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Virus Removal by Iron Coagulation Processes

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Abstract

Waterborne viruses account for 30% to 40% of infectious diarrhea, and some viruses could persevere for some months in nature and move up to 100 m in groundwater. Using filtration setups, coagulation could lessen virus charges as an efficient pre-treatment for reducing viruses. This work discusses the present-day studies on virus mitigation using coagulation in its three versions *i.e.*, chemical coagulation (CC), enhanced coagulation, and electrocoagulation (EC), and debates the new results of virus demobilization. The complexity of viruses as bioparticles and the process of virus demobilization should be adopted, even if the contribution of permeability in virus sorption and aggregation needs to be clarified. The information about virion permeability has been evaluated by interpreting empirical electrophoretic mobility (EM). No practical measures of virion permeability exist, a clear link between permeability and virion composition and morphology has not been advanced, and the direct influence of inner virion structures on surface charge or sorption has yet to be conclusively demonstrated. CC setups utilizing zero-valent or ferrous iron could be killed by iron oxidation, possibly using EC and electrooxidation (EO) methods. The oxidants evolution in the iron oxidation method has depicted promising findings in demobilizing bacteriophage MS2, even if follow-up investigations employing an elution method are needed to secure that bacteriophage elimination is related to demobilization rather than sorption. As a perspective, we could be apt to anticipate virus conduct and determine new bacteriophage surrogates following subtle aspects such as protein structures or genome size and conformation. The present discussion's advantages would extend far beyond an application in CC—from filtration setups to demobilization by nanoparticles to modeling virus fate and persistence in nature.

Keywords

Viruses, Chemical Coagulation (CC), Enhanced Coagulation (EnC), Electrocoagulation (EC), Electrophoretic Mobility (EM), Natural Organic Matter (NOM)

1. Introduction

Even in industrialized countries such as the US [1], waterborne viruses account for an evaluated 30% to 40% of infectious diarrhea [2]. Related to acute gastric and respiratory diseases and chronic conditions [3], some viruses could persevere for some months in nature [4] and move up to 100 m in groundwater [5] [6]. Various families/genera of waterborne viruses are in the US Environmental Protection Agency's (USEPA) Contaminant Candidate List (both CCL 3 and draft CCL 4) for potable water, showing the obligation for more investigation into presence and removal [7]. Also, the World Health Organization's (WHO) Guidelines for Drinking Water Quality [8] mention eight virus categories concerning potable water, all of which possess elevated tenacity and infectivity compared to different pathogens. Although several viruses somewhat tolerate traditional water treatment [8] [9], adenoviruses combat novel techniques like ultraviolet (UV) disinfection [10] [11]. Coagulation could be utilized to lessen virus charges and decrease the dose for disinfection [12]. Coagulation is an efficient pre-treatment for reducing viruses using filtration setups [13]-[18].

This work forms an opinion about the present-day studies on virus mitigation using coagulation. In addition, upcoming paths for investigation are debated based on the new results of virus demobilization in three coagulation techniques, *i.e.*, usual chemical coagulation (CC), enhanced coagulation (EnC), and electrocoagulation (EC) [19]. In traditional CC, metal hydrolytes are generated by dissolving a metal salt in water [1] [20] [21].

Aluminum and iron salts, e.g., $Al_2(SO_4)_3$ and $FeCl_3$, are frequently employed in water treatment [1]. At the same time, new coagulants, such as polyaluminum chlorides (PACls), have gained particular interest in virus reduction [22] [23] [24] [25]. Polymeric iron coagulants have also been developed [26] [27]; however, such chemicals have not been tried for virus reduction.

For example, the EPA's Disinfectants and Disinfection Byproduct Rule (DBPR) encouraged EnC before disinfecting potable water to avoid the generation of carcinogenic disinfection by-products (DBPs) [28] [29] [30]. EnC utilizes elevated injections of chemical coagulant and pH adjustment to efficiently reduce humic acids, fulvic acids, and other dissolved and suspended organic material (as a group named natural organic matter, NOM) [1] [31]. For such cases, EnC has been estimated for virus reduction in waters with increased NOM levels [16] [32] [33].

The literature suggests many captions for the pathways of neutralizing pathogens by electrochemical techniques [34] [35], which could be depicted in **Figure 1** and listed in **Table 1** [36] [37].

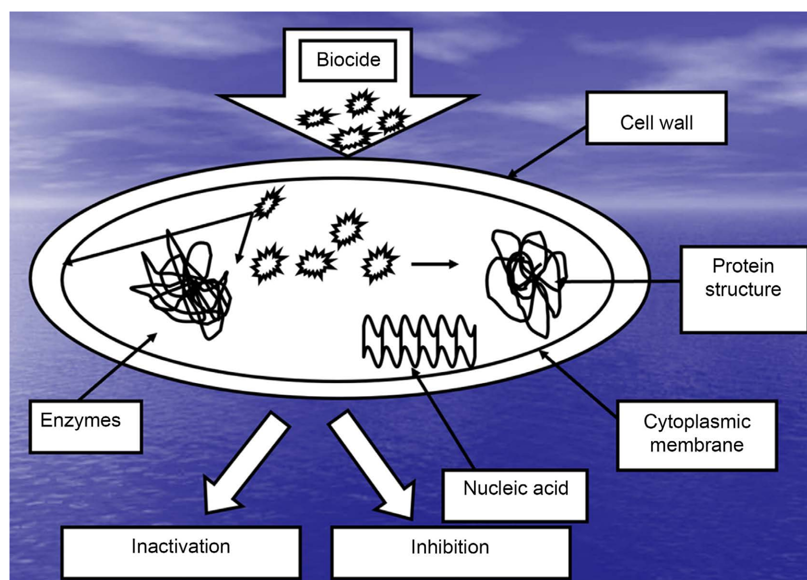

Figure 1. Targeted sites of biocides in microbial cells [37].

Table 1. Principal actions proposed to explain the deadliness of the electrochemical disinfection (ED) [37] [38].

Electrochemical Disinfection (ED) Tools	
Oxidants	Electric Field (EF)
Oxidative stress and cell loss of life.	1) Irreversible permeabilization of cell membranes. 2) Electrochemical oxidation of vital cellular constituents. 3) Electrosorption of negatively charged bacteria (e.g., *Escherichia coli* cells) to the anode surface + direct electron transfer reaction.

EC is the *in-situ* generation of coagulant by electrooxidation (EO) of a sacrificial electrode [39] [40] [41]. Fe and Al's sacrificial electrodes have been tried to reduce viruses [42] [43] [44] [45] [46]. In the EC process, in addition to the preceding paths (see **Figure 1** and **Table 1**), the bacteria can be inactivated due to the direct adsorption on the anode surface followed by electron transfer and physical elimination through floating pathogens with produced hydrogen gas and/or precipitating with the produced flocs [47] [48]. **Table 2** presents the detailed EC reactions of Fe and Al electrodes.

Table 3 shows a comparison among CC, EnC, and EC. Nowadays, coagulation could possess two versions: the first is accomplished by augmenting coagulant injection comparative to coagulation (optimized coagulation); the second is as for the first by augmenting coagulant injection with acidifying pH to attain more NOM elimination (EnC) [49]. EC injects coagulants electrochemically under an applied electric field (EF) [50] [51] [52]. When colloids exist single in the water, their elimination using coagulation is simpler than NOM (as the aquatic organic matter (OM) composition is very intricate [53] [54]). Also, when colloids and NOM exist together, their reduction becomes challenging because of the involved varying chemical reactions (**Table 3**).

Table 2. Electrocoagulation (EC) mechanisms using Fe (pH 2, 7, and 12) and Al (pH 7) electrodes [36].

