



The Role of Biophysical Stickiness on Oil-Mineral Flocculation and Settling in Seawater

Leiping Ye^{1,2*}, Andrew J. Manning^{2,3,4}, James Holyoke^{2,5}, Jorge A. Penaloza-Giraldo² and Tian-Jian Hsu²

¹ School of Marine Sciences, Sun Yat-sen University, and Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai, China, ² Department of Civil and Environmental Engineering, Center for Applied Coastal Research, University of Delaware, Newark, DE, United States, ³ HR Wallingford Ltd., Coasts and Oceans Group, Wallingford, United Kingdom, ⁴ School of Biological and Marine Sciences, University of Plymouth, Plymouth, United Kingdom, ⁵ Department of Civil, Architectural and Environmental Engineering, University of Texas at Austin, Austin, TX, United States

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*Correspondence:

Leiping Ye
yeleiping@mail.sysu.edu.cn

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Biophysical cohesive particles in aquatic systems, such as extracellular polymeric substances (EPS) and clay minerals, play an important role in determining the transport of spilled oil contamination and its eventual fate, particularly given that suspended sediment and microbial activities are often prevalent and diverse in natural environments. A series of stirring jar tests have been conducted to understand the multiple structures characteristics of the oil-mineral aggregates (OMAs) and EPS-oil-mineral aggregates (EPS-OMAs). OMAs and EPS-OMAs have been successfully generated in the laboratory within artificial seawater using: Texas crude oil (Dynamic viscosity: 7.27×10^{-3} Pa·s at 20°C), two natural clay minerals (Bentonite and Kaolin clay), and Xanthan gum powder (a proxy of natural EPS). A magnetic stirrer produced a homogeneous turbulent flow with a high turbulence level similar to that under natural breaking waves. High-resolution microscopy results show that EPS, kaolinite, and bentonite lead to distinguished oil floc structures because of the different stickiness character of EPS and mineral clay particles. With relatively low stickiness, kaolinite particles tend to attach to an oil droplets surface (droplet OMAs) and become dominant in small-sized flocs in the mixture sample. In contrast, the more cohesive bentonite particles stickiness could adsorb with oil droplets and are thus dominated by larger sized flocs. Biological EPS, with the highest stickiness, demonstrated that it could bond multiple small oil droplets and form a web structure trapping oil and minerals. Generally, adding EPS leads to flake/solid OMAs formation, and individual oil droplets are rarely observed. The inclusion of ESP within the matrix, also reduced the dependence of settling velocity on floc size and mineral type.

Keywords: biophysical stickiness, extracellular polymeric substance (EPS), oil-mineral aggregates (OMAs), flocculation, settling velocity

INTRODUCTION

With increasing human activities and industrial development within the marine environment, water resource quality, and subsequent recovery, from contamination by industrial dumping and oil spillages have been a major challenge (Doshi et al., 2018); especially in estuarine and coastal regions where they have high sensitivity to the ecosystem and public health (Barbier et al., 2011).

In recent decades, oil spill contamination has tended to occur more commonly in coastal water systems (Peterson et al., 2003; Radovič et al., 2012). In particular, the largest oil spill event in human history (at the time of publication) occurred in 2010, and was the Deepwater Horizon (DWH) oil spill. DWH released approximately 4.9 million barrels (or 779 million L) of crude oil into the Gulf of Mexico (Crone and Tolstoy, 2010; Atlas and Hazen, 2011). It was estimated that almost 1,773 km of the shoreline habitat was directly affected by the DWH spill (Michel et al., 2013). Besides the huge negative ecological impact in the nearby regions of the spill site and the shoreline, there was an unexpected sedimentation of oil-associated marine snow throughout the water column and down to the seafloor; this was an additional long-term impact on the benthic zones (Daly et al., 2016; Passow and Ziervogel, 2016; Romero et al., 2017).

Due to their lower relative density, most of the leaked oil contaminants were expected to float on seawater as the weathering processes proceeded. However, it was found that a considerable amount of oil droplets aggregated with the various suspended materials (Passow et al., 2012; Passow and Ziervogel, 2016), including: sediments, detritus, phytoplankton, and fecal pellets, and significantly facilitated by biologically secreted substances (Passow and Alldredge, 1995; Malpezzi et al., 2013), namely the Transparent Exopolymer Particles (TEP) or more generally the Extracellular Polymeric Substance (EPS). These oil-containing aggregates, called Marine Oil Snows (MOS) would finally settle to the seafloor as they were transported in the seawater (Daly et al., 2016; Passow et al., 2019).

