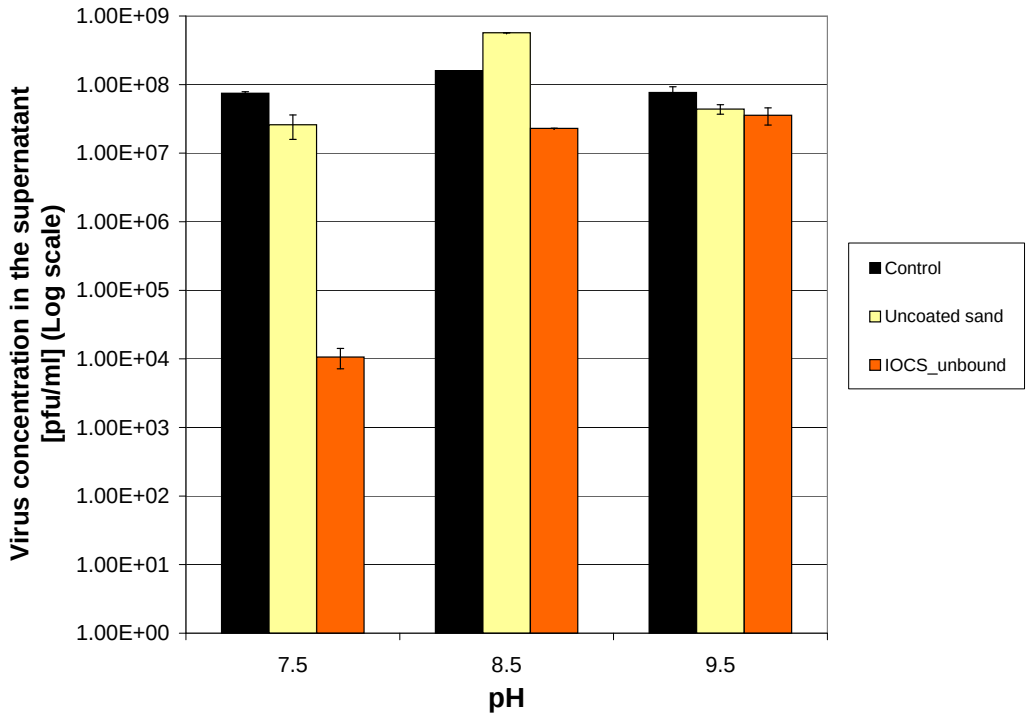

Figure 4.8: **Plot of the fr phages concentration in the supernatant after mixing with uncoated sand and iron oxide coated sand (IOCS) at an initial phages concentration of around 10^8 pfu/ml, in standard buffer (IS 20 mM) at pH 7.5, 8.5 and 9.5.**

3.5) and uncoated quartz sand (calculated pI around 2.5), confirmed our observations. Indeed, by testing pH from 3 to 9, they found near complete attachment of the phages at pH 3 and 5, while, at pH 7 and 9, they observed a little attachment (below 10 % of the phages). There was no significant decrease between 7 and 9.

Effect of the pH with IOCS. For fr phages, we observed a big increase of the unbound phages at pH 8.5 and pH 9.5. Thus, a strong increased repulsion between phages and IOCS took place at these pH values.
According to the DLVO theory (see section 2.2.2), the interactions between two particles begin to be mostly repulsive after a certain pH value that is called attachment edge. This attachment edge value is generally larger than the biggest pI value of the particles as illustrated by Loveland et al. [17]. In fact, in their study at 1-2 mM ionic strength, the attachment edge for PRD1 (calculated pI around 3.5) and uncoated quartz sand (calculated pI around 2.5) was around 5.5 while attachment edge between PRD1 and ferric oxyhydroxide-coated sand (calculated pI around 5.5) was around 7.
These results seem to be confirmed by Zhuang et al. [38] who found, in a column study, near complete removal efficiency of MS2 (pI around 3.9) and ϕX174 (pI around 6.6) to

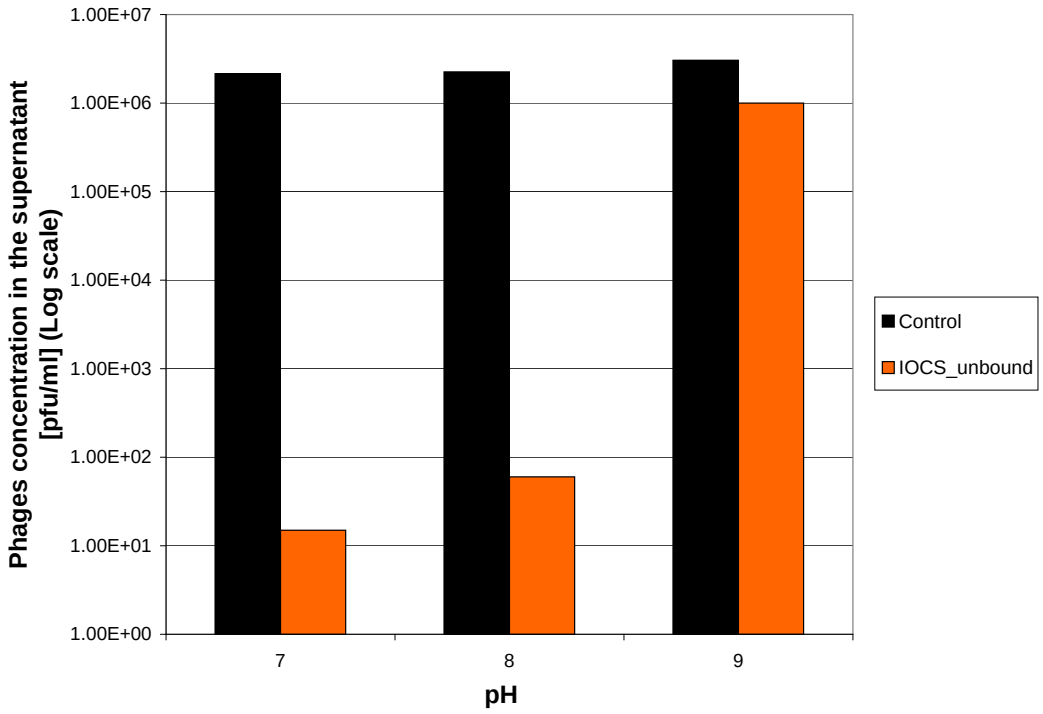

Figure 4.9: **Plot of the MS2 phages concentration in the supernatant after mixing with iron oxide coated sand (IOCS) at an initial phages concentration of around 10^6 pfu/ml, in standard buffer (IS 20 mM) at pH 7, 8 and 9.**