Fe Mechanisms	Medium	Reaction	
Mechanism # 1 (pH 2)	Anode	$2Fe_{(s)} - 4e^- \rightarrow 2Fe^{2+}_{(aq)} \left(E° = +0.447\ V\right)$	(1)
		$2H_2O_{(l)} - 4e^- \rightarrow O_{2(g)} + 4H^+_{(aq)} \left(E° = -1.229\ V\right)$	(2)
	Solution	$2Fe^{2+}_{(aq)} + 4OH^-_{(aq)} \rightarrow 2Fe(OH)_{2(s)}$	(3)
	Cathode	$8H^+_{(aq)} + 8e^- \rightarrow 4H_{2(g)} \left(E° = 0.000\ V\right)$	(4)
	Total	$2Fe_{(s)} + 6H_2O_{(l)} \rightarrow O_{2(g)} + 4H_{2(g)} + 2Fe(OH)_{2(s)}$	(5)
Mechanism # 2 (pH 7)	Anode	$2Fe_{(s)} - 4e^- \rightarrow 2Fe^{2+}_{(aq)} \left(E° = +0.447\ V\right)$	(1)
		$Fe^{2+}_{(aq)} - e^- \rightarrow Fe^{3+}_{(aq)} \left(E° = -0.771\ V\right)$	(6)
		$Fe_{(s)} - 3e^- \rightarrow Fe^{3+}_{(aq)} \left(E° = +0.037\ V\right)$	(7)
	Solution	$2Fe^{2+}_{(aq)} + 4OH^-_{(aq)} \rightarrow 2Fe(OH)_{2(s)}$	(3)
		$2Fe^{3+}_{(aq)} + 6OH^-_{(aq)} \rightarrow 2Fe(OH)_{3(s)}$	(8)
	Cathode	$8H_2O_{(l)} + 8e^- \rightarrow 4H_{2(g)} + 8OH^-_{(aq)} \left(E° = -0.828\ V\right)$	(9)
	Total	$3Fe_{(s)} + 8H_2O_{(l)} \rightarrow Fe(OH)_{2(s)} + 2Fe(OH)_{3(s)} + 4H_{2(g)}$	(10)
Mechanism # 3 (pH 12)	Anode	$Fe_{(s)} - 3e^- \rightarrow Fe^{3+}_{(aq)} \left(E° = +0.037\ V\right)$	(7)
	Solution	$2Fe^{3+}_{(aq)} + 6OH^-_{(aq)} \rightarrow 2Fe(OH)_{3(s)}$	(8)
	Cathode	$8H_2O_{(l)} + 8e^- \rightarrow 4H_{2(g)} + 8OH^-_{(aq)} \left(E° = -0.828\ V\right)$	(9)
	Total	$2Fe_{(s)} + 6H_2O_{(l)} \rightarrow 2Fe(OH)_{3(s)} + 3H_{2(g)}$	(11)
Al Mechanism (pH 7)	Anode	$Al_{(s)} - 3e^- \rightarrow Al^{3+}_{(aq)} \left(E° = +1.660\ V\right)$	(12)
		$2H_2O_{(l)} - 4e^- \rightarrow O_{2(g)} + 4H^+_{(aq)} \left(E° = -1.229\ V\right)$	(2)
	Solution	$Al^{3+}_{(aq)} + 3OH^-_{(aq)} \rightarrow Al(OH)_{3(s)}$	(13)
		$Al(OH)^-_{4(aq)} \rightarrow OH^-_{(aq)} + Al(OH)_{3(s)}$	(14)
	Cathode	$8H_2O_{(l)} + 8e^- \rightarrow 4H_{2(g)} + 8OH^-_{(aq)} \left(E° = -0.828\ V\right)$	(9)
		$Al_{(s)} + 4OH^-_{(aq)} - 3e^- \rightarrow Al(OH)^-_{4(aq)}$	(15)
	Total	$2Al_{(s)} + 8H_2O \rightarrow O_{2(g)} + 5H_{2(g)} + 2Al(OH)_{3(s)}$	(16)

Waterborne viruses are usually less than 100 nm in diameter [5] [56], rendering them among the smallest particles to eliminate via coagulation [1]. Due to coagulation, viruses could be mostly eliminated physically, even if coagulation has been lately noticed to make viruses noninfectious (inactivation pathway) [1] [57].

Table 3. Major pathways for chemical coagulation (CC), enhanced coagulation (EnC), and electrocoagulation (EC) [53]).

Process	Pathway
CC (*coagulant injection*)	Colloids' occurrence: 1. Charge neutralization (CN) of the negatively charged colloids via adsorption of positively charged coagulant species. 2. Enmeshment of colloids in precipitated $Me(OH)_{3(s)}$ flocs (e.g., $Al(OH)_{3(s)}$, $Fe(OH)_{3(s)}$).
	NOM's occurrence: 1. Complexation of NOM with dissolved metal coagulant species (Al^{3+} or Fe^{3+}), conducting to direct precipitation of a $Me\text{-}NOM_{(s)}$. 2. Complexation of NOM with dissolved coagulant species, conducting to adsorption of such complexed material onto precipitated $Me(OH)_{3(s)}$. 3. Direct NOM adsorption onto precipitated $Me(OH)_{3(s)}$.
	Colloids & NOM occurrence: 1. Dissolved coagulant species existing upon coagulant addition. 2. Existence of precipitated metal hydroxide solids. 3. Concentration of particles and NOM. 4. Chemical features of such pollutants and their responsiveness with dissolved coagulant species. 5. Coagulation's pH is influenced by the coagulant's chemistry and the water's alkalinity.
Optimized coagulation (*augmented coagulant injection*)	Colloids' occurrence: 1. More CN of the negatively charged colloids via adsorption of positively charged coagulant species. 2. More enmeshment of colloids in precipitated $Me(OH)_{3(s)}$.
	NOM's occurrence: 1. More complexation of NOM with dissolved metal coagulant species, conducting to direct precipitation of a $Me\text{-}NOM_{(s)}$. 2. More complexation of NOM with dissolved coagulant species, conducting to adsorption of such complexed mater onto $Me(OH)_{3(s)}$. 3. More direct NOM adsorption onto the precipitated $Me(OH)_{3(s)}$.
	Colloids & NOM occurrence: 1. More dissolved coagulant species exist upon coagulant introduction. 2. More occurrence of $Me(OH)_{3(s)}$. 3. Concentration of colloids and NOM. 4. Chemical features of such pollutants and their responsiveness with dissolved coagulant species. 5. Coagulation's pH is influenced by the coagulant's chemistry and the water's alkalinity.
EnC (*augmented coagulant injection & acidified pH*)	Colloids' occurrence: 1. Most CN of the negatively charged colloids by adsorption of positively charged coagulant species. 2. Less enmeshment of colloids in precipitated $Me(OH)_{3(s)}$.
	NOM's occurrence: 1. Most complexation of NOM with dissolved metal coagulant species, conducting to direct precipitation of a Me-NOM(s). 2. Most complexation of NOM with dissolved coagulant species, conducting to adsorption of this complexed mater onto precipitated $Me(OH)_{3(s)}$. 3. Less direct NOM adsorption onto the precipitated $Me(OH)_{3(s)}$.

Continued

	Colloids & NOM occurrence: 1. More dissolved coagulant species exist upon coagulant addition. 2. Less existence of precipitated metal hydroxide solids. 3. Concentration of colloids and NOM. 4. Chemical features of such pollutants and their responsiveness with dissolved coagulant species. 5. The coagulant's chemistry and the water's alkalinity affect the acidified pH of coagulation.
EC (*coagulant injection & electric field* (*EF*))	1. Migration to an oppositely charged electrode (electrophoresis) and aggregation due to CN. 2. Cations OH^- form precipitates with pollutants. 3. Metallic cation interacts with OH^- to form a hydroxide, which has high adsorption properties, thus bonding to the pollutant (*bridge* flocculation). 4. Hydroxides form larger lattice-like structures and sweep through the water (*sweep* flocculation) [55]. 5. Oxidation of pollutants to fewer toxic species. 6. Removal by sedimentation or electroflotation and adhesion to gas bubbles.

This work discusses new investigations on virus reduction using coagulation techniques regarding the comprehension of virus sorption and demobilization. Firstly, the forces affecting virus sorption and successive physical elimination are reported, comprising electrostatic forces, the hydrophobic effect, steric hindrance, hydrodynamics, and cation bridging. Also, environmental matrix impacts, like those of OM and divalent cations, are described. After, the virus's demobilization incident through coagulation methods is detailed, comprising procedures and dares to assess virus demobilization exclusive of physical removal. New results are scrutinized to suggest recommendations for future study orientations for reducing waterborne viruses via coagulation, involving convenient choice and usage of virus surrogates.

2. Virions' Physical Removal

Physical removal is mainly considered the controlling form of virus reduction inside coagulation techniques [1]. Consequently, several sources do not differentiate between physical removal and overall virus mitigation [21] [58]. Heffron [1] outlined virus coagulation investigations and concluded that 1) CC has been depicted to lessen viruses by 0.5 to 7 $\log_{10}$ (*i.e.*, 90% to 99.99% reduction), with typical mitigation of around 3 $\log_{10}$. In cases of CC with post-treatment microfiltration (MF), virus levels decreased to 8 $\log_{10}$, with typical mitigation of 5 $\log_{10}$. 2) EnC decreased virus levels by up to 4.5 $\log_{10}$ [33] and 7 $\log_{10}$ [16] with post-treatment MF. Nonetheless, EnC coagulation has not been investigated rigorously like CC and is employed for more demanding water sources. 3) EC with post-treatment MF has depicted encouraging findings in reducing bacteriophage MS2, excelling the EPA's Surface Water Treatment Rule (SWTR) of 4 $\log_{10}$ mitigation of viruses [42] [44] [46] [59].

Physical integration of viruses into flocs most probably occurs in one of two routes: integration into developing flocs (CN or bridging between particles) and

adsorption to the surface of forming flocs (*sweep* flocculation) [1]. Shirasaki *et al.* [14] noted a modest attenuation (<0.5 $\log_{10}$) of poliovirus on the preformed slices, which contrasted with an attenuation of about 3 $\log_{10}$ throughout flocs generation. Moreover, Shirasaki *et al.* [60] discovered that two phages could be physically reduced through rapid mixing, with insignificant or no reduction over flocculation and following settling. Identically, Kreißel *et al.* [23] observed that notable virus reduction occurred only through flocs formation. Applying EC to kill viruses, Tanneru *et al.* [44] established that *sweep* flocculation is the main pathway for reducing viruses following fluorescence microscopy and virus recovery from flocs. Such a difference could be related to the distinction in flocculation between EC and CC. CC is restricted by reaction kinetics and produces dense flocs, whereas the diffusion of coagulating ions restricts EC and creates dense flocs [61]. Furthermore, the coagulant is constantly liberated through EC; thus, the phenomenon could not be divided into rapid mixing coagulation and flocculation phases [62]. Consequently, deciding if the virus is integrated into the developing flocs or adsorbed on the floc surface remains hard [1] [63].