After oil spills occur, chemical dispersants are often initially used to treat the visible surface contaminants (Kujawinski et al., 2011; Lubchenko et al., 2012; McNutt et al., 2012). However, after the oil slick is dispersed into smaller oil droplets, there could be a higher probability that the oil droplets aggregate with other cohesive suspended materials in the water column to enhance MOS flocculation and settling (Fu et al., 2014; Kleindienst et al., 2015). Since cohesive sediment flocculation is extremely common in estuarine and coastal water systems with energetic flows (Manning and Dyer, 2007; Manning and Schoellhamer, 2013), oil droplets can readily flocculate with clay minerals, and also EPS from phytoplankton exudation, thus forming oil-particle aggregates (OPAs) (e.g., Zhao et al., 2014). These OPAs may significantly influence the entire subaquatic ecosystem (Teal and Howarth, 1984; Kingston, 2002; Fisher et al., 2016). Therefore, interactions between oil, cohesive minerals, and EPS play a notable role in the fate of oil spills in a natural ecosystem, and it becomes highly desirable to understand the formation, properties, and settling velocity of OPAs.

Various approaches have been reported to clean up oil-contaminated waters and these can be classified as: physical, chemical, and biological methods (Hoang et al., 2018). Physical methods are mainly using floating devices (e.g., booms) to gather an oil slick together to facilitate more effective removal or burning of a large amount of oil collected on the water surface. To accelerate the natural weathering processes, chemical dispersants are widely used to disperse the oil slick down to smaller oil droplets. These two types of methods may not be always effective and may even lead to secondary contamination. Therefore

biological methods, such as adding microbes to enhance the oil bio-degradation, could be the most environmental-friendly approach. However, bio-degradation by the microbes and their bacteria function, can be time-consuming and their overall effectiveness may be highly dependent upon other environmental factors such as: nutrient levels, salinity, temperature, and the types of bacteria present (Atlas and Bartha, 1992; Atlas, 1995); hence more fundamental research is needed.

In recent years, many biologically related oil aggregates studies have been predominantly focused on MOS though both field observations (e.g., Passow et al., 2012; Daly et al., 2016) and laboratory experiments (e.g., Passow et al., 2017). These investigations provide clear evidence that high concentration oil-associated marine oil snows (MOS) exist in the vicinity of surface oil and the sub-surface oil plumes. Moreover, riverine suspended mineral particles and soot (a by-product of burned oil) are also very likely to interact with oil to form sinking oil-mineral aggregations (OMAs) in addition to MOS (Daly et al., 2016). With the combination of OMAs and EPS (or more generally called OPAs), flocculation and settling processes become ever more complex.

Clay mineral particles, such as kaolinite and bentonite, have been proven to aggregate with oil, and form OMAs (Khelifa et al., 2002; Muschenheim and Lee, 2002; Stoffyn-Egli and Lee, 2002). The settling velocity of these OMAs can be up to several mm/s, and their characteristics are highly dependent upon the base mineral stickiness (Ye et al., 2020). As aggregation is a complex process involving encounter, contact, and sticking (Hill and Nowell, 1990) controlled by flow turbulence, by directly controlling turbulence level, the biological EPS functions on OMAs flocculation may be better understood in the controlled laboratory experiments. Parsons et al. (2016) has demonstrated that only a very small amount of EPS (<0.1%) can dramatically stabilize sedimentary beds, because EPS has an extremely high stickiness/stickiness. Therefore, the appreciable role of EPS in controlling the flocculation process requires improved quantification.

Many field studies measuring TEP concentration in the coastal/estuarine zone show TEP concentration as Xanthan gum equivalent concentration (e.g., Passow, 2012; Malpezzi et al., 2013). Although the Xanthan gum equivalent concentration is based on colorimetric determination, and hence it does not directly provide quantitative information on stickiness. A limited number of studies, such as Engel (2000), exhibit a positive correlation between TEP concentration and stickiness. In many laboratory sediment transport studies, pure Xanthan gum has been used extensively to model the benthic biota secreted EPS (e.g., Malarkey et al., 2015; Parsons et al., 2016), which is an important factor influencing the cohesive sediment dynamics (Tolhurst et al., 2002). Therefore, Xanthan gum has been used to simulate biological EPS in the present study.