goethite coated sand (calculated pI 7.1) at pH 7.5 and decrease of the removal efficiency at pH 9.3, with carbonate buffer of ionic strength around 150 mM.
In section 4.2, we concluded that the pI of fr phages should be below 7 according to our results. Therefore, as we have an attachment edge around pH 9, we can conclude that the pI value of our IOCS is between 7 (at least, according to Loveland et al.[17], but they worked with other phages and sand) and 9. Thus, the supposition that our IOCS is made of hematite-containing iron oxides is supported because the pI of hematite is between 8-9 (see section 3.1.1).
This conclusion was further supported by the experiment done with MS2 (see Figure 4.9). Indeed, we observed a large increase of unbound MS2 phages at pH 9.

Effect of increasing pH after adsorption onto IOCS. By using the pH and ionic strength conditions (standard buffer pH 7 and IS 20 mM) of the adsorption step, we created favourable conditions for the attachment of phages to the IOCS (see section 2.2.4).
Our results (see Figure 4.10) showed a decrease in the unbound fraction at pH around 9 and a significant increase of the unbound fraction at pH around 10. The decrease of

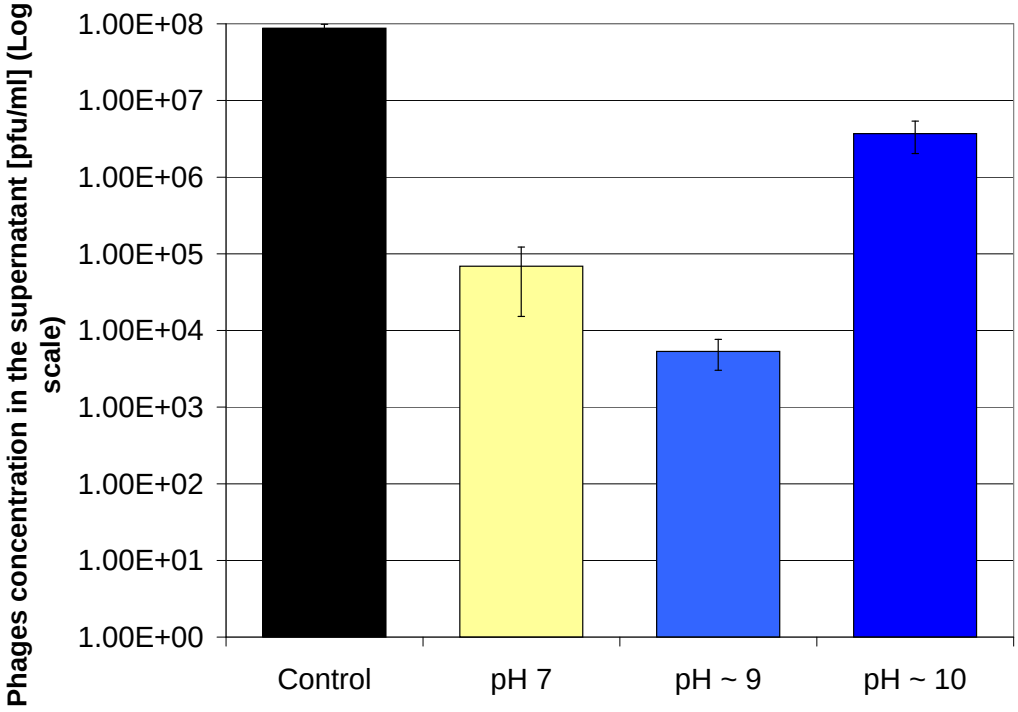

Figure 4.10: **Plot of the fr phages concentration in the supernatant after desorption without increasing the pH and by increasing the pH from 7 to around 9 and around 10, after a previous adsorption step of an initial concentration of 10^8 pfu/ml at pH 7 and IS 20 mM to iron oxide coated sand (IOCS).**

the unbound fraction at pH around 9 does not seem to be explainable, but the increase at pH around 10 can be described as follows: by increasing the pH, favorable sites of the IOCS, that have a pI between 8-9, become unfavorable to attachment. Thus, the detachment rate increased and the fraction of unbound phages at equilibrium increased. Loveland et al. [17] showed that release of phages from adsorption surfaces began at a pH value that is between 2-3 pH units higher than the pI of the mineral surface. This hypothesis may explain why we observed phage release starting at pH 10, i.e., around 2 pH units higher than the pI of the hematite.

4.4.3 *Ionic strength* influences on IOCS adsorption

a. Results

Results of phage adsorption when changing the ionic strength at the time of virus adsorption (see Figure 4.11). We saw a significant decrease of the unbound phages by increasing the ionic strength with uncoated sand. For the IOCS, we observed

an increase of the unbound phages by increasing the ionic strength from 20 to 100 mM and a decrease of the unbound part by further increasing from 100 to 300 mM.

