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Biophysical flocculation reduces variability of cohesive sediment settling velocity

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Biophysical cohesion, introduced predominantly by Extracellular Polymeric Substances (EPS) during mineral flocculation in subaqueous environments, plays important role in morpho-dynamics, biogeochemical cycles and ecosystem processes. However, the mechanism of how EPS functioning with cohesive particles and affects settling behaviors remain poorly understood. We measure initial flocculation rate, floc size and settling velocity of mineral and artificial EPS (Xanthan gum) mixtures. Combining results from these and previous studies demonstrate coherent intensification of EPS-related flocculation compare with those of pure mineral and oil-mineral mixtures. Importantly, the presence of EPS fundamentally changes floc structure and reduces variability of settling velocity. Measured data shows that ratios of microfloc and macrofloc settling velocity for pure mineral flocs is 3.9 but greatly reduced to a lowest value of 1.6 due to biological EPS addition. The low variability of settling velocity due to EPS participation explains the seemingly inconsistent results previously observed between field and laboratory studies.

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Mud in subaqueous environment generally consists of organic materials (e.g., extracellular polymeric substances (EPS)[1]) and inorganic mineral clays[2]. They are transported as flocs with a wide variety of physical properties, such as settling velocity and porosity/density that are of interest in understanding coastal and estuarine morphodynamics[3,4], biogeochemical nutrient cycling[5,6], carbon and pollutant transport[7,8], and ecosystem functions[9]. A good understanding of flocculation dynamics is also crucial to predict the impact of erosion and sedimentation when being confronted with sea level change, land-use shortage, waterway navigability, and scour around engineering infrastructure[10,11].

Particle settling velocity is one of the most important quantity in the study of sediment transport[12–14]. Due to flocculation, estimating settling velocity of cohesive sediment is difficult and it is usually measured by video cameras or laser diffraction devices[15–17]. Most laboratory studies focus on inorganic clay/silt particles that show large variability of measured settling velocity as function of floc size and mineral types[18–20]. On the contrary, many field observations in coastal environments have reported that a floc settling velocity of around $1\,mm{\cdot}s^{-1}$ is a robust estimate[21,22] implying a rather low variability of floc settling velocity[23,24]. In fluvial environment, Lamb et al.[25] report that clay minerals and silt particles may settle as flocs with a nearly uniform settling velocity of $0.34\,mm{\cdot}s^{-1}$. They further conjecture that the reason why floc can form in freshwater is possibly due to high organic content via sticky EPS.

Microbial EPS has been considered one of the natural flocculants[26], however, its effect on settling velocity of floc are not fully understood[27]. EPS is products of biochemical secretions and can make up as much as 50~90 % of the organic matter content of microbial aggregates[28,29]. EPS has distinctive characteristics in composition and densities that are different from the minerals or other organic particles in suspension[30]. However, being anionic polyelectrolytes, EPS have somewhat similar flocculation mechanism with synthetic anionic flocculants such as polyacrylamide. It has been reported to cause bioflocculation via the divalent cation bridging (DCB) mechanism, whereby divalent cations such as Ca^{2+} and Mg^{2+} form bridges between anionic groups of EPS and negatively charged particles[31] and reduce the electrostatic repulsion between the particles due to the compressed double layer. Similar mechanism of DCB has also been reported for anionic polyacrylamide flocculation of mineral (kaolinite) particles[32,33].

In natural water environments, flocculation dynamics involve complicated mixtures of multiple types of cohesive particles[34]. Most clay minerals and EPS possess charges or functional groups that promotes floc formations[35]. It is well-established that floc formation is controlled by the intermolecular and surface forces between clay particles and EPS molecules, including van der Waals attractions[36], Coulomb forces[37], hydrogen bond[38], and ion-dipole forces[39], among others. These clay-EPS interactions are further complicated by water chemistry, clay surface's electrical double layers, and associated clay-clay interactions[40–42]. The EPSs act as polymeric bridges between particles to build large, settlable flocs[43], which may otherwise be kept apart by the double layer repulsion[44,45]. Therefore, the geometrical conformation and length of EPS molecules also affect clay-EPS interactions. The multivalent cations can further enhance the formation of polymeric bridges by connecting between the functional sites of EPSs and the negatively charged sites of mineral particles[46]. The EPSs and binding cations mitigate the overall negative surface charge of particles, thereby increasing the flocculation potential of particles[47–49]. A group of such clay particles bridged by EPS molecules make up a loosely associated floc. As a result, the size of clay-EPS flocs depends on these interactions as well as the physical and chemical properties, mainly 'stickiness', of the two types of constituents, clay minerals, and EPSs. Clearly, EPSs play an important role in flocculation and are a key component in the heterogeneous composition of flocs.