EPS has the potential to become a practical and viable substance for stabilizing oil droplets in the aquatic environment, when accompanied by natural clay minerals. Scientifically, as natural EPS and sediments are ubiquitous in the marine environment, understanding the role of EPS in controlling the

flocculation of sediment and stabilize oil droplets is a critical element to the understanding of the fate of spilled oil.

The primary goal of this study is to deduce the influence EPS has on oil-mineral flocculation. As a first step, the experiments are designed without considering the chemical dispersant, in order to better quantify the effect of EPS on oil-mineral flocs by making a direct comparison with earlier work on oil-mineral flocs without EPS (Ye et al., 2020). Relatively higher concentrations (~ 500 mg/l) of suspended minerals and EPS are applied artificially in controlled laboratory experiments in order to obtain discernable differences for inter-comparison. Data obtained from controlled laboratory experiments of six cases are analyzed with three main objectives: (1) To understand the EPS-OMAs structures formed with two different types of common clay minerals, using high-resolution digital microscopy; (2) to measure physical characteristics of EPS-OMAs, such as their sizes and settling velocities using a high magnification floc video camera; and most importantly, (3) synthesize measured and statistically analyzed data to gain insights into how EPS participation influences oil containment within flocs, floc structure, and settling dynamics. The EPS-OMA parameters derived from this laboratory research can be applied to the predictive modeling framework for oil spills; a preliminary endeavors can be found in Cui et al. (2021) in this issue.

MATERIALS AND METHODS

Laboratory jar test experiments were undertaken at the Center for Applied Coastal Research, University of Delaware utilizing a magnetic stirring system, with a self-designed turbulence monitoring frame attached (see Ye et al., 2020). The resultant OMAs were measured with a LabSFLOC-2 (the 2nd version of Laboratory Spectral Flocculation Characteristics instrument, Manning et al., 2010) aside of the jar test apparatus. Each experimental run adopted a uniform stirring speed of 490 rpm (equivalent to turbulence dissipation rate at $\varepsilon = 0.02 \text{ m}^2/\text{s}^3$, and shear rate parameter $G = \sqrt{\varepsilon/\nu} \sim 140 \text{ s}^{-1}$, Ye et al., 2020). As discussed in Ye et al. (2020), this was a relatively high shear rate parameter and similar to the highly turbulence conditions experienced under breaking waves. To prepare each sample, 1 Liter of artificial seawater was used for each run and the salinity was set (using laboratory grade sodium chloride) nominally to 35 ppt. White Kaolin clay ($92.3 \pm 2.5\%$ Kaolinite), Wyoming sodium Bentonite clay ($85.2 \pm 2.3\%$ Montmorillonite), Xanthan gum, and raw Texas crude oil (Dynamic viscosity: $7.27 \times 10^{-3} \text{ Pa}\cdot\text{s}$ at 20°C) were each added to the artificial seawater solution using a mini pipette.

To generate the flocs, the oil-to-sediment (or EPS) ratio was 2:1, and a sediment (or EPS) concentration of 500 mg/l was used. This oil-to-sediment ratio was utilized, in order to maximize the oil trapping efficiency in the mineral particles/flocs present. Pre-test assessments were initially conducted to deduce the optimal duration required for flocs to reach the equilibrium phase in the stirring jar tests (>2 hrs). Six OMA samples were selected and studied here (see Table 1, C01–06). Cases C04, C05, C06 are the primary focus of this study, which is to investigate

the effect of EPS on OMAs. Cases C01, C02, and C03 are the corresponding baseline OMA samples, and part of these OMA results (already reported in Ye et al., 2020) are also presented in order to demonstrate the contrasting effects of EPS.

Each experimental jar test run had a nominal 2 h duration before they were collected for analysis using the LabSFLOC-2 system (Manning et al., 2007, 2017). As demonstrated by Ye et al. (2020), for the given turbulence level, the flocculation process reaches equilibrium within the 2 hrs experimental run time. The total floc population characteristics were observed using the LabSFLOC-2 system which comprised a high-resolution (one pixel $\sim 5 \mu\text{m}$) video camera that observes and records individual flocs whilst settling in a dedicated settling column. The analyzed digital floc data provided by LabSFLOC-2 includes complete population mass-balanced: floc numbers, floc size, floc effective density (floc bulk density minus the fluid density), settling velocity, and fractal dimensions. Each floc data size spectrum was segregated into 12 size bands (SB) from <20 microns in SB1 to >640 microns in SB12.

