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Characterizing Settlement of Activated Sludge Based on AI-Assisted Analysis of Moving and Still Images

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Abstract

Morphological and physical characteristics of activated sludge (AS) from 6 different wastewater treatment plants by moving-image and still-image analysis were compared, and important factors for improving the sedimentation of AS and suspended solids in treated water were examined. There were samples in which AS of sufficient size for recognition in the moving image remained above the interface when the interface settles, and the samples in which particles that could not be recognized in the moving image remained. Minute particles above the interface may be removed according to the shape characteristics of AS or otherwise remain in the supernatant and eventually degrade the quality of the discharged water. The shape of minute particles which remain in the supernatant and/or the contact opportunity with AS which is larger and easier to sink than the minute particles may be important for the water quality improvement of the effluent. Although it is not yet known whether the behavior of solids in the container actually occurs in the secondary clarifier due to the difference in system size, the use of light-processing and AI-supported image analysis techniques simplifies the introduction to plant, and may be used for objective AS characterization without relying on skilled staff.

Keywords

Deep Learning; Solid–Liquid Interface; Quantitative Image Analysis; Sludge Volume Index; Suspended Solid

Synopsis

- 34 Frame-subtraction-based image analysis with a low processing load and AI-assisted microscopic
- 35 image analysis characterized settlement of different AS.

Introduction

Solid–liquid separation of activated sludge (AS) is responsible for effluent water quality. Therefore, it is important to investigate the parameters affecting AS settleability in secondary clarifier effluent (SCE) and to improve effluent quality (suspended solids: SS). Digital-microscope- and smartphone-based technologies capturing moving and still images of AS are effective because they can be easily implemented in wastewater treatment plants (WWTPs) in developing countries and small plants. In addition, it is important to analyze the images with the support of artificial intelligence (AI) technology to diagnose the state of AS by obtaining objective and non-arbitrary analysis results that do not require skilled employees or human observers.

The settleability of AS is generally evaluated by the sludge volume index (SVI), using the height of the AS interface after leaving AS to stand in a cylinder for 30 minutes¹. Sedimentation velocity of the AS/supernatant interface^{2,3}, sedimentation velocity of a single sludge floc by direct measurement⁴, and theoretical sedimentation velocity based on shape⁵ are also methods for evaluating settlement. However, from the viewpoint of reducing SS in treated water, it is necessary to evaluate the movement of the interface, the characteristics and behavior of solid particles above the interface, and the consolidation process of AS occurring below the interface^{6,7}. Determination of the settling velocity of AS below the interface by ultrasonic waves has been previously reported⁸. However, smartphone-based technology^{9,10}, which continues to increase in prominence, is also necessary to improve accessibility of AS analysis techniques. Therefore, this study was conceived to quantitatively visualize the behavior of AS not only at the interface but also above and below it by photographing AS sedimentation in a container with a short depth and by analyzing the whole visual field.

Video (moving image) analysis of AS, a technique for determining the amount of sludge particles in a subject based on the temporal change in color space, has been reported¹¹. However, this technique was not for tracking the behavior of sludge particles. In recent years, moving-image analysis using machine learning has been used in various fields as a technique for tracking the behavior of a subject. Among them, object detection by YOLO (You Only Look Once) algorithm^{12,13} is one of the general analytical methods. However, when this technique is applied to the study of recognizing AS flocs and tracking their motion, there are cases where the behavior cannot be tracked because of overlapping and aggregation.

On the other hand, in moving-image analysis using inter-frame subtraction, all objects that have moved between frame intervals in the field of view (FoV) are reflected in the analysis results. Although the inter-frame subtraction method is susceptible to background noise, it is simple and quick, and it is one of the most common video analysis methods^{14,15}. If imaging can be performed such that AS settling is the only variable factor in the FoV, then it is expected that quantitative output can be obtained from the behavior of all flocs by analysis based on frame subtraction. Li and coauthors⁷ collected aerobic granular sludge at different stages of time from the same reactor and analyzed the video of settling in a cylinder. They conducted analysis using the gray level co-occurrence matrix (GLCM) technique, succeeding in quantifying the concentration below the interface and the behavior of particles on the interface. In our present study, we used software to analyze frame subtraction, which can divide the FoV into arbitrary meshes and determine the degree of change of elements in the mesh between frames without much processing power.

Previous studies that have found the relationship between the two-dimensional shape of AS captured by microscopic images and sedimentation properties have reported that SVI correlates with the amount of mixed liquor suspended solids (MLSS) per unit area (density, ρ), aspect ratio

(AR), roundness (RN), fractal dimension (FD), equivalent diameter of high-density floc (D_{eqHD}), etc..^{16,17,18,19,20,21} Here, the area is not the actual surface area but the projected area as recognized in the two-dimensional microscope image. The equivalent diameter is the radius of the circle that has the same area as the projected area of one AS floc, and D_{eqHD} measures only the core part of the AS extracted by image processing. It has been reported that the amount of filaments correlates with sedimentation properties when the predominance of filamentous bacteria is a major factor in the deterioration of settleability²². However, except in the case of filamentous bulking, no common shape parameter that determines settleability has been found for AS with characteristics that depend on the WWTP source.