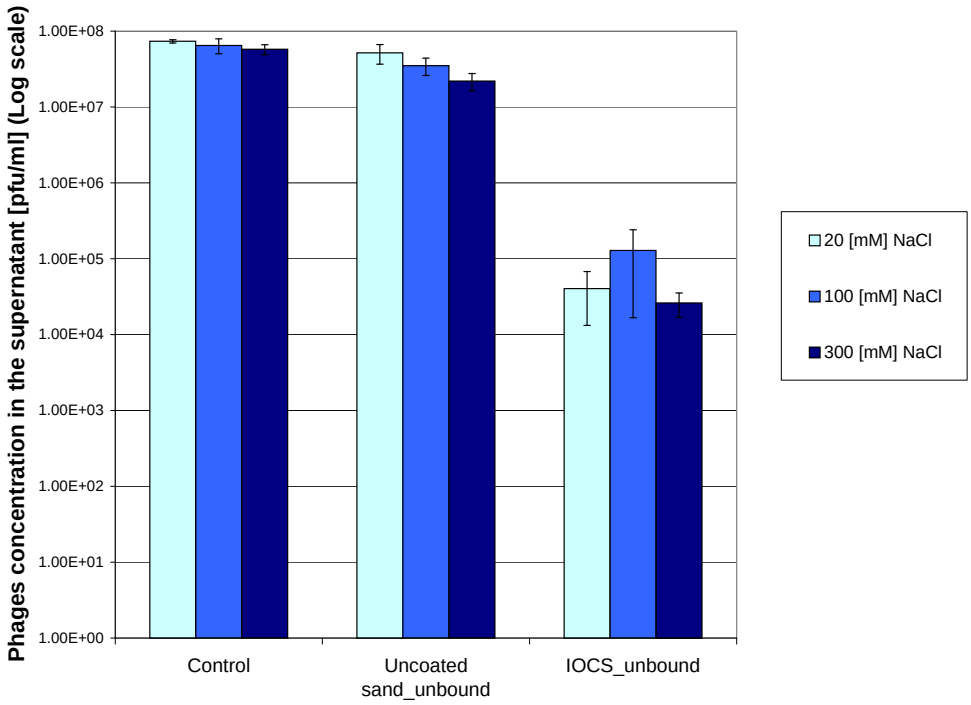

Figure 4.11: **Plot of the fr phage concentration in the supernatant after mixing with uncoated sand and iron oxide coated sand (IOCS) at an initial phages concentration around 10^8 pfu/ml, in standard buffer (pH 7) at ionic strength(or NaCl concentration) of 20, 100 and 300 mM. Note that the error bars are here 95% confident intervals, obtain from 9 measured values.**

Results of changing the ionic strength after adsorption in a standard buffer. We observed no variations of the unbound phages by increasing the ionic strength after adsoprtion with the standard buffer (see Figure 4.12).

b. Discussions

Effect of the ionic strength with uncoated sand. The unbound phages decreased when we increased the ionic strength (see Figure 4.11). Penrod et al. [25] obtained the same tendencies with MS2 and lambda phages by increasing IS from 10 to 100 mM, however, by further increase from 100 to 300 mM, they observed an attenuation

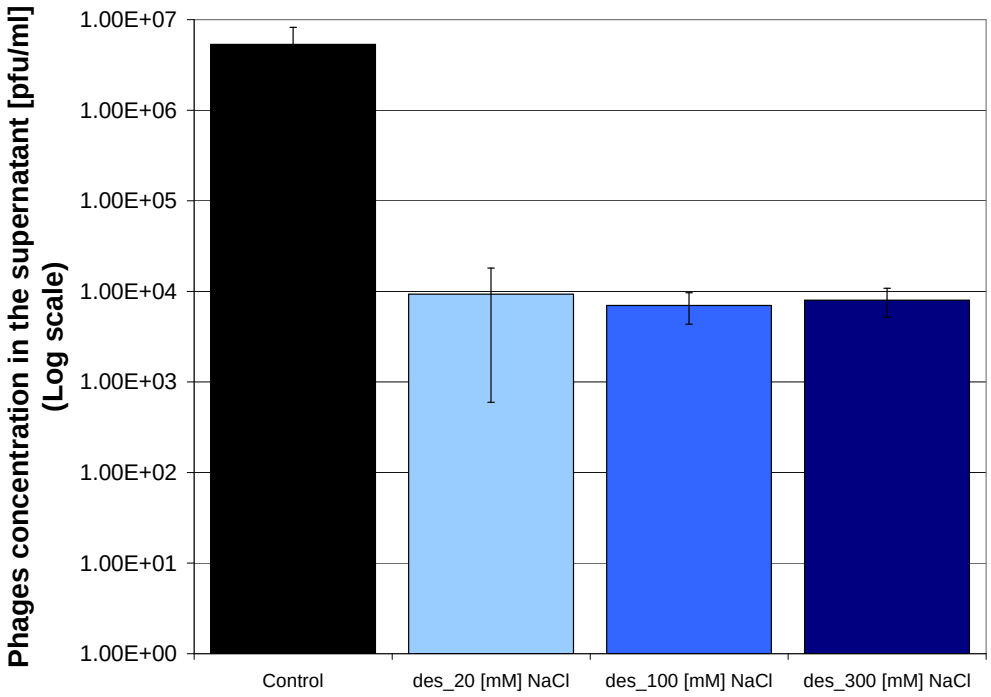

Figure 4.12: **Plot of the fr phage concentrations in the supernatant after desorption without increasing the ionic strength and by increasing the ionic strength (or NaCl concentration) from 20 to 100 and 300 mM, after a previous adsorption step of an initial concentration of 10^8 pfu/ml at pH 7 and IS 20 mM to iron oxide coated sand. (IOCS).**

of the attachment efficiency for lambda and a decrease of the attachment efficiency for MS2. They concluded, according to the DLVO theory, that the salt concentration had an effect on the compression/expansion of the electrical double layer surrounding the phages. Thus, by increasing the ionic strength, the electrical double layer is compressed and the surface potential of the phages is reduced. In other words, at the same separation distance between a phage and a grain of sand (see Figure 2.2 A), the electrical double layer repulsion (potential energy) is lower when the solution contains more ions. Ions in the aqueous solution act like shields between like-charged particles and, thus, promote attachment of phages to unfavorable sites.