Without coagulants, elevated virus levels can destabilize through environmental circumstances, producing aggregates. The aggregate generation is crucial in terms of theoretical and empirical points of view. Because of their critical size and lower surface charge, aggregates are more accessible to remove than monodispersed virions. Aggregates are more vulnerable to handling methods [64]. Aggregation frequently remains the consequence of laboratory techniques and does not certainly represent natural circumstances [1].

Many parameters affect the physical reduction of viruses by flocs, comprising electrostatic and van der Waals forces (which are modeled by Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory [65] [66]), besides non-DLVO parameters like hydrophobic effects [67], structural incompatibility between virus and adsorbent (steric hindrance), and interactions with each other (aggregation), and components in the aqueous matrix [1]. The effect of such parameters is a function of the virus itself (like its structure, surface charge, or permeability level) [68], the type of adsorbent (floc features), and the composition of the aqueous matrix. The influence of such factors on virus adsorption has been examined in more detail through porous media [69] [70] [71] [72] [73]. Nonetheless, many acquired considerations also apply to coagulation [1].

2.1. Electrostatic Interactions

Electrostatic forces can influence the adsorption of virions to surfaces such as flocs [74]. Researchers utilize various methods to estimate electrostatic forces [1]. The isoelectric point (IP) is the pH at which a particle or surface in an electrolyte solution has a neutral charge. If pH > IP, the surface is negatively charged; the surface is positively charged if pH > IP. Electrophoretic mobility (EM) is a measure of particle motion in the occurrence of an EF and can be employed to deduce the potential close to the particle surface. Electrostatic forces

generally control virion adsorption because of the long-range electrostatic effects and the low IP of most enteroviruses, suggesting a strong negative potential close to the particle surface at neutral pH. Nonetheless, EF shielding suppresses electrostatic forces at high ionic strength.

2.1.1. Virion Permeability Effect on Isoelectric Point (IP)

Studies are indecisive about whether the electrostatic charge of the virion is dictated exclusively by the capsid surface or whether the deeper capsid functional group and internal genomic compartments likewise influence the electrostatic interaction between the virion and its environment. Schaldach *et al.* [75] and Langlet *et al.* [76] developed models considering virion permeability. The Langlet *et al.* [76] model is built on the Duval and Ohshima [77] model of "soft" (permeable) colloids. Schaldach *et al.* [75] and Langlet *et al.* [76] models affirm that the acidic IP of the genome has a more significant effect on the overall IP as virion permeability augments [75] [76] [78]. To examine such a hypothesis, Langlet *et al.* [79] estimated phage MS2 and Qβ reduction rates on hydrophilic membranes. Both phages were identical in size and measured IP; nevertheless, Qβ had a larger genome. MS2 was mainly reduced. Langlet *et al.* [79] deduced that the genome of Qβ endows the virion with a more significant negative charge density, thereby repelling the membrane. Nevertheless, the distinction in reduction is not inevitably related to the difference in genome size. Qβ is more hydrophobic than MS2 [80], so less adsorption on hydrophilic membranes is awaited [1].

Bacteriophage MS2 was used as a specimen of the impact of the viral genome on the IP [1]. Based on the charged total capsid moieties, the theoretical IP of MS2 is around 7 - 9 [81] [82], whereas the IP of the ribonucleic acid (RNA) genome of MS2 is around 3 [81]. It is usually assumed that the measured MS2 IP is between 3 and 4, closer to the RNA IP than the capsid IP [83]. Employing a different procedure for calculating capsid IP, Penrod *et al.* [69] precisely estimated the measured IP of MS2 by estimating most of the charged structures exposed on the capsid surface. Schaldach *et al.* [75] proposed a better correlation with experimental EM data considering capsid permeability than the Penrod method.

However, Dika *et al.* [78] juxtaposed MS2 bacteriophage and virus-like particles (VLPs) and supported Penrod *et al.*'s model [69] for anticipating IP. VLPs are assembled by expression of the viral coat proteins in a bacterial host, even if they lack the viral genome. Instead of possessing an IP between 7 and 9, as anticipated, MS2 VLPs had a measured IP between 3 and 4. Dika *et al.* [78] supposed that negatively-charged host material was enclosed with the VLPs throughout propagation. Considering the intricate, optimized packing of the viral genome into the capsid throughout anticipated bacteriophage propagation [84] [85] and evidence from electron micrographs [78], VLPs probably do not hold sufficient host material in their interior to develop a negative charge density similar to whole virions. To adopt the explanation of viruses as soft colloids, we have to see at least some elevation in the IP of VLPs juxtaposed to bacteriophag-

es to mirror the genome effect. The permeability model could also be more viable for some virions than others [1].

2.1.2. Virion Permeability Effect on Electrostatic Interactions

Numerous scientists established that accounting for permeability did not yield better virion sorption or aggregation predictions. Gutiérrez *et al.* [86] found that the modeled permeability of rotavirus was sufficiently low that a hard colloid formalism would suffice. Yuan *et al.* [87] determined that the energy barrier to MS2 adsorption was better predicted by the DLVO model for hard (impermeable) colloids than when permeability was considered. Nguyen *et al.* [88] noticed that MS2 bacteriophages whose RNA genomes had been decomposed at high pH did not significantly differ from intact MS2 bacteriophages regarding aggregation or adsorption to the water surface at the air-water interface and concluded that internal RNA had minimal effect on sorption. Dika *et al.* [89] responded with an investigation illustrating that the virus purification method employed by Nguyen *et al.* [88] (*i.e.*, polyethylene glycol (PEG) precipitation) hides differences between viruses and VLPs.

Investigators also focused on the influence of the genome on virus-virus sorption phenomena in charge of aggregation. Dika *et al.* [89] noticed that MS2 aggregates produced at pH 4 did not re-disperse when the solution was acidified to pH 2. Different trials of MS2 aggregation employing pH titration underline such a trend [1]. On the other hand, MS2 VLPs aggregated only near the IP and dispersed at lower pH. The distinction in aggregation reversibility was supposed to be due to the attractive impact of the genome. Nonetheless, VLPs did not aggregate at any pH at high ionic strength, while entire virions did. At high ionic strength, the effective distance of electrostatic forces decreases; consequently, VLPs and entire virions must behave more likewise if permeability affects surface charge. Heffron [1] noticed that the MS2 and MS2 VLPs acted more likewise at low ionic strength. In addition, it remains ambiguous why the relative absence of RNA in VLPs would clarify aggregation [1]. In contrast, Dika *et al.* [78] used residual RNA in VLPs to demonstrate the VLP IP measured.

Different trials observed identical patterns of irreversible aggregation for somatic bacteriophages PRD1 and ΦX174 and F-specific bacteriophages Qβ and GA [89] [90]. Bacteriophages PRD1, Qβ, and GA all have measured IP between 2 and 4 reported in the literature [83]; thus, aggregation in such a pH span is not unusual. From an evolutionary standpoint, enteric viruses and bacteriophages may gain a selective advantage by aggregating to avoid inactivation by proteases in the stomach ($pH < 4$ [91]) and dispersing in the near-neutral pH of the intestines for the most excellent chance of infection [1]. In addition, the aggregation has been shown to inhibit virus inactivation by chemical disinfectants [92] [93].

Nevertheless, aggregation below pH 4 is unpredicted for ΦX174, which has a generally accepted IP of 6.6 from capillary isoelectric focusing and aggregation investigations [83]. If the IP of ΦX174 was more than pH 4, aggregation occurred only in a pH span where virions must possess a positive net charge. Re-

searchers [1] [72] lately mentioned lower IP for ΦX174 (4.4 and 2.6, respectively) founded on electrokinetic measurements.

2.2. Non-Electrostatic Sorption Phenomena

When electrostatic interactions are repulsive or neutral, van der Waals and non-DLVO phenomena (such as the hydrophobic effect and steric hindrance) and interactions with constituents in the water matrix could lead to dissimilarities in virion sorption. In addition, as discussed below, van der Waals and non-DLVO forces are inclined to notably change the impacts of electrostatic forces when electrostatic forces are reduced (e.g., by electrostatic screening or near the IP of the particle) [1].

Arising from electronic resonance between surfaces, van der Waals interactions form an attractive force proportional to the polarizability of the virion and the abiotic surface [1]. Van der Waals forces could not be measured independently of electrostatic and non-DLVO phenomena [90]. On the other hand, Armanious *et al.* [94] noticed a minimal effect of surface polarizability on bacteriophage adsorption. Nonetheless, the two surfaces juxtaposed also differed in hydrophobicity.