In marine environments, EPS has been observed to form large-sized flocs (>5 mm), or called "marine snows", on the sea up to several meters in size[50], which aggregate with other particles in suspension such as minerals and pollutant-like spilt oil[51,52]. The physical interparticlle bonds provided by EPS are observed to increase the tensile strength of the mud fraction[53] and enhance mixed biophysical cohesion[54]. Therefore, quantification of the stickiness between specific types of cohesive particles becomes crucial for determining floc properties (size, density, and settling velocity) and flocculation dynamics[55,56]. Previous studies generally utilize an empirical cohesion coefficient to represent the stickiness value of particles[57,58]. However, multiple particles mixtures may behave very differently in aggregate characteristics during flocculation process. Most importantly, the main characteristic of EPS is to enhance aggregation of suspended cohesive particles dramatically[59] although the natural EPS may show much complexity in various fractions such as protein and polysaccharide due to microorganism species. Exsiting studies on the quantification of cohesion and the resulting floc properties due to the participation EPS is rare. The research gap regarding how the participation of EPS in the formation of flocs of cohesive sediment can modify the floc size, density, and the resuting settling velocity need to be further addressed.

The objective of this study is to quantify the effect of EPS on the variability of floc settling velocity. We provide quantitative evidence that bio-cohesion greatly enhances the stickiness during flocculation, which dominates over the London-van der Waals force and electrostatic bonding forces and hence the effect of clay type and salinity becomes unimportant. As a result, bio-cohesion drastically reduces the variability of floc settling velocity as a function of floc size and clay type. Our findings explain the field observed low variability of floc settling in fluvial and coastal environments due to the dominance of bio-cohesion.

Results

Quantifying stickiness of minerals and EPS flocculation. Eight cases of different collective mixture of minerals and EPS (C01 to C06 in saltwater with salinity 35 psu and C07 to C08 in freshwater condition, Table 1) show distinctly different flocculation rates during the first 3~5 minutes of floc generation. Here, the initial flocculation rate (IFR) is utilized to quantify the stickiness of each case, which is proportional to the magnitude of IFR. For the saltwater cases without EPS addition (C01 to C03), mineral particles themselves show a wide range of IFR or stickiness (Fig. 1a, b). The lowest stickiness is Kaolinite (C01) with IFR =

Table 1 A summary of samples used in each case of the jar experiment.

Cases/Samples		Kaolinite clay (K)	Bentonite clay (B)	Xanthan gum (EPS)	Salinity
		mg	mg	mg	‰
C01	K	500	/	/	35
C02	B	/	500	/	35
C03	K + B	250	250	/	35
C04	K + EPS	500	/	500	35
C05	B + EPS	/	500	500	35
C06	K + B + EPS	250	250	500	35
C07	K + EPS	500	/	500	0
C08	B + EPS	/	500	500	0