Effect of the ionic strength with IOCS(see Figure 4.11). The variations of the unbound phages due to the increase of the ionic strength of the solution shows no tendencies. In fact, we have the same unbound phages concentrations at 20 mM and at 300 mM. At 100 mM, it seems that there is more unbound phages. But if we look at the statistical error, we see that the variation of the results is large. As we were

expected a linear behaviour, the results of the 100 mM measurements does not seem to be significant.
However, Zhuang et al. [40] worked with aluminium oxides coating (pI around 9) and ϕX174 and MS2 phages at pH 7.5, and found a decrease of the attachment efficiency when increasing the ionic strength (from 2 to 163 mM). But, they worked with phosphate buffer that contained a mix of NaCl and KCl with 0.25 and 20 mM of Na_2HPO_4 and carbonate buffer that contained a mix of NaCl and KCl with 0.8 and 60 mM of $NaHCO_3$. These two substances dissociate to carbonate (HCO_3^-) and phosphate (HPO_4^{2-}) in solution. The influence of these anions on phage-mineral interactions is not well understood. For example, You et al. [36] found that anions like HPO_4^{2-} or SO_4^{2-} competed with MS2 for binding sites at a concentration 25 mM.
Therefore, it is not clear if the ionic strength has an influence on the attachment efficiency between oppositely-charged particles. Furthermore, in our experiment, we do not know the impact of Tris-HCl buffer on the ionic strength of the solution.

Effect of increasing ionic strength after adsorption onto IOCS. We did not observe any release of phages by increasing the ionic strength of the solution (see Figure 4.12). According to the previous results where we did not see a significant influence of ionic strength on the attachment of phages to IOCS, this result seems to be logical.

4.4.4 *Humic acid* influences on IOCS adsorption

a. Results

Results of phage adsorption when changing the humic concentration at the same time (see Figure 4.13). For the uncoated sand, we observed no increase or decrease of the unbound concentration upon the addition of HA. For the IOCS, however, we saw a small decrease of the unbound phages for 1 and 10 mg/l and nearly no adsorption of the phages with a humic acid concentration of 100 mg/l.

Results of changing the humic acid concentration after adsorption in a standard buffer (see Figure 4.14). We observed release of the attached virus at a humic acid concentration of 30 mg/l. Then, between 50 and 250, the release increased slightly, but without reaching a complete release of the phages.

b. Discussions

Effect of the humic acid with uncoated sand. Humic acid had no relevant effect on the adsoprtion of phages to uncoated sand. Indeed, because the interactions between phages and porous media are unfavorable, the humic acid, that commonly compete with phages for favorable attachment sites[30], did not disturb the low level of adsorption that normally takes place in its absence.

Effect of the humic acid with IOCS. In between 10 and 100 mg/l, near com-

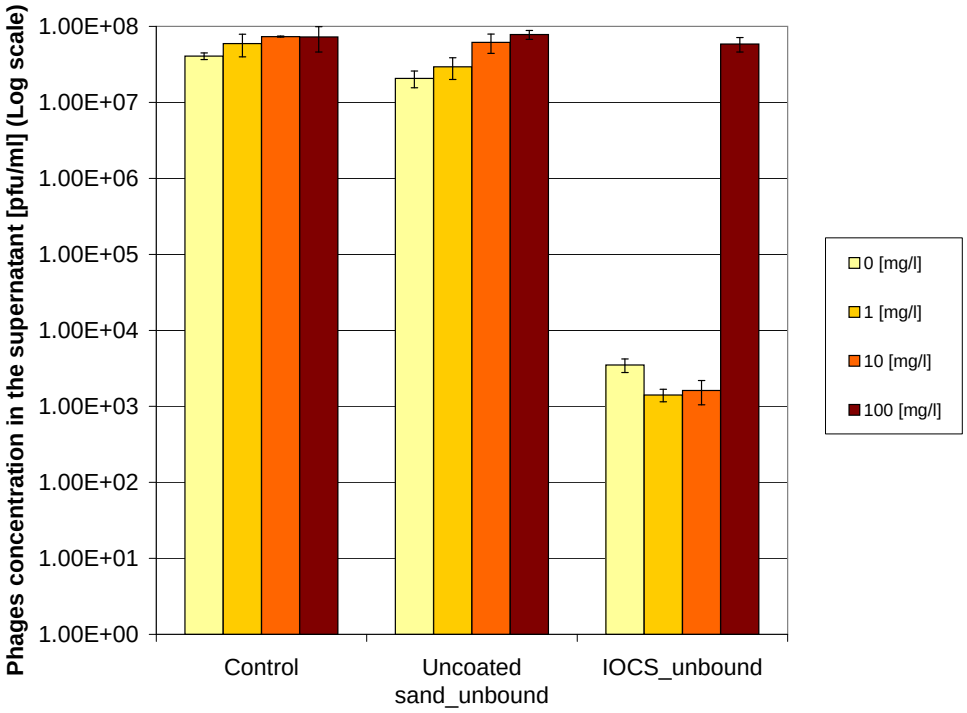

Figure 4.13: **Plot of the fr phages concentration in the supernatant after mixing with uncoated sand and iron oxide coated sand (IOCS) at an initial phages concentration of around 10^8 pfu/ml, in standard buffer (pH 7 and IS 20 mM) with 0, 1, 10 and 100 mg/l of humic acid content.**

plete release of phages into solution takes place (see Figure 4.13). Thus, humic acid, that is thought to compete with phages for binding sites, is present at a concentration that occupies almost all of the attachment sites. Zhuang et al. [39] observed the same tendency with MS2 and sand with a very low iron content (32.5 mg/kg against 5000 mg/kg for our sand) with phosphate buffer at pH 7.5. Indeed, they noted a significant amount of unbound phages at a humic acid (purchased from the International Humic Substance Society) concentration of 5 mg/l. They explained that both electrostatic interactions and specific sorption (covalent binding) are involved in the binding of organic matter, like humic acid, to mineral particles. Therefore, organic matter sorption is more favorable and potentially stronger than phage sorption. Thus, humic acid may out-compete the phages for attachment sites.
Furthermore, the adsorption of humic acid on the sand increases the negative charge on the solids and provides an electrostatic barrier would hinder subsequent virus attachment.
However, according to other authors, Zhuang et al. considered that humic acid could provide hydrophobic sorption sites. Although MS2 is highly hydrophobic compared to