In order to also examine the detailed structures of the OMAs and EPS-OMAs and the capability of these flocs to contain oil droplets, representative equilibrium floc samples were collected at the completion of each run and subsequently analyzed using a digital microscope (AmScope Inc.). Analysis of the microscopy images from the equilibrium stage (C01–06, Table 1), enabled the number of free oil droplets, total flocs, and OMAs to be measured and statistically assessed. These values were counted from a snapshot digital image obtained from the microscope for a given sample. For each sample, nominally 30 images were analyzed and various statistics, including mean ($\bar{N}$) and median values of all identical flocs, and the lower and higher 25% values from the mean were all obtained. See Ye et al. (2018, 2020) for further information on the control experiments for OMA cases without EPS.

RESULTS

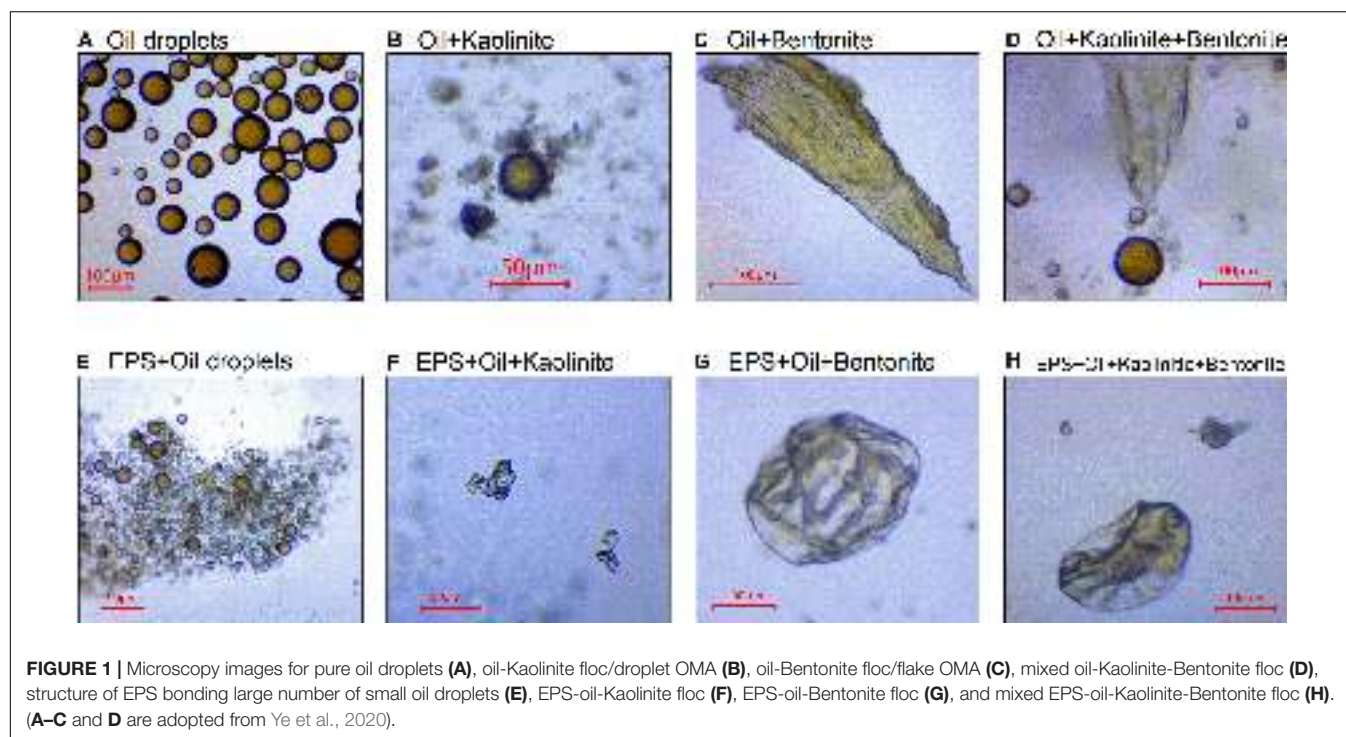
Aggregates Structures

Multiple types of equilibrium EPS-OMAs structures were examined using high-resolution microscopy, and compared with the well-known droplet OMAs and flake/solid OMAs (Stoffyn-Egli and Lee, 2002) formed by oil droplets and mineral. A key finding reported by Ye et al. (2020) regarding the existence of droplet OMA and flake/solid OMA, is their dependence on the mineral stickiness. Under the applied high turbulence level, the averaged droplet size was calculated to be about $57 \mu\text{m}$ (see Figure 1A). The less sticky individual kaolinite particles and micro-flocs, were seen to directly attach to the oil droplets forming droplet OMAs (see Figure 1B). In contrast, oil tended to be directly adsorbed into the high stickiness bentonite aggregate structures forming flake/solid OMAs (see Figure 1C), and the individual droplets were no longer observed. In the equally mixed kaolinite-bentonite sample, both types of OMAs were observed (see Figure 1D), but the overall settling velocity statistics are closer to the stickier bentonite-oil characteristics.