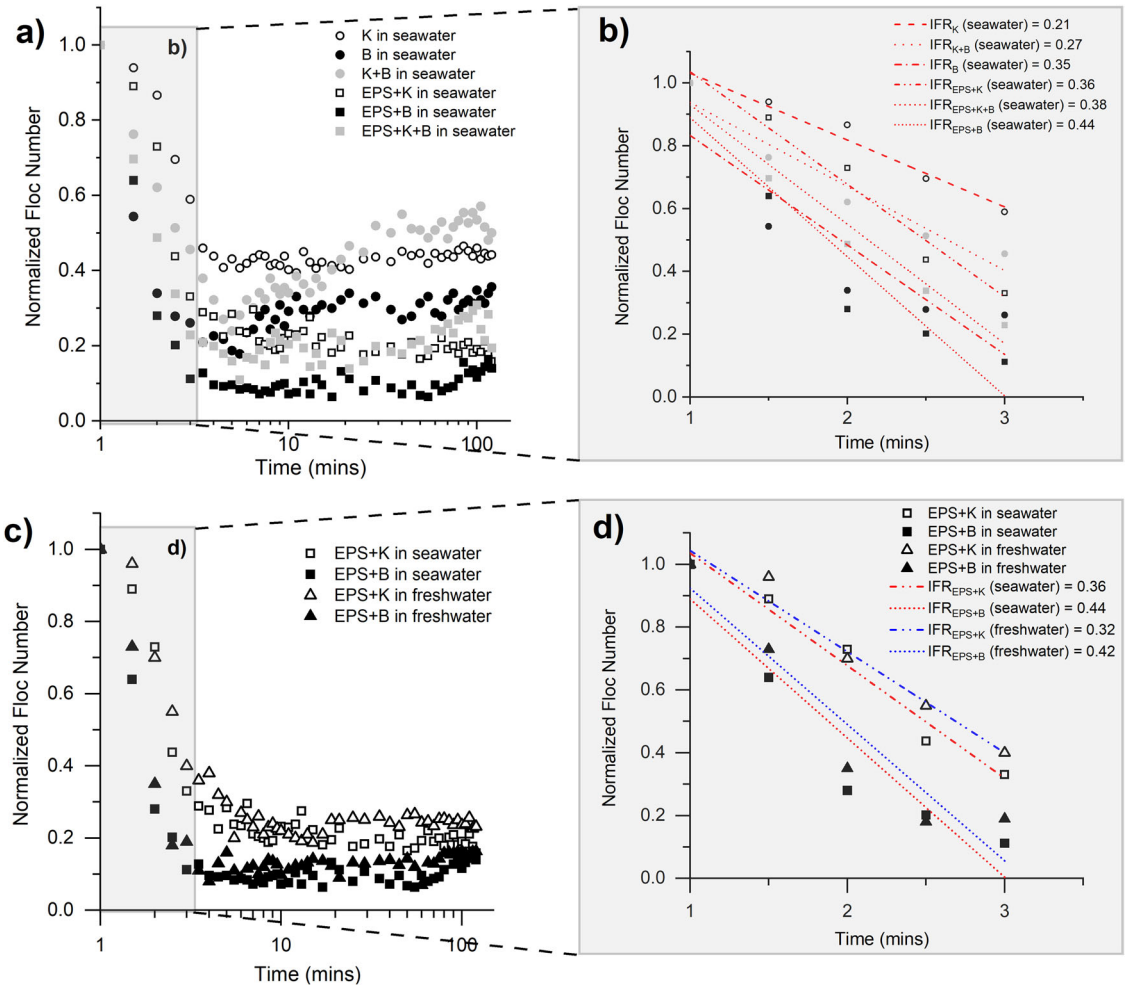


Fig. 1 Temporal evolutions of normalized floc numbers in each studies case. a, **b** are temporal evolutions of normalized floc numbers for six saltwater cases: Kaolinite (K, blank circles, C-01), Bentonite (B, solid circles, C-02), 1:1 mixed Kaolinite and Bentonite (K + B, gray circles, C-03), EPS-Kaolinite (EPS + K, blank squares, C-04), EPS-Bentonite (EPS + B, solid squares, C-05), EPS-Kaolinite-Bentonite (EPS + K + B, gray squares, C-06); **c**, **d** show that the temporal evolutions of two freshwater samples for EPS-Kaolinite (C-07) and EPS-Bentonite (C-08) are similar to their saltwater counterparts. The initial particle number of each case measured from high-resolution image was used for number normalization. The shadowed area **b** and **d** represents the zoom-in of the initial 3 minutes emphasizes the initial floc numbers changes, and the slopes quantify the initial flocculation rates.

0.21 while the highest is Bentonite (C02) with IFR = 0.35 (Fig. 1b). Therefore, these two mineral types cause a factor 0.35/0.21 = 1.67 change in stickiness in seawater.