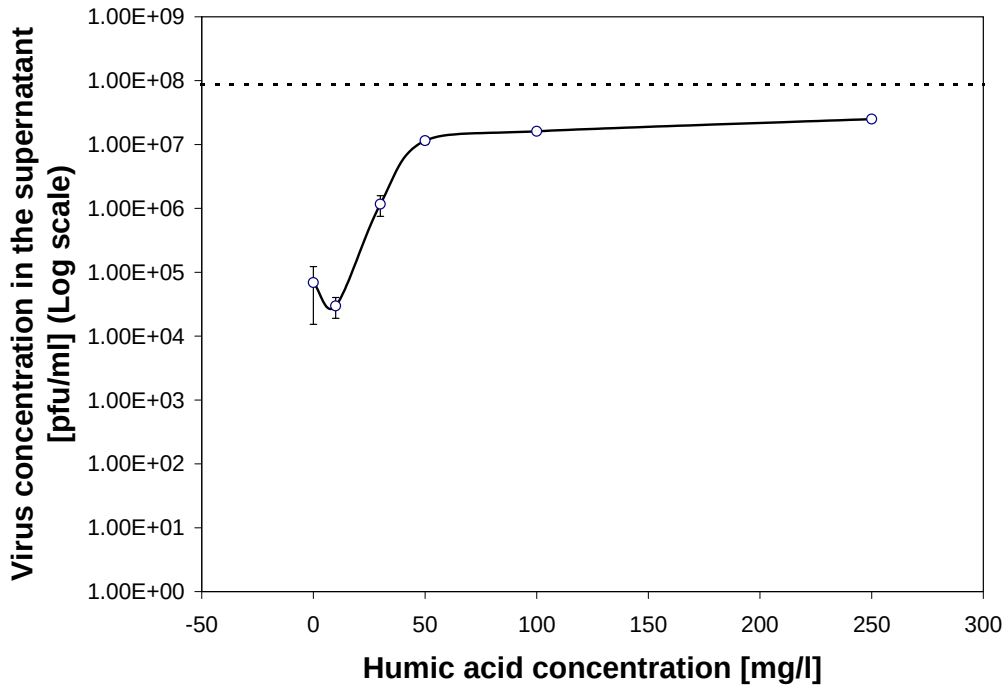

Figure 4.14: **Plot of the fr phages concentration in the supernatant after desorption without adding humic acid and by adding 1, 10, 30, 50, 100 and 250 mg/l of humic acid, after a previous adsorption step of an initial concentration of 10^8 pfu/ml at pH 7 and 20 mM to iron oxide coated sand (IOCS) compared to the control concentration (dotted line).**

other phages (see section 2.2.3), it seems that the loss of attachment sites by competition with humic acid is more important than the increased hydrophobic attachment to the humic acid.

Effect of increasing humic acid concentration after adsorption onto IOCS. In line with the earlier HA experiments, we observed the first release of previously adsorbed phages at 30 mg/l (see Figure 4.14). At 50 mg/l, further increase of the humic acid content only slightly increased the release fraction. Thus, it seems that a certain fraction of the phages remained attached or not out-competed by the humic acid, even if it is present in high concentration (until 250 mg/l). There is no clear explanation except that they were not out-competed by the humic acid during their attachment phase and a fraction of them are apparently so strongly attached that humic acid cannot compete them anymore.

4.5 Adsorption behaviour of aged iron oxide coated sand

The goal of this step was to observe the behaviour of the adsorption of the IOCS after "aging" it by exposing it to buffer solution or humic acid for a given period before the experiment.
The experiment of aging with the *standard buffer solution* was made in order to simulate the behaviour of the IOCS during continuous flow experiment, where the porous media is washed by buffer flowing through.
The experiment of the aging with the *humic acid* was done in order to simulate if the humic acid creates a coating on the IOCS that interactes with the sorption of phages.
Both experiments were also done to observe if the environmental conditions have an effect on the behaviour of the iron oxides coating. In fact, iron oxyhydroxides are subjected to dissolution and transformation in solutions [5]. *Dissolution* can proceed by a variety of pathways, e.g. protonation, complexation with inorganics and organics ligands (e.g. humic acid) and photochemical and biological reduction. *Transformation* can also proceed by a variety of pathways, e.g. dissolution/precipitation, reduction/oxidation, thermal or mechanical dehydroxalation[1], dehydration. Under oxic conditions, like in our experiments, goethite and hematite are thermodynamically the most stable compounds and are, therefore, the terminal products of many transformation routes.

a. Results and discussions

Aged IOCS with the buffer solution. We observed about 1 log decrease of the adsorption capacity of the aged IOCS compared to the IOCS used during the experiment (see Figure 4.15). However, the removal of the phages was still around 3 logs i.e., 0.1-0.2% of the phages remain in the solution (0-0.05% remains in the solution for the IOCS). However, these results should be interpreted carefully because we do not know the variation of the results (we did not do triplication). Thus, a small fraction of our iron oxides coating seems to disappear by dissolution or our iron oxides coating could have partially transformed to iron species that are less favorable to attachment. Lukasik et al. [18] did not detect any ferric in their column effluents over a long period of time. Therefore, the second hypothesize previously proposed is more relevant,even if hematite is considered as a stable form of the iron under oxic conditions. Anyway, the effect of the buffer solution on the IOCS adsorption properties is very low and, thus, can be considered negligible.