The hydrophobic effect emerges from hydrogen bonds that preferentially form between water molecules to exclude nonpolar molecules—the hydrophobic effect conducts to the tendency of nonpolar substances to partition out of the aqueous phase [1] [67]. Armanious *et al.* [94] discovered that the hydrophobic effect moderated electrostatic repulsion to permit the adsorption of bacteriophages fr, GA, MS2, and Qβ to nonpolar surfaces. Likewise, they proposed a manner for quantifying hydrophobicity founded on the size and number of nonpolar patches on the capsid surface. They anticipated a pattern of decreasing hydrophobicity: Qβ >> fr > GA >> MS2. Armanious *et al.*'s manner was qualified to clarify significant trends in bacteriophage sorption to hydrophobic surfaces, even if isolating the hydrophobic influence from other phenomena remains impossible. Different investigators [1] empirically suggested a relative hydrophobicity of GA > Qβ > MS2.

Investigators [89] noted that surface hydrophobicity could decipher dissimilarities in the sorption of bacteriophages. They juxtaposed the hydrophobicity of bacteriophages MS2, Qβ, and GA with known hydrophobic and hydrophilic surfaces. Hydrophobicity affected virus sorption to surfaces even in low ionic strength solution (1 mM $NaNO_3$), where electrostatic forces are anticipated to control [89]. Likewise, bacteriophages MS2, PRD1, and ΦX174 were juxtaposed. Despite varying charge densities among the three bacteriophages in low ionic strength electrolytes, Dika *et al.* [90] established identical EM at high ionic strength (100 mM). However, the bacteriophages diverged in their affinities for membranes of varying hydrophobicity. In addition, hydrophobicity has been established to affect virus sorption to finely powdered activated carbon positively [95].

On the other hand, the molecular-level structure of virus capsids and the sorbent surface can hinder virion adsorption at close range. Such a steric hindrance happens when interactions between the adsorbent and adsorbate are restricted by the spatial orientation of their molecular structures. Many scientists have established steric hindrance in virus sorption. For example, Penrod *et al.* [69] discovered that steric interactions (here considering all non-electrostatic repulsion to be steric) might conduct to increased MS2 mobility in porous media when electrostatic forces are screened (*i.e.*, at high ionic strength). Likewise, Armanious *et al.* [94] proposed that the variable topography of bacteriophage fr and MS2 capsids could have led to poor adsorption to a gold surface compared to bacteriophages Qβ and GA. Despite similar surface hydrophobicity, Dika *et al.* [89] found that bacteriophages preferentially sorbed to stainless steel over the glass. Such a tendency was more evident at high ionic strength, which conforms with the theory that surface roughness affects electrostatic interactions when the roughness is on a scale comparable to the Debye length (a measure of the effective range of electrostatic forces) [89]. As a rule, steric hindrance seemed to abate sorption in circumstances of identical electrostatic charge and hydrophobicity rather than primarily defining sorption behavior [1].

2.3. Influence of Water Matrix Composition on Virus Sorption

Suspended and dissolved matters in the water matrix (e.g., NOM and dissolved salts) could considerably influence virion sorption. Due to the heterogeneous charge distribution and polarity of OM in the aquatic medium, the impact of NOM on virus sorption implicates electrostatic forces, hydrophobicity, and steric interactions. Usually, NOM carries polar and nonpolar moieties and possesses a negative charge at neutral and high pH due to the deprotonation of carboxyl and phenyl groups [70] [94] [96]. In porous media filtration trials, investigators [70] observed that MS2 breakthrough was quicker in sorbed or dissolved OM; however, ΦX174 breakthrough was comparatively unaltered. They concluded that NOM competes for sorption sites on the media and improves the nonpolar virions' sorption by generating hydrophobic sites. Scientists [94] detected high sorption of bacteriophages GA and Qβ at pH 6 on a NOM-coated surface; however, MS2 and fr sorption was negligible. GA and Qβ sorption diminished considerably from pH 6 to pH 8, probably because of electrostatic repulsion emerging from the deprotonation of carboxyl groups on the NOM and capsid surfaces. When ionic strength was elevated from 10 mM to 100 mM to screen electrostatic forces, Qβ sorption was high even at pH 8; however, MS2 sorption was quantifiable, even so low. Such findings again depict that the hydrophobic effect controls only when electrostatic forces are weak. Investigators [87] noted that MS2 deposition on silica was more significant than on NOM-coated surfaces, even at ionic strengths high enough to screen electrostatic charges efficiently. They concluded that the findings could be related to steric hindrance, by which NOM surface structures avoid binding in contrast to the even surface of silica [1].

Deposition tests have illustrated that cation bridging may considerably augment virion sorption to like-charged surfaces [86] [97] [98]. In cation bridging, divalent cations (like Ca^{2+} and Mg^{2+}) form complexes with negatively-charged moieties on both the capsid and the solid surface [1]. The Ca^{2+} and Mg^{2+} occurrence significantly augments the sorption of viruses to repulsive surfaces in comparison to monovalent ions, beyond the expected increase due to the screening of electrostatic forces [86] [98]. On the other hand, rotavirus adsorption to an oppositely-charged (non-repulsive) surface was independent of Ca^{2+} or Mg^{2+} levels [86]. The influence of cation bridging may be notable at Ca^{2+} and Mg^{2+} levels typical of potable water sources [86]. For the bacterium *Pseudomonas aeruginosa*, cation bridging considerably improved sorption to repulsive surfaces at levels as low as 10^{-5} M Ca^{2+} or Mg^{2+} [1]. Interactions between MS2 virions have been depicted to transition from repulsive to attractive between 10 mM Ca^{2+} and 50 mM Ca^{2+} [97].

Ca^{2+} ions have been established to possess a more significant positive effect on virus sorption to repulsive surfaces than Mg^{2+} ions [86] [98]. Ca^{2+} ions are large and possess weakly bound hydration spheres, permitting inner-sphere complexation with carboxyl groups on the virus capsid and the solid surface [86] [98]. On the other hand, Mg^{2+} ions have tightly-bound spheres of hydration that could let only outer-sphere complexation. The mechanism for the relatively weak sorption in the occurrence of Mg^{2+} may not be bridging but rather CN by complexation with negatively-charged moieties on either the virion or the surface [98]. The potential to generate bonds with carboxyl groups renders cation bridging very significant in the sorption of negatively charged viruses to NOM [1]. Scientists [98] discovered that Ca^{2+} enhanced the deposition of MS2 on a NOM-coated silica surface to a far greater extent than on a bare silica surface. However, the bare silica was more negatively charged than the NOM-coated surface. For juxtaposition, employing NOM from the same source yet in a monovalent electrolyte, scientists [87] observed poorer adsorption of MS2 on a NOM-coated surface than on a silica surface. Investigators [97] observed that a lower level of Ca^{2+} was needed to destabilize MS2 in the occurrence of NOM (10 mg/L total organic carbon; TOC).

2.4. Consequences of Electrostatic and Non-Electrostatic Phenomena on Virus Aggregation

Electrostatic repulsion participates in virion stability; thus, aggregation usually happens at high ionic strength or pH domains near the virion IP [1]. Likewise, Non-DLVO forces could affect virus aggregation. Scientists have pointed out that protein loops extending from the capsid surface could increase virions' stability by steric hindrance [88] [97]. Virus aggregation is more considerable in the presence of the divalent cation, even if not in the regular Ca^{2+} and Mg^{2+} levels span in potable water [86] [88] [97]. Hydrodynamic forces could as well impact aggregation. Investigators proposed [1] suggested that the low EM of virion ag-

gregates can be because of hydrodynamic drag. Aggregates could display more significant hydrodynamic drag because of permeability. Due to such a drag, aggregates remain aggregated once constituted [1]. On the other hand, the hydrodynamic drag of individual virions due to capsid permeability could counteract the repulsive electrostatic forces of surfaces and neighboring virions, leading to aggregation.

2.5. Consequences of Electrostatic and Non-Electrostatic Phenomena on Coagulation

Although porous media investigations give worthy understandings, not all lessons could be presumed to be relevant to virus coagulation [1]. Unlike sorption to solid surfaces, coagulation could happen by sorption to solid flocs and complexation of the virion surface by dissolved coagulant (CN or inter-particle bridging). Also, metal oxide flocs diverge in structure, charge, and polarity from porous media. Deciding which parameters are necessary and sufficient to describe virion sorption during coagulation/flocculation remains hard.

Hydrophobicity is improbable to highly influence coagulation in several situations since $Al(OH)_{3(s)}$ and $Fe(OH)_{3(s)}$ are polar [1]. Nevertheless, in NOM, hydrophobicity could be a crucial partitioning factor for several viruses. Scientists [44] discovered that $Al(OH)_{3(s)}$ became more hydrophobic following the NOM sorption. Consequently, NOM could improve the sorption of very hydrophobic virions. Because of the irregular, fractal structure of $Al(OH)_{3(s)}$ and $Fe(OH)_{3(s)}$ [1], steric hindrance could likewise contribute to flocs' sorption.

In 2019, Heffron [1] noted that definite proof of the impact of divalent cations on virus sorption to metal hydroxide flocs (as opposed to electrostatically repulsive and nonpolar surfaces) does not exist. In 1958, investigators [99] concluded that Ca^{2+} and Mg^{2+} could have inhibited virus mitigation. Nonetheless, the juxtaposition was performed between trials utilizing synthetic *vs.* raw water sources; therefore, the dissimilarity in virus reduction could not be conclusively ascribed to divalent cations, as opposed to, e.g., NOM. Investigators [100] depicted that alum coagulation of bacteriophage T4 was unaffected by Ca^{2+} and Mg^{2+} levels up to 330 mg/L as $CaCO_3$, even if they employed synthetic water free of NOM. Microbalance tests of virus deposition on $Al(OH)_{3(s)}$ and $Fe(OH)_{3(s)}$ surfaces, identical to those performed on silica and NOM-coated surfaces, may better recognize the significance of surface charge, hydrophobicity, and roughness, as well as divalent cations and NOM levels [1].