However, few studies have combined video analysis and microscopic image analysis for investigating the characteristics of AS that can provide clean supernatant in the secondary clarifier⁷. Moreover, most of the studies above, which analyzed moving-images of AS sedimentation or AS's microscopic still-images and compared them with physical properties such as settleability, were focused on AS from a common WWTP or reactor. In this study, parameters related to the morphology obtained from magnified still images of AS under a digital microscope and AI-based technology were compared with results from moving-image analysis, and AS collected from six different WWTPs were classified according to sedimentation characteristics. The purpose of this study is to extract physical factors that determine the settleability of AS and SS in treated water based on the characteristics of different sludge.

Materials and Methods

AS and SCE were collected from six WWTPs (A-, B-, C-, E-, F-, and G-WWTPs) located in Hokkaido, Japan. These WWTPs apply a conventional AS method. Sampling date, treatment

population and treated water volume are summarized in Table 1. In each WWTP, AS was collected at the end of the reaction tank. A-, B-, and C-WWTPs are described in previous publications^{20,23,24,25}, while A- and E-WWTPs are described in previous research²⁶ under the names S- and E-WWTPs, respectively. For each sample, MLSS concentration in the AS reaction tank (*MLSS*), SS concentration in the SCE (*SS_{SCE}*), and SVI after 30 min (*SVI₃₀*) in a 1 L graduate cylinder were measured according to a standard method¹ on the day of sampling. MLSS represents the numerical value of the concentration of sludge immediately after sampling of AS, and SS represents the numerical value of the concentration of solids in the supernatant after settling of AS

Table 1. Sewer system, treatment population, and treated water volume of A-, B-, C-, E-, F-, and G-WWTPs in 2021.

WWTP	Sampling date (YYYYMMDD)	Sewer system	Treatment population (persons)	Treated water volume (m ³ /d)
A	20231222	Combined	213,400	144,000
B	20230621	Combined+Separated	341,600	238,000
C	20231227	Combined+Separated	373,800	186,000
E	20221117	Separated	13,360	7,900
F	20230929	Separated	23,300	16,000
G	20221220	Separated	16,100	14,700

In order to obtain morphological values for each sludge, 154 still images covering a preparation of 50 μ L of AS (18 mm $\times$ 18 mm) were taken using a tabletop digital microscope, automated XY stage, and software, and then they were analyzed by a method reported by previous works^{20,25,26}. Density [$\rho_{M/A}$ (M is mass, A is area)] was calculated by dividing the mass of AS in a preparation (calculated by $MLSS \times 50 \mu L$) by the total area on the preparation.

Aspect ratio (AR), roundness (RN), fractal dimension (FD), and equivalent diameter of high-density flocs (D_{eqHD}) were evaluated for each sample by Eq. 1–4 on the basis of the same calculations as in previous research²⁰:

$$AR = \frac{4[Radius_{max}(mm) - Radius_{min}(mm)]}{\pi \times Radius_{min}(mm)} + 1 \quad \text{Eq. 1}$$

$$RN = \frac{Perimeter^2 (mm^2)}{4\pi \times Floc \text{ area } (mm^2)} \quad \text{Eq. 2}$$

$$Floc \text{ area} \propto Perimeter^{FD} \quad \text{Eq. 3}$$

$$D_{eqHD}(\mu m) = 2 \sqrt{\frac{Area \text{ of high-density part } (\mu m^2)}{\pi}} \quad \text{Eq. 4}$$

Here, AR of a sample is determined by averaging AR obtained from $Radius_{max}$ and $Radius_{min}$ (maximum and minimum radii, respectively) of each recognized floc. AR describes the elongation of an object; the AR of a circular particle should be 1, and it would take on higher values for more elongated and irregular objects. RN was obtained from $Perimeter$ and $Floc \text{ area}$ of each floc and then averaging over all flocs for one sample. In this definition, the roundness of a circular object is 1, and that of other shapes is greater than 1. FD of each sample was obtained from the slope of the double logarithmic plot obtained from $Perimeter$ and $Floc \text{ area}$ of all of the recognized flocs. FD describes the boundary of an object, with values ranging from 1 to 2 for a two-dimensional object. It is generally higher when an AS floc exhibits a smooth and regular boundary as well as a compact shape. Floc area per floc (A/F) was determined by averaging the area of all flocs captured in one preparation (154 images), and it is a remarkable parameter for size. D_{eqHD} , another representative parameter of floc size, was also obtained by using *Area of high-density part*. This is calculated from an image's core part of AS, which is darkened by a close image-filtering process²⁰.

Aggregated flocs (AFs) probability score (P_{AF}) was determined from the output value of an AI-based classifier for AS images based on the similarity to training data including images of aggregated and dispersed AS. This classifier has been constructed by the Inception v3 [convolutional neural network (CNN) architecture] model, which has been trained and validated by AS images from A-WWTP and E-WWTP captured by the same setup as the present study. Details of training data, architecture, and performance metrics have been described in previous research²⁶. Images from A-WWTP and E-WWTP were respectively labeled as 1 (aggregated) and 0 (dispersed). Therefore, P_{AF} for AS that is well-aggregated and similar to A-WWTP is expected to be close to 1, and that for dispersed AS similar to E-WWTP is expected to be close to 0.