Aged IOCS with the humic acid solutions. We observed no significant decrease or increase of the IOCS adsorption capacity compared to the IOCS used during the experiment (see Figure 4.16).
However, we tested 1 and 10 mg/l that are relatively low concentrations of humic acid.

[1]Process that remove one or more hydroxyl groups out of a compound.

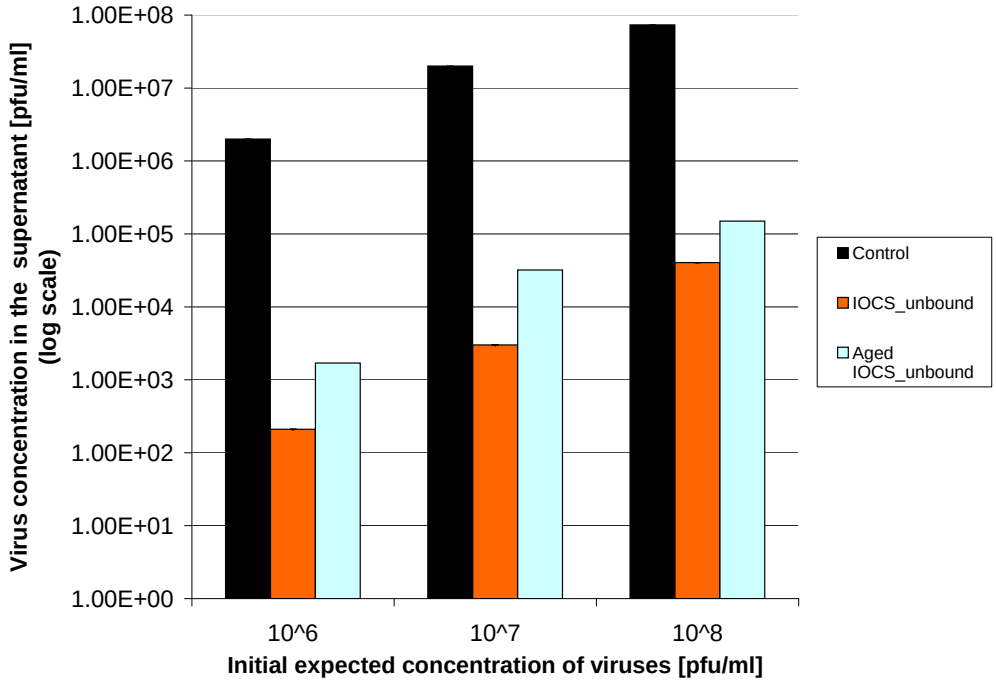

Figure 4.15: **Plot of the fr phage concentration in the supernatant after mixing with iron oxide coated sand (IOCS) and aged iron oxide coated sand with standard buffer solution (Aged IOCS) at different initial phages concentrations, in standard buffer (pH 7 and IS 20 mM).**

Actually, because our IOCS was thought to be used for post-treatment of wastewater plant effluents, we decided to test a maximal humic acid concentration of 10 mg/l. In fact, in the Swiss law [41], the maximal allowed dissolved organic carbon concentration of the wastewater treatment plant effluent is 10 mg/l. Furthermore, Imai et al. [13] observed that hydrophobic (typically humic substances) and hydrophilic acids dominated more than 55% of the DOM as DOC in all wastewater effluents tested.
Zhuang et al. [39] tested the effect of organic matter (OM) coating on sand (with relatively small amount of iron 32.5 mg/kg against 5000 mg/kg for our sand). The organic matter coating was made by mixing OM concentration of 100 g/l with 200 g of sand. They observed that adsorption of MS2 was reduced from 99.2% to 29% removal by OM coating of the sand.

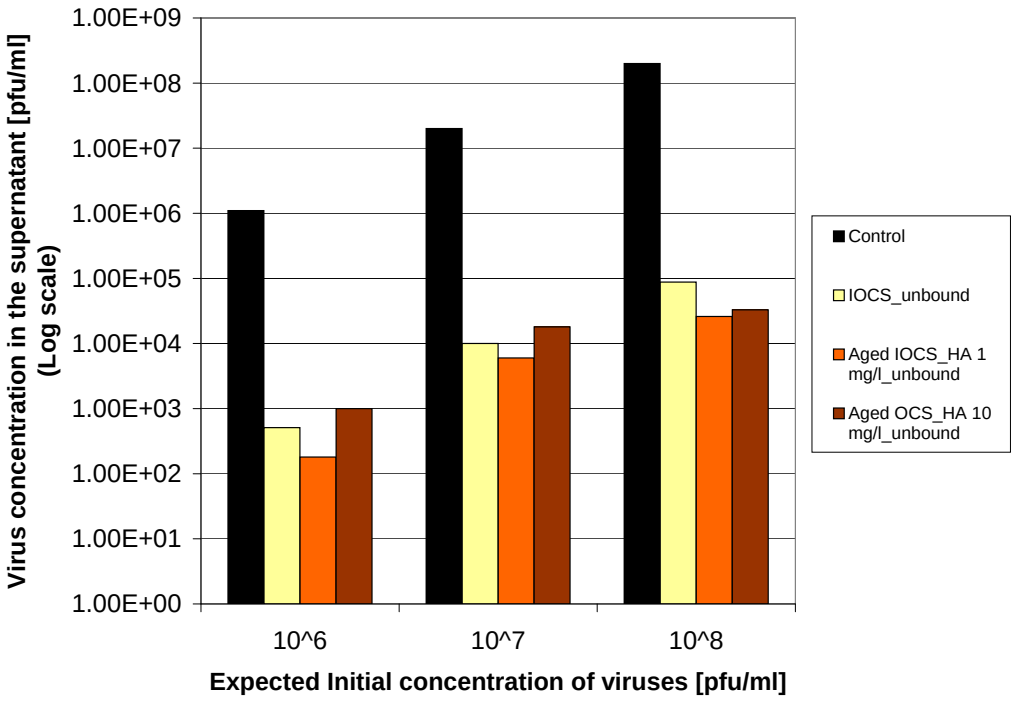

Figure 4.16: **Plot of the fr phage concentration in the supernatant after mixing with iron oxide coated sand (IOCS) and aged iron oxide coated sand with standard buffer solution + humic acid at concentration of 1 and 10 mg/l (Aged IOCS_HA) at different initial phages concentrations, in standard buffer (pH 7 and IS 20 mM).**