Adding EPS to mineral flocs clearly increases the resulting floc stickiness in seawater (Fig. 1b). For instance, looking into the IRF of Kaolinite cases (C01 and C04), including EPS increases stickiness of Kaolinite floc by more than 70 % in seawater. For Bentonite floc (compare C02 and C05), the stickiness increases by only 26 %. As a result, adding EPS reduces the resulting the variability of particles stickiness due to mineral type. Considering the higher stickiness in EPS-Bentonite mixture (IFR = 0.44) and the lower stickiness in EPS- Kaolinite mixture (IFR = 0.36), a considerably lower variability of factor 0.44/0.36 = 1.22 is obtained (compare to 1.67 for a much larger variability without EPS participation). Essentially, the EPS effect on increasing stickiness for Kaolinite particles is nearly three times greater than that for Bentonite. As Bentonite particles behave much stickier in seawater than Kaolinite, adding EPS only slightly increases its stickiness. On the contrary, for the less cohesive Kaolinite particles, EPS plays a dominating role in determining the overall stickiness.

In freshwater condition, the addition of EPS to Kaolinite and Bentonite show similar IFRs to those in seawater (Fig. 1c, d). For Kaolinite and EPS flocculation in freshwater (C07), the IFR reduces only slightly by around 10 % compared to saltwater, while for Bentonite and EPS (C08), the IFR reduction is only 5 %. The flocculation functions by EPS is clearly dominant in subaqueous environments. Ye et al.[60] reported that the physical properties of flocs can be quite different for specific mineral types when the cohesion of mineral particles controls floc aggregation and breakups. With the presence of EPS dominating the cohesion during flocculation, the corresponding floc characteristics may be importantly modified.

How dose EPS change floc structures? High-resolution microscopy images show unique structures of different mineral and EPS-mineral flocs. These distinct floc structures are resulted from each type of mineral particles' properties and bio-cohesion and they may be directly associated with stickiness. The pure Kaolinite particles having the lowest stickiness tend to form much smaller-sized flocs with a more compact structure (Fig. 2a) than those of Bentonite flocs (Fig. 2b). Examining a small number of larger Kaolinite floc, it is evident that they are of much lower porosity (Fig. 2a) than the Bentonite flocs where the higher transparency porous structure can be seen (Fig. 2b). The 1:1