3. Demobilization through Coagulation Techniques

Besides physical removal by sorption and co-precipitation, many investigations have focused on bacteriophage demobilization by coagulation techniques [1]. Viruses can be demobilized by damage to the virion protein capsid and the viral genome. Damage to viral proteins emerges as an inability of the virus to attach to the host cell and inject the genome. In contrast, genomic damage stops the

replication and proliferation of the virus in the host [101]. If viruses are physically eliminated or demobilized remains a challenging question. Coagulation techniques augment the formation of sludge that must be correctly treated. If high degrees of virus demobilization could be attained, sludge treatment would be safer and more cost-effective. Secured sludge treatment stays severe for decentralized water treatment; thus, we should be convinced that coagulation does not just gather pathogens.

Confirmation of demobilization has been reported for both CC and EC [1]. For Al coagulants, polynuclear Al_{13} and Al_{30} species are supposed to oxidize virions chemically [23] [102]. Whereas soluble, monomeric aluminum species are mainly anionic above pH 6 [1], soluble Al_{13} and Al_{30} species are cationic near neutral pH [23] [102]. As most virions possess negative surface charges [83], the polynuclear cations could interact with and oxidize virions more than monomeric anions. PACls form more polynuclear hydrolytes in solution than simple aluminum salts [13] [102].

Similarly, the most proof of virus demobilization has been noticed with PACl coagulation [1] [13]. Coagulation with usual Al and Fe salts (like $Al_2(SO_4)_3$, $AlCl_3$, $FeCl_3$, and $Al(NO_3)_3$) has depicted only restricted virus demobilization [1]. Polynuclear iron coagulants have equally been suggested; however, such coagulants have not been assessed for reducing viruses [1].

Like in sorption investigations, numerous scientists noted that CC demobilization happened simultaneously with floc generation, with weak to no demobilization when viruses were spiked in a solution with pre-formed flocs [1] [23] [25]. However, scientists [23] observed that demobilization was more considerable when viruses were exposed to soluble PACl at pH 4.5, demonstrating that demobilization could be linked to soluble species rather than insoluble flocs. Therefore, different investigators have proposed alternate demobilization pathways, like the deformation of virions by forces at the interphase boundary [103] and inhibition of infection by irreversible adsorption of coagulant polymers to the capsid surface (like at binding sites) [22].

EC has been established to kill algae and bacteria, but scientists' findings frequently do not distinguish between physical removal and demobilization [47] [104] [105]. Some investigations have been dedicated to reducing viruses using EC [33] [42] [43] [44]. In EC, disinfection happens by oxidizing chloride to free chlorine [43] [106] [107]. For Heffron [1], EC has only been depicted to demobilize viruses in the occurrence of Cl^-. Since the production of free chlorine is an oxidative process at the anode [108] [109], chlorine generation is a secondary and competing reaction to the oxidative dissolution of the anode itself. Investigators [43] observed that bacteriophage demobilization needed a prohibitively long residence period because of the minimum levels of free chlorine produced in their case, *i.e.*, <0.1 mg/L. In addition, incorporation in flocs protects viruses from demobilization by free chlorine [43].

Scientists [43] observed damage to the MS2 genome and proteins following EC with expanded treatment periods. They noticed conformational alterations

to proteins and an elevation in the level of protein oxidation by-products using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Likewise, they utilized quantitative reverse transcription polymerase chain reaction (qRT-PCR) amplification to examine damage to the MS2 genome directly [43]. They amplified a 77 bp section of the maturation protein coding region [110] and compared it between treated and initial samples [43]. The short length of the amplicon likely makes this method a conservative indicator of total RNA damage. The same scientists [43] noticed a fast decrease in copy number during the first sixty minutes of EC, similar to the decline observed when utilizing a culture-based plaque assay. They did not observe augmented genome demobilization with the more extended residence period, even if overall demobilization continued to increase. Such findings propose that for EC, the pathway of demobilization could change during application. Changing demobilization pathways could be at play, even for disinfectants [1]. Investigators [101] detected that free chlorine attacked MS2 proteins and genome, and demobilization occurred as an inability to inject the viral genome into the host cell. Their procedure could present a less conservative approximation of RNA damage since about half of the viral genome was analyzed.

Because of the dissimilar supposed demobilization pathways (*i.e.*, generation of free chlorine during EC *vs.* polynuclear cations in CC), Tanneru *et al.*'s [43] outcomes could not be expanded to CC. Investigators [101] found that chemical oxidants (*i.e.*, O_2, Cl_2, ClO_2) differ in their demobilization pathways. Also, the demobilization pathway probably varies between free chlorine and the large polynuclear cations hypothesized to be in charge of demobilization due to CC (like Al_{13} and Al_{30}). Employing a procedure identical to that of researchers [43] [101] would lead to explaining the demobilization pathway by CC. As an introductory assumption, polynuclear aluminum species may broadly attack capsid surface proteins since size and charge would restrict access to the internal structure (particularly juxtaposed to Cl_2) [1].

The following sections examine obstacles in estimating virus demobilization at the lab level. Because of the price and duration of cultural assays, molecular methods emerge as an exciting choice. Nevertheless, investigation is needed to establish the validity of molecular methods for quantifying demobilization [1]. Likewise, a given degree of demobilization could be significant to treatment efficacy but still hard to quantify because of its comparative insignificance juxtaposed to different treatment fates. Virus aggregation also frustrates attempts to quantify demobilization, and no satisfactory method is available to ensure against aggregation of treated samples.

3.1. Quantification of Virus Demobilization

Measuring virus demobilization constitutes an empirical defy [1]. Scientists [22] [25] [43] used a cultural plaque assay to express the number of infectious viruses in suspension and those sorbed to solids employing a recovery protocol. Such a

procedure could be considered a "plaque-forming unit (PFU) balance." Viruses are sampled in the supernatant following gravitational separation and recovered from the floc. The total virus recovery is juxtaposed to the untreated control sample to calculate demobilization. A PFU balance differs from a mass balance since it is a discrete count of PFUs, not a continuous measure of mass. Like any plaque assay, the PFU balance may distinguish between infectious and inactive viruses, not between a single virus and an aggregate [1]. Furthermore, juxtaposing recovered PFUs with the initial concentration permits the evaluation of virus demobilization within the expected method recovery efficiency [43]. Nevertheless, the PFU balance's procedure needs twice the number of plaque assays to analyze the concentrations of viruses in suspension and adsorbed to flocs, augmenting cost, and time inputs.

Other investigators [13] [14] [23] [60] [103] employed qPCR (qRT-PCR for RNA viruses) to contrast copy number decreases with PFU counts. Juxtaposed with plaque assays, qPCR is somewhat fast, and aggregation does not influence qPCR findings. Oppositely to plaque assays, qPCR estimates the total number of intact viral genomes in the sample, in any case, infectivity, permitting a comparison between plaque assay and qPCR findings to evaluate complete demobilization. Nonetheless, there are many worries with juxtaposing molecular and cultural techniques. Because of genome damage, molecular procedures cannot distinguish between physical elimination and demobilization [1]. The copy number of even short amplicons could lessen throughout demobilization [43]. During estimating chemical oxidation using dissolved PACl, researchers [23] likewise noticed a reduction in copy number (around 1 $\log_{10}$ from the initial concentration). However, if the depressed recovery is an artifact of the procedure or indicative of genome destruction remains ambiguous. Such a decrease in copy number during coagulation research would be indiscernible from a lowering because of physical elimination, as depicted in **Figure 2**. Consequently, qPCR could amplify the physical removal significance and minimize that of demobilization.

Identically, a few parts of demobilized viruses are possibly retained with the flocs and thus considered as physical removal. Demobilized viruses can unexpectedly be disproportionately eliminated. Destruction of viral proteins could considerably modify virion structure and genome packing [111]. The influence of morphological alterations on sorption could not be minor. If infectious viruses are more easily retained in flocs, then qPCR analysis of treated water would be suitable for assessing total viruses (infectious + inactive). If inactive viruses are easily included in the floc phase, qPCR analysis would once more systematically underreport demobilization [1].