Chapter 5

Futures perspectives

5.1 Continuous flow experiments

As discussed in section 2.5, continuous flow experiments are the only way to quantify the sorption behaviour of viruses during transport through a porous media or to measure the kinetics of virus adsorption.
Thus, the next step of this work will be to test phage sorption and inactivation in a continuous flow system.
To date, all laboratory studies have performed vertical flow experiments in columns [34][7][38]. Fields studies generally utilize horizontal flow experiments [32][28].
Because we would like in the futur to examine the effect of Fenton and photo-Fenton reaction processes (see section 5.2), we need to implement laboratory-scale, horizontal flow systems so that we can apply sunlight or simulated sunlight to the exposed upper surface (see Figure 5.1).

After the calculations of the hydrodynamic parameters of our systems, it will be possible to fit a theoretical model (e.g., a two-site kinetic model [31]) to determine the attachment/detachment behaviour and the inactivation kinetics (see section 2.4).

Given the difficulty synthesizing IOCS reproducibly, the IOCS that we are going to use for the continuous flow experiments will probably not be identical to the one used for the batch experiments. In fact, both the size of the initial uncoated sand and the coating procedure are not the same.
Indeed, in order to have a sand with a sufficiently high hydraulic conductivity, we will use Ottawa sand purchased from Fisher Scientific (Folson, US) that has a relatively large average diameter (0.5 - 0.8 mm).
The washing procedure will be the same for the sand used in the batch experiments (appendix A).
Unfortunately, using the same coating procedure for different batches did not lead to identical IOCS. Indeed, when we tried to reproduce the coated sand that we used for the batch experiments, we obtained sand that was less coated. Thus, in order to find a

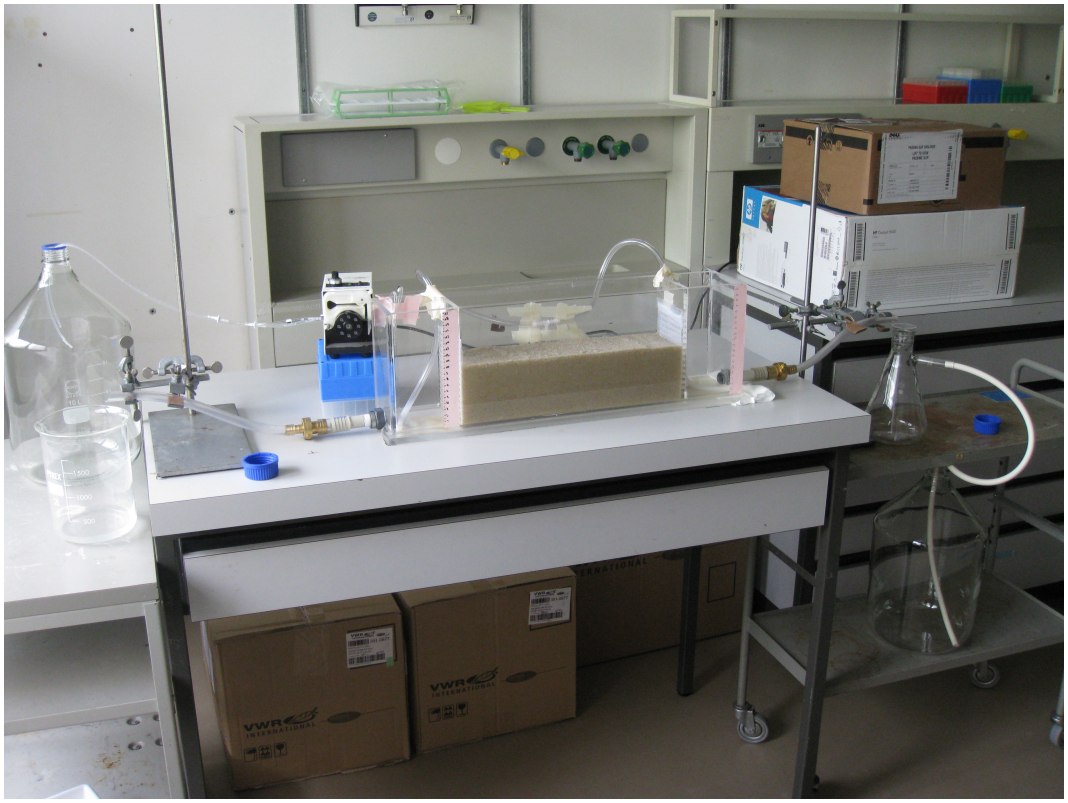

Figure 5.1: **View of the continuous flow experimental set-up.**

method that is easily reproducible and that provides an amount of iron coating around 5 [mg of Fe/g of iron oxides caoted sand], we will test other coating procedures. For example, Lo and Chen [16] created a method with which they obtained a high degree of iron coating (around 8 [mg Fe/g sand]) by pH optimisation of the coating solution.

5.2 Enhancement of virus inactivation by Fenton and photo-Fenton reaction

It was reported that the inactivation rate of the phages attached to solids was three times slower than the inactivation of phages in solution [28]. However, it was also reported that inactivation rate of the phages attached onto the solids was as fast or faster than the inactivation of the phages in the solution by a factor of up to five [29].
Anyway, inactivation rates in the solution or onto the surface are ten to hundred times much slower than detachment rates to the unfavorable fraction of attachment sites of a porous medium [29]. Thus attenuation of the phage concentration in water during soil passage occurs mainly because of adsorption behaviour (attachment/detachment) rather than because of inactivation.