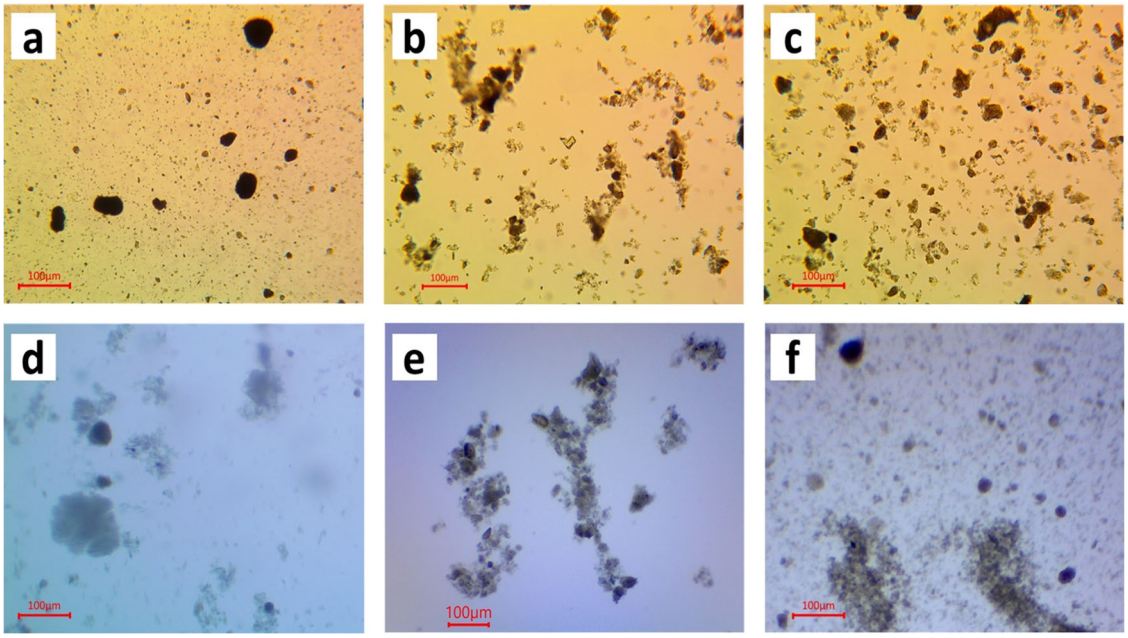


Fig. 2 Floc images obtained from the high-resolution microscopy analysis. a Kaolinite floc (C-01); **b** Bentonite floc (C-02); **c** mixed Kaolinite and Bentonite floc (C-03); **d** mixed EPS and Kaolinite floc (C-04); **e** mixed EPS and Bentonite floc (C-05); **f** mixed EPS, Kaolinite and Bentonite floc (C-06).

mixed Kaolinite and Bentonite flocs appear to consist of both types of floc structures (Fig. 2c).

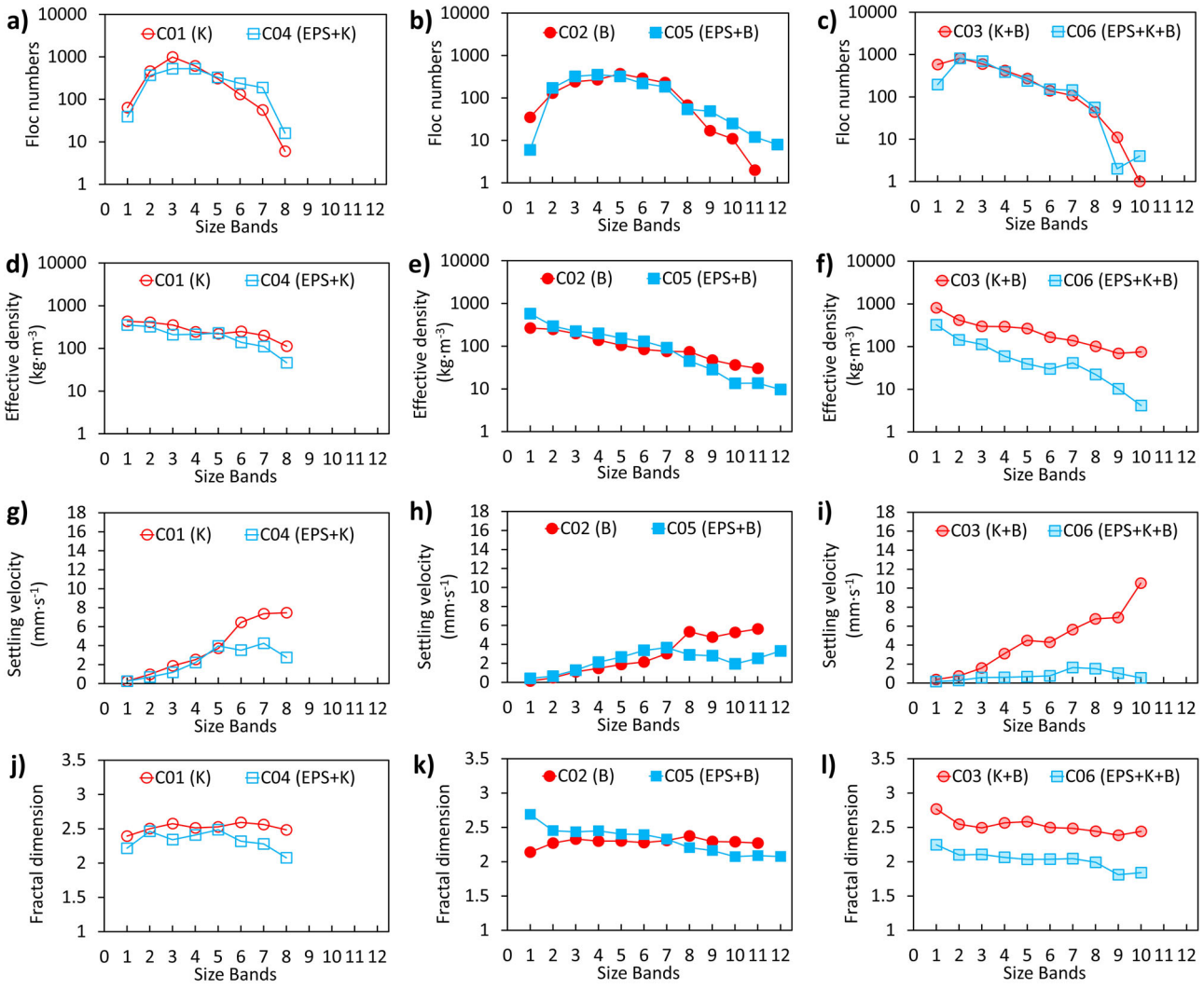
With the addition of equivalent amount of EPS, all three mineral cases show the formation of considerably larger and porous flocs (Fig. 2d–f). Although Kaolinite and Bentonite clay show evident differences in mineral particles' stickiness and thereby floc structures (Fig. 2a, b), the same amount of EPS addition enlarges floc size and porosity in both clay types (Fig. 2d, e). Particularly for Kaolinite floc, a large number of small flocs disappear after the addition of EPS and the resulting large-sized floc are dominated by the higher porosity EPS-web structure (Fig. 2a, d). Meanwhile, the shape and structure of the EPS-Bentonite flocs appear to be qualitatively similar to those of pure Bentonite, except that the floc number (floc size) is dramatically reduced (increased) (Fig. 2b, e). For the 1:1 mixture of Kaolinite and Bentonite, adding EPS tends to bond together many smaller mineral flocs to form large porous floc bodies (Fig. 2c, f). These floc images reveal the role of sticky EPS in homogenize the aggregate structure of different clays, and this distinct effect is expected to modify the physical properties of floc to be quantified in the next section.