Researchers [60] examined the liquid phase and dissolved floc of treated water using qPCR and plaque assay (essentially performing both a PFU balance and a copy number balance). Despite significant decreases in amplicons in the liquid phase, they recovered roughly all MS2 and Qβ amplicons from the floc. The elevated recovery designates that MS2 and Qβ demobilization by genome destruction was below detection [60]. Such absence of genome demobilization may mirror

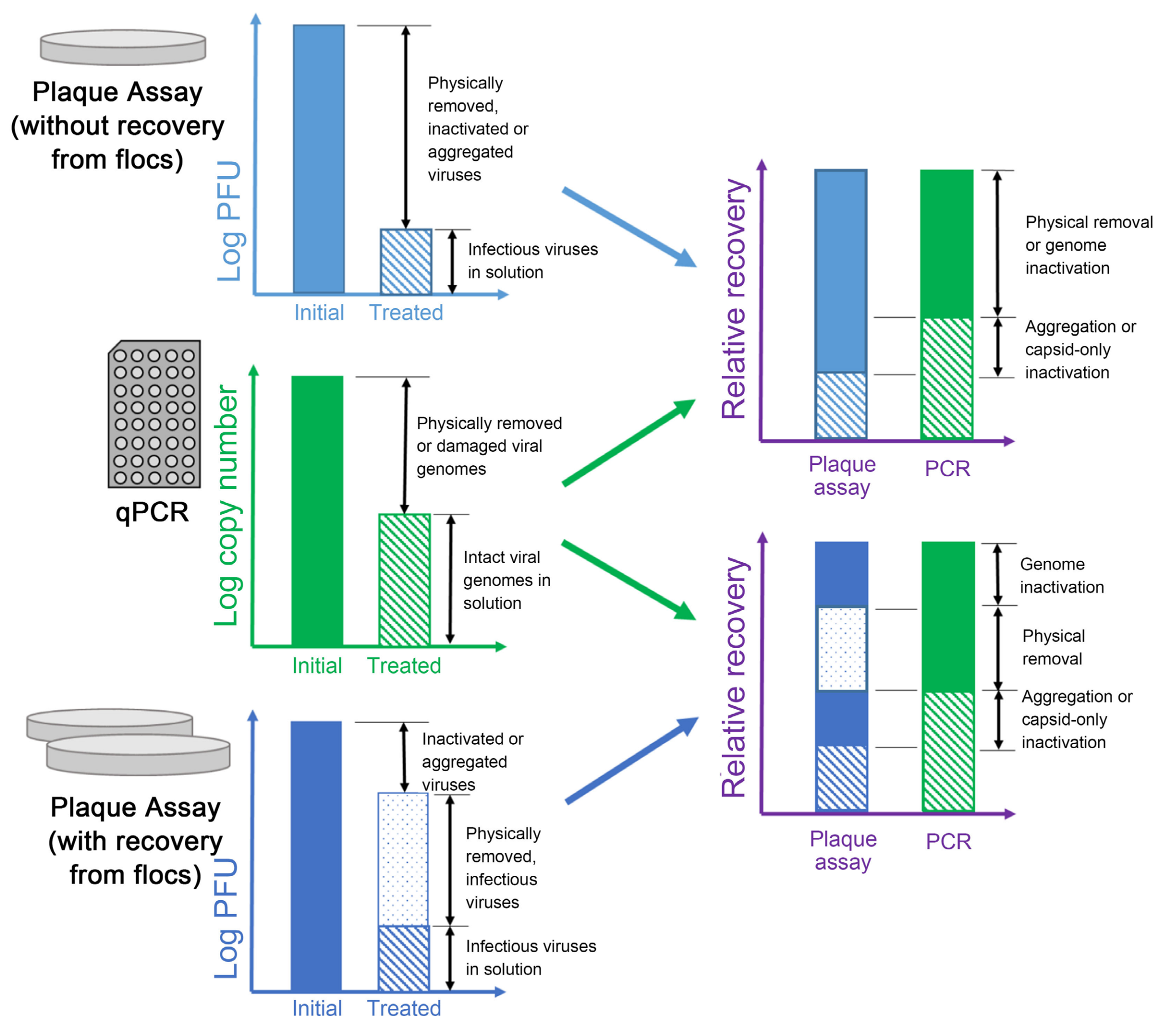

Figure 2. The most comprehensive theoretical categorization of probable behavior employs three quantification procedures alone and in integration. The relative values depicted in stacked columns were chosen only for visual clarity. Resolving numerous such amounts could be practically out of the question since amounts could vary in value and by several orders of magnitude [1].

the demobilization pathway of PACl. Nonetheless, Qβ demobilization was evident in the floc and liquid phases, showing the retention of demobilized viruses in flocs. For both bacteriophages, a more considerable disparity between molecular and cultural quantification was noticed in the liquid phase than in the floc phase. The more noticeable difference in the liquid phase may be related to aggregation, mainly considering that the liquid phase was only centrifuged (2000 × *g*, 10 min), not dissolved and agitated for resuspension like the floc phase [1]. Nonetheless, such findings [60] propose that genome demobilization could not considerably affect qPCR findings for several bacteriophages and treatment techniques. On the other hand, employing qPCR without recovery from flocs could under-represent demobilization related to the sorption of demobilized viruses in the floc, like in the situation of Qβ. Following the appointed study remains needed to determine a firm methodological basis before utilizing qPCR and plaque assays without recovery from flocs.

The scope of data that could be acquired utilizing the quantification procedures examined above is recapitulated in **Figure 2**. Integrating plaque assay with recovery from flocs furnishes the most detailed account of virus fate; nonetheless, one or more of such fates are possibly to be undetectable in practice. A plaque assay with recovery likewise gives more pertinent data (*i.e.*, the level of infectious viruses in the sludge) than qPCR and plaque assay without recovery [1]. If several fates could be seen as inessential for a specific usage (esp., demobilization related to genome damage), integrating qPCR and plaque assay without recovery would be comparable to plaque assay with recovery.

3.2. Detecting Low Degrees of Demobilization

Dealing with increased degrees of virus lowering (*i.e.*, logarithmic representations are usual) likewise constitutes a problematic question. Whether quantified by molecular or cultural methods, demobilization is assessed by subtracting a concentration of recovered PFUs or amplicons from an initial higher concentration of several orders of magnitude. As the error of each quantity is relative to the concentration, demobilization can only be assessed to a statistical degree of certainty when inactivation is a primary reduction mechanism, as illustrated in **Figure 3** [1].

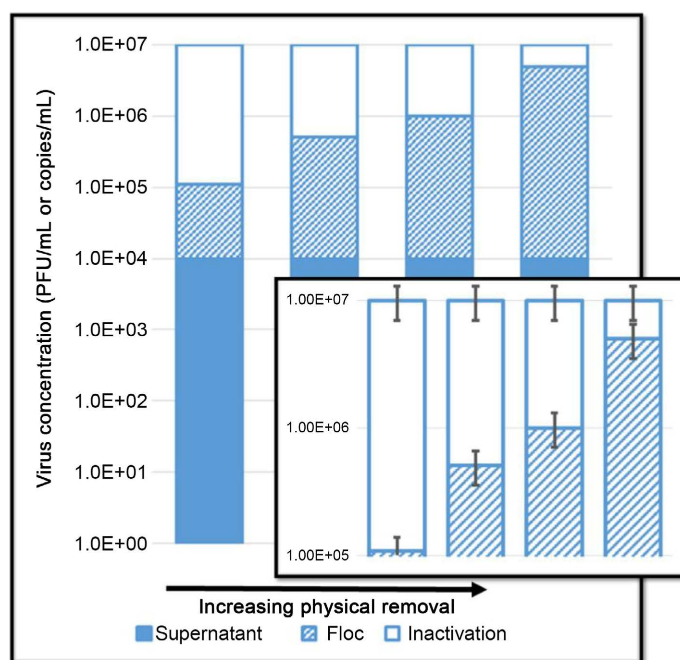

Figure 3. Certainty in estimating decreasing quantities of demobilization. In such a hypothetical case, the recovery of bacteriophages from the supernatant stays constant for all bars (104 PFU/mL); however, the number of bacteriophages recovered from the floc augments from 10^5 to 5×10^6 PFU/mL. The standard error of the mean for all measurements is set as 30% (error bars, inset). The amount recovered from the supernatant does affect demobilization. Nonetheless, as demobilization decreases to near 0.5 log10 reduction, the certainty intervals begin to overlap, and demobilization cannot be distinguished from the analytical uncertainty [1].

Also, demobilization is commonly seen as independent from physical removal rather than additive. If demobilization works as a polishing step, small numbers of demobilized viruses could have a considerable influence. For instance, if an additional 0.09% of the initial virus concentration is demobilized beyond the 99.9% that can be retained in flocs, that minimal reduction signifies the difference between satisfying the EPA's SWTR requirements and not [59]. The restricted data obtainable advocates demobilization might have such a polishing impact [1]. As debated in Section 3.1, scientists [60] noticed more significant demobilization of MS2 and Qβ in the liquid phase than in the floc phase, which significates that demobilization may participate in virus removal beyond the potential of physical removal alone (*i.e.*, demobilized viruses would not necessarily have been physically retained were they not demobilized). After physical retention, demobilization's "polishing" impact would greatly diminish the level of viruses remaining in the treated water. Nevertheless, such a modest virus removal would be lost on the scale of the original spiked concentration. Consequently, even if crucial for disinfection, the degree of demobilization would be hard to recognize practically. If the demobilization cannot be precisely determined, demobilization parameters cannot be adjusted.

3.3. Assessment of Virus Aggregation

As illustrated in **Figure 2**, aggregation stays quantitatively indiscernible in all methods from at least some demobilization—aggregation guides to artificially low plaque counts since each originates from numerous viruses instead of one [1]. Founded on aggregate size, researchers [112] observed that aggregation could be responsible for more than 4 $\log_{10}$ lowering in PFUs (from an initial concentration of around 10^{11} PFU/mL). Nevertheless, extra control could furnish some "insurance" against aggregation for the plaque assay with the recovery method. The efficiency of the recovery method could be examined below parameters of minimal demobilization (like adsorbing viruses on pre-formed flocs or quenching oxidants with sodium thiosulfate). The recovery efficiency depicts those viruses can be recovered from the flocs and that the viruses in the treated water are no more aggregated than in the initial virus suspension. Scientists [60] [78] have likewise relied on electron micrographs to illustrate the occurrence or absence of aggregates qualitatively.