Therefore, from an engineering point of view, improving the inactivation and attachment rates are important ways to enhance the removal efficiency of viruses from water. For this purpose, the use of iron oxide coated sand seems to be a solution because of its high adsorption capacity at natural pH conditions [30] and its catalytic function in the creation of active oxygen species like hydroxyl radicals via both the Fenton and photo-Fenton reactions [10][9].
Iron minerals can catalytically produce hydroxyl radicals in the presence of sunlight via a photo-Fenton mechanism. This involves the photo-reduction of structural Fe(III) and subsequent re-oxidation by H_2O_2 (Eqn.1.1 and 1.2):

$$Fe(III) + light \longrightarrow Fe(II) \tag{5.1}$$

$$Fe(II) + H_2O_2 + H^+ \longrightarrow Fe(III) + OH^* + H_2O \tag{5.2}$$

In fact, inactivation by photo-Fenton reaction could enhance the inactivation rate of the virus in the solution or on the solids.

However, before studying the effect of the photo-Fenton reaction on the inactivation of the phage, we have to characterize precisely the sorption and inactivation capacity of the iron oxide coated sand that we use (see section 5.1).

Chapter 6

Conclusion

Concerning the interactions between our synthesized IOCS and fr phages we can conclude that:

- The iron oxide coating provides a great number of attachment sites or a high adsorption capacity for our sand, even if it occupies only a small fraction of the quartz grain surface area.
- There is no inactivation due to the attachment to the IOCS at the time scale of our experiments (1 - 2 hr).
- The effect of pH is predominant, especially when the pH approaches the isoelectric point (8-9) of our IOCS. Furthermore, we see a release of adsorbed phages when we increase the pH from 7 to at least 10. However, most of the phages remain attached.
- The effect of ionic strength is significant in the case of like-charged particles (e.g., with uncoated sand). Thus, we see more adsorption by increasing the ionic strength. But for our IOCS, the influence on the adsorption of the phages has no influence on either the adsorption or the release of the phages.
- Humic acid concentrations begin to have a significant influence from around 30 mg/l, mainly due to competition. Furthermore, the addition of humic acid after phage adsorption led to the release of a large amount of attached phages. However, a part of them remain attached even if we further increased the concentration of humic acid.

With the aged IOCS experiment, we can conclude that the adsorption capacity of our IOCS did not change after contact with buffer solution or with a 10 mg/l humic acid solution. However, continuous flow experiments are required to have a better understanding of the aging effect on our IOCS.

Other parameters like e.g. redox potential of the solution, water saturation, microbial activities, competitions with other substances (e.g. phosphate, organic pollutants, viruses) for the binding sites, should be examined in order to have a more complete knowledge of the adsorption behaviour of phages onto our IOCS.
However, the results observed in our batch experiments provide a good knowledge concerning the behaviour of phages with IOCS, and the effect of the main environmental parameters. In particular, the desorption experiments by changing environmental parameters tell us that changes in environmental parameters can lead to the re-release of infective viruses. This would be important for the functioning of a wetland used to treat water that contains infective viruses. In fact, if we design wetlands to simply remove viruses by adsorption without causing inactivation, then we have not eliminated the public health threat.
Finally, with our results, it will be easier to determine the important parameters to vary to obtain relevant results in further experiments (e.g., continuous flow experiment). Furthermore, the results of these subsequent experiments will be easier to interpret with the knowledge gained during batch experiments.

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Appendix A

Procedure to remove metals from sand

Reactant needed:

- sodium citrate ($Na_2C_6H_5O_7$*$2H_2O$)
- citric acid ($H_2C_6H_5O_7$)
- sodium dithionite ($Na_2S_2O_4$)

Material:

- 2l polyethylene centrifuge bottle per 300 g of the water-washed sand
- 2l beaker to make the sand and solution settle.

Procedure to wash 300g of sand with acid:

- Verification of the presence of impurity in sand by measuring the turbidity of the rinsing water.
- Prepare citrate buffer solution in a beaker with:
  - 1l of demineralised water.
  - 42.816g/l of sodium citrate ($Na_2C_6H_5O_7$*$2H_2O$),
  - 10.5 g/l of citric acid ($H_2C_6H_5O_7$)
- Put in a plastic bottle:
  - 300g of sand
  - 500ml of buffer solution
  - 15g of sodium dithionite ($Na_2S_2O_4$) (weight and add it under a fume cupboard as it could produce some toxic gazes with acid).

- Heat at 80°C in water bath for 10 min.
- Then shake for 20 min in a rotative homogeneizer.
- Remove the solution and throw it as a toxic waste.
- Take the settled sand and clean it again 3 more times from the third step of the procedure.
- Rinse the sand with demineralised water until the rinsing water is close to 6.1μSiemens (measured electroconductivity of distilled water) and from pH of distilled water, at least 6 times.
- Dry the sand in an oven at 105°C for 2 days.

Appendix B

Procedure to coat sand

Reactant needed:

- $FeSO_4$*$7H_2O$
- $NaHCO_3$

Material:

- A 2 liter glass bottle
- A cap with a hole to permit aeration. Procedure to coat 300g of sand:
- Replace the oxygen dissolved in 1 liter of distilled water with nitrogen by bubbling.
- Add 13.9g of unoxidised crystals of $FeSO_4$*$7H_2O$ in the solution.
- Add 300g of acid wash sand and 110 ml of 1 M $NaHCO_3$ solution.
- Put the bottle on a rotating plate (170 rpm for 48h) with aeration to oxygenised the solution.
- After 48hours, Rinse the sand with distilled water until the rinsing water has a pH and an electric conductivity close to distilled water.