TABLE 1 | A summary on proportions of mineral clay, Xanthan gum and oil in each experimental run.

Case	Kaolinite clay (mg)	Bentonite clay (mg)	Xanthan gum (mg)	Texas crude oil (mg)	Saline water (ml)
C01	500	/	/	1,000	1,000
C02	/	500	/	1,000	1,000
C03	250	250	/	1,000	1,000
C04	500	/	500	1,000	1,000
C05	/	500	500	1,000	1,000
C06	250	250	500	1,000	1,000

/: none mineral or EPS added.



For the EPS and oil mixture (Figure 1E), a large number of oil droplets flocculated to form large-sized (several hundred micrometers) structures. Interestingly, those droplets absorbed into the EPS structure did not exceed 10–20 μm in size, which is 3–4 times smaller than the size of pure oil droplets (Figure 1A) generated under the same turbulent dissipation rate. This reduced oil droplet size with EPS is comparable to the size-scale of the smallest microflocs (i.e., smallest disaggregated floc size fractions typically observed in a very high turbulent flow; Keyvani and Strom, 2014). In other words, the structure of oil droplets in EPS, even though they can still be discerned, have reduced nearly two orders of magnitudes in volume.

Through the scrutiny of a plethora of microscopy images from the EPS-oil-Kaolinite floc sample (e.g., Figure 1F), the flocs show the presence of oil, EPS and minerals all closely aggregated together forming a structure similar to solid/flake OMAs, and no individual droplet OMA were observed. This highlights the key difference in the flocs resulting from the addition of the extremely sticky EPS to the suspension matrix. The results suggest that EPS significantly reduces the droplet size (see Figure 1E) down to a

size-scale similar to those of diminutive microflocs, and hence prevents the prospect of mineral floc bonding. Moreover, we can speculate that the high stickiness of EPS, may encourage the oil to be absorbed into the aggregate structure in a similar way to the effect of high stickiness depicted by bentonite. Following this concept, it is unsurprising and logical to observe numerous sizeable EPS-oil-Bentonite flocs formed in Figure 1G; these are similar to the compact dense oil-Bentonite OMA (Figure 1C), but encompassing supplemental EPS. The EPS-oil-kaolinite-bentonite flocs (see Figure 1H) also shows a consistent solid/flake structure in which no droplet OMA were observed, in contrast to their no-EPS counterpart (Figure 1D).

Oil Droplet Numbers

Motivated by the distinctive floc structures exhibited in Figure 1 (due to different mineral clays and the addition of EPS), it is anticipated that these flocs may also contain a dissimilar amount of oil droplets. The oil trapping efficiency for different samples is quantified by counting the number of oil droplets present in each sample (Khelifa et al., 2002) on attaining quasi-equilibrium