Dynamic Light Scattering (DLS) is a procedure for estimating the electrokinetic response of colloids and the size distribution of particles in suspension. Several investigations [23] [60] [90] [103] [112] employed DLS analysis to regulate circumstances where virions aggregate. Nevertheless, DLS analysis needs very high virus concentrations (>10^9 PFU/mL) [43]—higher than even the usual spiking concentrations utilized for testing (commonly 10^7 - 10^8 PFU/mL). Consequently, scientists cannot directly evaluate aggregation in the same samples to be tried by plaque assay and/or qPCR. As an alternative, investigators should attempt to establish whether or not aggregation happens in circumstances identic-

al to those tried [1].

An important volume of virus stock suspension is requisite for DLS to reach the needed concentrations. Such stock suspensions could possess higher ionic strengths and diverge considerably in composition from natural waters. Electrolyte composition may greatly influence electrokinetic responses such as aggregation [83]. Aggregation is more considerable in phosphate-buffered saline (PBS), which is usually utilized for virus stocks, than in deionized water or bicarbonate solution [87]. Ideally, virus stocks must be purified and spiked into the identical water matrix employed for coagulation tests. Nonetheless, the procedure of virus purification could likewise considerably impact virion characteristics. As discussed above, scientists [89] juxtaposed three approaches of MS2 purification: PEG precipitation, successively dialyze in deionized water and 1 mM $NaNO_3$, and ultracentrifugation in a CsCl gradient. PEG precipitation led to a larger hydrodynamic radius of unaggregated viruses, with aggregation detected at pH 6. Dialysis led to aggregation at pH 4; however, viruses separated in a CsCl gradient did not aggregate at any pH. Each procedure possesses disadvantages: PEG seems to adhere to the capsid surface, dialysis retains viral and non-viral particles based only on membrane exclusion, and cesium ions may permanently deform protein structures [89]. Nevertheless, the test does not define which purification best approximates virus behavior in the environment [1].

3.4. Demobilization via Ferrous Iron Oxidation

Coagulation techniques implying zero-valent or ferrous iron possess the gathered complexity of redox reactions. Different from Al, Fe has several stable valence states. The products generated by mixed-valent iron precipitation change from ferrous minerals (e.g., green rust and magnetite) to ferric minerals (e.g., ferrihydrite, lepidocrocite, and goethite) [113] [114] [115] [116]. The unique precipitation products generated are a function of the degree of the ions in suspension; however, the oxidative circumstances finally control the valence state of iron [114] [115] [116]. Fe oxidation via dissolved oxygen (DO) can catalyze the oxidation of other metals and organic compounds [1] [117] [118] [119]. Ferrous oxidation has also been examined for disinfection implementations [120] [121]. Therefore, one possible pathway of virus reduction via ferrous chloride coagulation/iron EC is demobilization due to ferrous iron oxidation.

Researchers [120] [122] established virus demobilization using iron oxidation due to Fenton's reagent and ferrous/zero-valent iron alone. Disinfection was more significant at pH 5.5 - 6), possibly related to an augmented residence period with ferrous ions and more excellent radical oxygen species formation. They observed greater MS2 abatements employing zero-valent iron nanoparticles than ferrous ions, and the nanoparticles were less dependent on DO. They supposed that viruses might have been demobilized by surface interactions without oxygen to form hydrogen peroxide, leading to Fenton-like reactions [1] [120] [123].

Employing enzyme-linked immunosorbent assay (ELISA) to reveal a reduc-

tion in antigenicity and qRT-PCR to reveal chromosomal destruction, researchers [120] discovered that capsid deterioration (lower antigenicity) was a route of MS2 disinfection for ferrous ions. At the same time, qRT-PCR did not detect genomic harm. Zero-valent nanoparticles were observed to demobilize viruses by both capsid and genome destruction. Even if the investigation utilized ELISA and qRT-PCR besides cultural procedures to emphasize virus demobilization, the participation of virus aggregation in log lowering in PFUs could not be completely ruled out since the MS2 phages were not eluted following treatment. Also, regardless of being adopted mainly as a surrogate virus for filtration treatment units, MS2 could be more vulnerable to some forms of disinfection than other likely bacteriophage surrogates for human viruses of interest [1] [124].

3.4.1. Formation of Intermediate Oxidants through Ferrous Iron Oxidation

The routes of virus demobilization by Fe oxidation remain efficiently known. Nevertheless, learnings could be drawn from the investigations on iron oxidation of chemical species. Fe-based oxidation possesses an extensive record in the Fenton process, which utilizes ferrous iron and hydrogen peroxide at pH ≈ 3 to produce oxidants [1]. The emergence of reactive oxygen species (ROSs) using Fe via the Fenton process has been well-detailed [125] [126] [127]. Paradoxically without introducing hydrogen peroxide, oxidation of zero-valent iron by DO has been depicted to produce Fenton's reagent (Fe(II) and H_2O_2), as well as ROSs related to the Fenton reaction, like hydroxyl- ($^{\bullet}OH$) and superoxide ($^{\bullet}O^{2-}$) radicals [119] [120]. The apparent resemblance between the Fenton reaction and autooxidation of ferrous iron without the occurrence of hydrogen peroxide is helpful as far more research has been dedicated to the former. Nevertheless, learnings drawn from the Fenton reaction should be implemented carefully for the oxidation of Fe by DO because of the presence of hydrogen peroxide in the Fenton reaction.

The Fenton reaction is usually ineffective close to neutral pH [119]. However, scientists [119] [128] [129] have elucidated the oxidative impacts of zero-valent and ferrous iron close to neutral pH. Around neutral pH, oxidant production emerges essentially from the oxidation of ferrous iron by DO rather than zero-valent iron to ferrous iron [118]. Oxidation at circumneutral pH is frequently related to the generation of ferryl ions ($Fe^{IV}O^{2+}$) [129] [130]; however, the subject persists to be a matter of debate [1] [128].

Ferryl iron is an unstable intermediate of ferrous oxidation [117], with an oxidation potential of about 1.4 V for the Fe^{3+}/Fe^{4+} couple [131]. As early as the 1930s, ferryl iron was supposed to decompose hydrogen peroxide in the Fenton process [130]. However, since ferryl species are ephemeral, direct Fe(IV) detection constitutes experimental defies. The gold standard of high-valent detection, Mössbauer spectroscopy, demands fast freeze-quenching of samples before analysis in liquid nitrogen or helium [1] [132]. Scientists [132] asserted via Mössbauer spectroscopy that the oxidation of ferrous ions through ozone formed

$[(H_2O)_5Fe^{IV}{=}O]^{2+}$ in acidic solutions and that the primary, intermediate oxidant at pH 1 was $^{\bullet}OH$. Since samples must be frozen for milliseconds to quantify iron (IV) and (V), trials are usually performed at low temperatures not representative of standard conditions [1].

Efforts have also been performed to define oxidant species produced by the Fenton reaction or iron autoxidation employing specific organic probes. Both the ability of the probe to quench oxidation and the oxidation by-products produced can assist in identifying the occurrence of known oxidants. Nonetheless, such procedures can only infer the identity of new oxidants (like ferryl species). Superoxide dismutase, an $^{\bullet}O_2^-$ scavenger, has been discovered to impede the slow phase of the Fenton reaction, in which oxidants are only generated by regenerating iron (II) from iron (III). Nevertheless, superoxide dismutase does not impede the initial, rapid reaction caused by the initial oxidation of ferrous ions [122]. Consequently, superoxide is not a relevant oxidant to ferrous oxidation at neutral pH [1].

At low pH, $^{\bullet}OH$ is the primary oxidant generated; however, $^{\bullet}OH$ is not an essential intermediate of Fe oxidation above pH 5 [118] [119] [120]. By employing a spin quencher with electron spin resonance (ESR) spectroscopy, scientists [129] noticed that $^{\bullet}OH$ decomposition products were generated in the Fenton technique at pH 7.4; however, not in the autooxidation of ferrous iron (without the presence of hydrogen peroxide). Oxidizing zero-valent iron depicts variable quenching with selective $^{\bullet}OH$ probe compounds at high and low pH [119] [128] [133], giving more proof of a switch to an oxidant other than $^{\bullet}OH$ [1].

Researchers [125] developed a model by which ferryl iron emerges as the central intermediate in the Fenton technique around neutral pH 6 - 7. Such a model has been founded on a pH-dependent change in dimethylsulfoxide (DMSO) oxidation by-products; however, oxidation related to $^{\bullet}OH$ at low pH was supplanted by a shorter-lived, less reactive oxidant at high pH. Nonetheless, the half-life of ferryl species is commonly seen to be much longer (on the order of seconds) than that of $^{\bullet}OH$ (on the order of nanoseconds) [1] [134]. Scientists [128] reached the opposite deduction that ferryl iron is not an intermediate oxidant in the Fenton reaction at neutral pH, considering the failure of zero-valent iron species to form appropriate by-products in the occurrence of a methyl phenyl sulfoxide probe.