How does EPS change floc density, settling velocity, and fractal dimension? The results analyzed by the 2nd version of Laboratory Spectral Flocculation Characteristics instrument (LabS-FLOC-2 system, see the Methods section) for floc numbers (Fig. 3a, c), effective density (Fig. 3d, f), settling velocity (Fig. 3g–i) and fractal dimension (Fig. 3j–l) in 12 size bands (Fig. 3m) reveal the vital role of EPS in controlling physical properties of flocs. EPS addition clearly increases the floc numbers in large-size bands (SB) of Kaolinite (SB6-8) and Bentonite (SB8-12) (Fig. 3a, b). Since the total floc mass must conserve, increased floc number in larger SB causes a reduction of floc number in smaller SB. Specifically, adding EPS reduces the floc number in size bands smaller than 160 μm in Kaolinite (Fig. 3a) and 80 μm in Bentonite (Fig. 3b). This is consistent with the image shown in Fig.2d, e that EPS produces large flocs by connecting smaller flocs via its mucus web structure. A similar but less pronounced trend is observed by adding EPS to the Kaolinite and Bentonite mixture (Fig. 3c).

Since EPS has a very lower effective density (~33~200 $kg{\cdot}m^{-3}$)[61] than the mineral effective density (~1650 $kg{\cdot}m^{-3}$), the presence of EPS can importantly reduce the effective density of large Kaolinite flocs (floc size >160 μm) (Fig. 3d), and their settling velocity is also reduced by a factor 2~3 (Fig. 3g). Specifically, for flocs larger than 160 μm, pure Kaolinite has a settling velocity ranging from 4~8 $mm{\cdot}s^{-1}$, while the EPS and Kaolinite mixture shows a much narrower range of 3~4 $mm{\cdot}s^{-1}$ (Fig. 3g). Adding EPS also reduces the density of large Bentonite flocs (floc size >320 μm) while contrarily, slightly increases the density of smaller Bentonite flocs (floc size <320 μm) (Fig. 3e). This results in an even lower settling velocity variability as a function of floc size (Fig. 3h). For floc size greater than 120 μm, pure Bentonite settling velocity ranges from 1.5~6 $mm{\cdot}s^{-1}$, but Bentonite and EPS mixture shows a much narrower range of 2.5~4 $mm{\cdot}s^{-1}$ (Fig. 3h). Adding EPS to Kaolinite and Bentonite mixture reduces floc density throughout the entire floc spectrum with a more important reduction for floc size larger than 320 μm in SB8-10 (Fig. 3f). This also narrows the variability of floc settling velocity to between 0.5~2 $mm{\cdot}s^{-1}$ (Fig. 3i) for floc size greater than 120 μm while the pure Kaolinite and Bentonite mixture shows a very wide range of settling velocity between 2~11 $mm{\cdot}s^{-1}$.