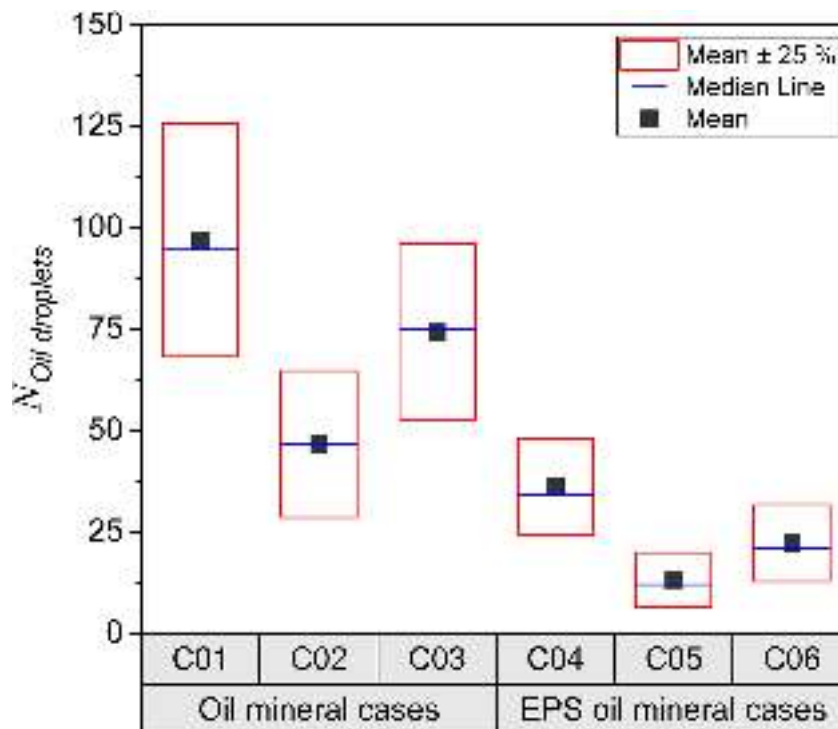


FIGURE 2 | Box charts of oil droplets numbers, analyzed by microscopy images collected from the bulk sediment mixture samples at the equilibrium. Each dot represents number obtain from an image and each sample was analyzed by 30 images randomly taken under microscope covering thousands of individual flocs statistically.

(see **Figure 2**). The case of oil and kaolinite (C01) has the highest number of free oil droplets remaining after 2 h of agitation (mean value $\bar{N} = 95$), whilst the bentonite case (C02) shows almost half the number of oil droplets ($\bar{N} = 47$). This result indicates that bentonite is approximately twice as effective in containing oil droplets in OMAs.

With the addition of EPS to the kaolinite and bentonite samples (C04 and C05), both cases show significant (nearly a factor 3–5) reductions in the number of free oil droplets ($\bar{N} = 32$ and $\bar{N} = 10$ for C04 and C05, respectively) compared to those without EPS (C01 and C02). In particular, the EPS-oil-bentonite sample (C05 in **Figure 2**) has the highest capability to contain oil droplets, with the fewest free oil droplet still present in the sample on completing a 2 h flocculation jar-test. Both of the samples with kaolinite-bentonite mixtures, revealed the remnant number of oil droplets ($\bar{N} = 72$ for C03 and $\bar{N} = 20$ for C06) were lower than kaolinite related samples (C01 and C04, respectively), but greater than bentonite related samples (C02 and C05, respectively); while adding EPS reduced the free oil droplets almost by a factor of 3 (i.e., comparing C03 and C06). More analyses confirming these finding will be discussed in the section “How the Aggregate Structures Determine the Oil Trapping Efficiency?”

Total Floc Characteristics

Key floc population characteristics (including floc size, settling rate, and density) were provided by the LabsFLOCS-2 system. To illustrate the effects of EPS on the resultant flocculation,

the samples have been grouped into three for inter-comparison: Group #1) oil-Kaolinite floc (C01, OMA_K) and EPS-oil-Kaolinite (C04, EPS-OMA_K) samples; Group #2) oil-Bentonite (C02, OMA_B) and EPS-oil-Bentonite (C05, EPS-OMA_B) samples; and Group #3) oil-Kaolinite-Bentonite (C03, OMA_{K-B}) and EPS-oil-Kaolinite-Bentonite (C06, EPS-OMA_{K-B}) samples.

OMA_K and EPS-OMA_K (Group #1)

The oil-Kaolinite flocs (OMA_K) have a size range from 20 to 350 microns (more than 85% were smaller than 160 micron—the nominal microfloc:macrofloc size division boundary) and settling velocity range from 0.04 to 4 mm/s (**Figure 4A** floc data measured using the LabSFLOC-2 instrumentation). Adding EPS (EPS-OMA_K) expands the floc size range from 18 to 500 microns in size (with more than 80% smaller than 160 microns) and 0.02 to 8 mm/s in settling velocity (**Figure 4B**). Generally, adding EPS widens the range of observed floc sizes and settling velocities.

The distribution of the number of floc in the kaolinite cases reveal that the OMA_K and EPS-OMA_K have the most flocs present in SBs2–3 (~990 flocs). In fact, the most notable effect of adding EPS is the new existence of the largest flocs in SB8 (~40 flocs) and SB9 (~6 flocs), indicating observed floc sizes in the range of 320 microns to 480 microns. The effective density trend generally decreases from small-sized flocs down to larger-sized flocs for both oil-kaolinite and EPS-oil-kaolinite cases. Moreover, adding EPS increased the effective density of small flocs (see EPS-OMA_K in SB2–4), but the effective density of the macroflocs in