One potential cause for the large conflict between investigations is that few empirical perturbations could influence Fe oxidation products. Even the kind of intermediate oxidant formed may be affected by the composition of the water matrix. As an illustration, scientists [125] observed that $^{\bullet}OH$ was formed via the Fenton technique in phosphate buffer from pH 6.1 to 8 yet not in amine buffers over the identical pH span. Researchers [133] noted that arsenic oxidation via the Fenton technique augmented with the bicarbonate concentration. In the OM presence, organic radicals could be produced instead of $^{\bullet}OH$ [1]. Besides, only modifying the rate of ferrous addition to the system could greatly change the yield of oxidation by-products [125].

3.4.2. Sequential Fe Electrocoagulation (EC) – Electrooxidation (EO)

Iron EC is a developing technique to which ferrous-founded disinfection could contribute [135] [136] [137]. A sacrificial, zero-valent iron electrode is oxidized in iron EC by passing an electric current across the cell [138] [139] [140]. Fe is liberated into the solution as ferrous cations [1], further oxidizing in DO [113] [117] [141]. The Fe precipitates as solids, such as green rust and magnetite in anoxic conditions or lepidocrocite in oxygenated conditions [113] [115]. EC has first been viewed for the physical elimination of pollutants comprising viruses. Nonetheless, arsenite oxidation via EC has been reported [117]. Demobilizing bacteriophage MS2 via iron EC has likewise been suggested, even if the pathways and implementation are not explored [1] [46].

EO utilizes non-sacrificial electrodes to oxidize pollutants via two likely pathways: the formation of oxidants in solution (indirect oxidation) and electron exchange at the electrode surface (direct oxidation) [109] [142]. Disinfecting electrochemically using EO has been tested broadly for bacteria [143] [144] [145], even if less interest has been in killing viruses employing EO [146] [147] [148]. Viruses could be more rebellious to electro-disinfection (ED); bacteriophage MS2 and recombinant adenovirus depicted poorer reduction than *E. coli* [149] and *Enterococcus* in a toilet-water ED setup featuring a semiconductor anode [148]. Consequently, reducing viruses using EO constitutes a sensible lack in the ED literature [1].

Boron-doped diamond (BDD) electrodes are frequently utilized in EO experimentation since they are highly resistant to chemical and thermal degradation [150] [151]. Likewise, BDD possesses a wide solvent window, signifying that the electrode reacts with solvents only at high positive and negative electrode potentials. Especially for electroanalytical methods, BDD has a high oxygen (O_2) overpotential that helps in reversible cyclic voltammetry. For water treatment, the elevated O_2 overpotential implies that oxygen formation competes for less with the anodic oxidation of pollutants [1].

ED using BDD EO usually happens thanks to generating ROSs from DO or free chlorine and chlorine dioxide from chloride [152] [153]. In the absence of chloride, $^{\bullet}OH$ is the key oxidant species [154]. Numerous investigators [152] [153] [155] [156] noted that chloride boosts BDD ED, sowing that chlorine formation yields more excellent disinfection than ROSs alone. In addition, chloride has been observed to augment ROSs produced by BDD EO [152]. As ROSs are short-lived, oxidation occurs mainly at the electrode surface [154] [157]. Consequently, microorganisms should be transported to the electrode surface for successful killing. Transport can happen via either electrophoresis or convection/diffusion. Electrophoresis is the movement of charged species in an applied EF [50] [51] [62]. While charged pollutants are subject to electrophoresis and convection, uncharged pollutants should only be transported from the bulk solution to the electrode surface by diffusion [1].

EO and EC possess an elevated possibility of harmonizing techniques; both require electrical power with compactness and portability. Besides, the occur-

rence of residual iron due to EC can improve oxidation via EO. Investigators noticed that ferrous-catalyzed zonation is more performant than ozonation single in oxidizing organic contaminants and chemical oxygen demand [158] [159]. Further, scientists observed that ferric iron has identical, even if probably lesser, catalytic impacts for ozonating organic pollutants [1] [159] [160]. Even though disinfection researches employing iron-enhanced oxidation stay rare, researchers [161] discovered that TiO_2 photocatalysis attained an additional 2 $\log_{10}$ reduction of MS2 when augmented with 2 μM ferrous sulfate. An oxidation process (like ozonation) may also regulate Fe oxidation to increase disinfection and reduce soluble Fe residuals. Moreover, EC efficiently reduces NOM and turbidity [162] [163] [164]. Consequently, EC may work as a pretreatment step for EO by eliminating NOM and turbidity, thus decreasing the oxidant demand. In addition, acidifying water to pH 4 - 5 before EC can reduce NOM by boosting CN [165] [166].

4. Conclusions

At this level in the coagulation survey, viruses can no longer be supposed to be inert nanoparticles. Instead, the complexity of viruses as bioparticles and the process of virus demobilization should be adopted. However, the contribution of permeability in virus sorption and aggregation stays precisely ambiguous. The information about virion permeability has been evaluated by interpreting empirical electrophoretic mobility (EM) [81]. Heffron [1] concluded that no practical measures of virion permeability exist, a clear link between permeability and virion composition and morphology has not been advanced, and the direct influence of inner virion structures on surface charge or sorption has not been conclusively demonstrated.

Non-DLVO forces should also be considered to interpret and anticipate virus sorption conduct. Hydrophobicity stays a substantial contributor to sorption, particularly for nonpolar virions. Different details, like steric interactions and hydrodynamics, possibly have a crucial contribution when electrostatic forces are repulsive or minimal (e.g., at high ionic strength or near the virus or floc IP). Also, the composition of the water matrix has possibly an active contribution to numerous viruses. NOM could be involved in sorption sites on flocs when repulsive electrostatic charges govern NOM-virion interactions. NOM can work as a sorbent to boost the flocculation of hydrophobic virions. Ca^{2+} and Mg^{2+} increase the sorption of viruses to similarly-charged species like NOM, either by cation bridging or surface complexation. Sorption varies by both virion and environmental conditions [1].

In coagulation technologies, the capacity for demobilization stays a promising approach for water treatment experimentation. Demobilization muddles unit treatment efficacy testing with artificially high reduction rates. Nonetheless, future coagulation setups may be adjusted for demobilization. Coagulation setups utilizing zero-valent or ferrous iron could be killed by iron oxidation, with poss-

ible usages in methods such as electrocoagulation (EC) and electrooxidation (EO). The oxidants evolution in the iron oxidation method has depicted promising findings in demobilizing bacteriophage MS2, even if follow-up investigations employing an elution method are needed to secure that bacteriophage elimination is related to demobilization rather than sorption. Moreover, the technique has not yet been tried on other bacteriophages or human viruses [1].

The applied investigation must comprise at least two bacteriophage surrogates with changing vulnerability to physical reduction and demobilization. To inform surrogate election and let the design of ameliorated treatment devices, the demobilization pathway by CC should be defined. Determining a surrogate by physical similarities may be inappropriate if viruses are demobilized by capsid protein deterioration. Such facts indicate the necessity for a fundamental survey into coagulation that directly compares human viruses of interest and bacteriophages. Additional comparisons between bacteriophages are furthermore requested [1].

Plaque assays with recovery from flocs stay the gold standard for assessing demobilization. However, more testing remains requested to emphasize the credibility of integrating quantitative reverse transcription polymerase chain reaction (qRT-PCR) and plaque assay without recovery from flocs. Merging qRT-PCR and plaque assays may be suitable and cost-saving for some viruses; however, only if future studies depict that the procedure does not underreport demobilization. Also, continued investigation is required to define how demobilization influences total virus removal via coagulation. If the demobilization of viruses is a polishing stage for coagulation, little demobilization would be decisive for satisfying treatment objectives. More investigations juxtaposing the recovery of viruses from flocs by plaque assay and qRT-PCR may assist in delineating the link between coagulation and demobilization. Further, demobilization should be separated from aggregation; however, a quantitative estimation of virus aggregation in treated samples is impossible [1]

Kept exploration into the viruses' physicochemical features will let us anticipate sorption and demobilization conduct. Such kind of modeling will also help to determine bacteriophage surrogates better. Presently, surrogates are usually chosen following characteristics such as size and IP. However, the complexity of virus sorption and demobilization evades such easy procedures. Consequently, designing relationships between virus morphology and physical chemistry is vital. Significant steps in this direction have been mentioned in this discussion, like Langlet *et al.*'s model of virus electrokinetic [76], Sigstam *et al.*'s model of virus capsid susceptibility to inactivation [167], and Armanious *et al.*'s method for assessing hydrophobicity from virion surface structure [94]. Nevertheless, such models stay under study and cannot confidently predict the viruses' conduct. By juxtaposing morphologically comparable bacteriophages, we may comprehend more about how little modifications in structure influence sorption and demobilization features. As a perspective, we could be apt to anticipate virus

conduct and determine new bacteriophage surrogates following subtle aspects such as protein structures or genome size and conformation. The present discussion's advantages would extend far beyond an application in coagulation—from filtration setups to demobilization by nanoparticles to modeling virus fate and persistence in nature [1].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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