Our finding that the addition of EPS importantly reduces the settling velocity variability may be a remarkable implication for natural flocs with mixed types of cohesive materials. The general recommendations that the floc settling velocity is around 1 $mm{\cdot}s^{-1}$ in estuaries[21,22,62] and is around 0.34 $mm{\cdot}s^{-1}$ in the fluvial environments[25] are very likely due to bio-cohesion. The settling process of cohesive particles in natural subaqueous systems may be much stable and predictable with the participation of pervasive biological sticky materials.

Fractal dimension is a useful parameter to quantify the porosity and shape of flocs[63]. A fractal dimension as low as 2 or smaller indicates a high floc porosity and typically a elongated shape[64]. Adding EPS to Kaolinite, the fractal dimension is reduced from 2.5 to about 2.2~2.4 (Fig. 3j). Interestingly, adding EPS to Bentonite slightly increases the fractal dimension of small-sized flocs (Fig. 3k). EPS addition results in the loose small Bentonite floc structure becoming more compact. However, for large-sized



m)

Size Band	1	2	3	4	5	6	7	8	9	10	11	12
Size Range (μm)	20-40	40-80	80-120	120-160	160-200	200-240	240-320	320-400	400-480	480-560	560-640	>640

Fig. 3 Floc properties obtained from LabSFLOC-2.0 analysis of saltwater cases. a–l Show the size spectra, expressed in 12 size bands (see **m**), of floc numbers (**a**–**c**), effective densities of flocs (**d**–**f**), settling velocities of flocs (**g**–**i**), and fractal dimension (**j**–**l**) in Kaolinite-related cases (**a**, **d**, **g**, **j**), Bentonite-related cases (**b**, **e**, **h**, **k**), and mixed kaolinite and bentonite cases (**c**, **f**, **i**, **l**), respectively.

Bentonite flocs, adding EPS shows an expected reduction of fractal dimension from 2.4 to 2.2 (Fig. 3k). Introducing EPS to a Kaolinite-Bentonite mixture, the fractal dimension is importantly reduced from 2.5 to 2.0 (Fig. 3l). Again, the reduction of fractal dimensions is consistent with the EPS web and gel structures bonding with mineral flocs, which influences the porosity and shape of the flocs especially in large size bands that generally contain more EPS constituent.

Discussion and conclusions

In cohesive sediment studies, the flocs are often classified by microflocs and macroflocs with a demarcation floc size set to be 160 μm[65,66]. The mean values of the floc settling velocity for each case are summarized in Table 2. Results again reveal that the pure mineral flocs generally show dramatic differences in settling velocity between macroflocs and microflocs (a ratio of 2.7, 2.4, and 3.9 for Kaolinite, Bentonite, and Kaolinite-Bentonite mixture, respectively). When EPS participates in the flocculation process, the mucus structure provides unique capabilities with a much greater stickiness to connect mineral particles/flocs that dominates the mineral cohesion and reduces the difference of settling velocity between microflocs and macroflocs (a ratio of 2.1, 1.5, and 1.6 for Kaolinite, Bentonite, Kaolinite-Bentonite mixture, respectively). In natural subaqueous environment, flocculation process can be highly influenced by multiple environmental factors, such as salinity and bio-cohesion[67,68]. In freshwater, flocculation occurs primarily due to bio-cohesion[25] (Fig. 1c). Algae bloom has been considered as the main contribution to the formation of large-sized macroflocs[69]. After all, biological EPS fraction may vary in natural water, but with generally high EPS production in estuarine and coastal ecosystems, its role in the flocculation process can importantly affect natural cohesive flocs settling characteristics and their depositional prediction[66].

Results presented here also provide an explanation of the recent work by Ye et al.[27], who show the participation of EPS in oil-mineral aggregates (OMAs)[70,71] importantly reduces the variability of settling velocity as a function of floc size. Using IFR to quantify stickness in the pure mineral and EPS-mineral samples in saline water and freshwater, this work provides