For obtaining moving images of AS settling, an acrylic container 40 cm in length, 20 cm in width, and 3 cm in depth was placed in front of an LED light (artificial lightning) with the sides shielded (Figure 1a and 1b). About 2 L of AS was poured into the container, and a moving image of AS settling for 30 minutes was acquired by a smartphone (Figure 1c). The side walls were prepared to prevent the reflection of human movement in the laboratory, and the upper part was not shielded because the equipment was installed in a position where the reflection of the laboratory lighting did not enter the smartphone lens. The distance between the smartphone and the container was set at 45 cm, and all videos were acquired with the same dimensions, as they were taken without zooming. The video was analyzed using Insect Eye²⁷ (Energy Gateway Incorporated, Japan) (Figure 1d), which is a commercial video analysis software to quantify the time change of the subject in the video including all elements such as color, shape, and position based on inter-frame subtraction. In this paper, it was used to divide the video that captured the sedimentation of the AS into several meshes, calculate the amount of change (AoC) of the elements in each mesh in the depth direction at certain intervals, and quantitatively obtain the time change.

It is a commercially available lightweight video analysis engine, and according to the description on the website of the provider, it calculates the change of the elements in the video using inter-frame subtraction, so one can obtain the analysis results regardless of the resolutions and lighting conditions of the input video, and does not require any special preprocessing.²⁷ The ability to obtain analytical results with little preprocessing of the input data is similar to the characteristic advantages of AI assisted technology in general, and the software may be called a non-learning AI.

The processing procedure of moving images is as follows. First, the moving image was divided into arbitrary meshes, and the elements in the meshes were sequentially compared at an arbitrary frame interval. Subsequently, parameters called “difference” (AoC) and “coefficient” (degree of changes) were calculated for each mesh at each time set as a frame interval. They can be output in the .csv file format. Although the specific calculation method is not disclosed by the software developer, we confirmed the following by asking the developer: the difference indicates the magnitude of change of elements including color when the frame difference of one mesh is taken. It takes on values from 0 to 255, with larger values indicating more change. The coefficient indicates the degree of pattern matching between frames, taking on values from -1 to 1 ; smaller values indicate less matching, that is, more pixels have changed. Since the specific calculation formula was not available because it was related to the basis of the software, it was difficult to quantitatively compare the output values from different moving images. However, since the shooting conditions such as illumination and resolution are in principle the same in the same moving image, this software was used to visualize the settlement state of AS in a single figure for each WWTP.

For visualizing the parameters, a threshold value can be set for the difference and the coefficient, and a mesh with only a small difference or coefficient due to noise between certain

frames can be considered as having no change. The edge of each mesh is colored as colorless (no change), blue (small change), yellow (medium change), or red (large change) according to the threshold value set for the difference or coefficient. The processed moving image can be output in the .avi file format, and a mesh with a large change in a moving image can be visualized. In this study, the inside of the container was divided into 68 cells vertically and 20 cells horizontally. The frame interval was set to 1 second, and a 30-minute moving image capturing AS settling was analyzed as a continuous image of 1800 frames. For a certain frame, a continuous image was obtained by coloring the edges of the mesh as colorless if the difference in the mesh was 0 to 2, blue if it was 2 to 4, yellow if it was 4 to 6, and red if it was 6 or more compared to the previous frame (1 second), and a moving image of 30 frames per second was obtained. In other words, a moving image of 30 minutes of settling was compressed into a processed moving image of about 60 seconds (30 times speed). These threshold values were set so that the color change of the edge of mesh corresponding to the behavior of the AS was most remarkable in the moving image output from the Insect Eye. In short, the software does not recognize the shape of the AS, but quantitatively calculates the light and dark changes of the image for each mesh.

The AS/supernatant interface was traced by edge detection. Here, the moving image was binarized into a black-and-white moving image, and the entire FoV was slightly blurred to reduce the influence of noise from lighting and vibration. For each captured frame, a vertical luminance gradient was obtained over the entire FoV. This was calculated as the difference in luminance between two pixels directly above or below. Near the interface, points had a large range in luminance gradient values. To average these points, the luminance gradient was integrated in the horizontal direction of the FoV to yield a luminance gradient value for each position in the vertical direction of the container. The position where this value was at a maximum was defined as the

AS/supernatant interface, and its height was recorded. However, since the AS was reflected toward the upper end of the FoV at the beginning of the moving image, this calculation could not correctly recognize that the interface was at the upper end. Therefore, only at the beginning of the moving image, the background image was virtually combined with the upper end of the moving image. The interface position was obtained by considering the number of pixels from the water surface and the number pixels for the bottom of the container and then converting into height (cm) from the water surface. Finally, changes in the interface position over time were traced, and a precipitation image in which the calculated interface position was emphasized by a horizontal line was produced (30 times speed) (Figure 1e).

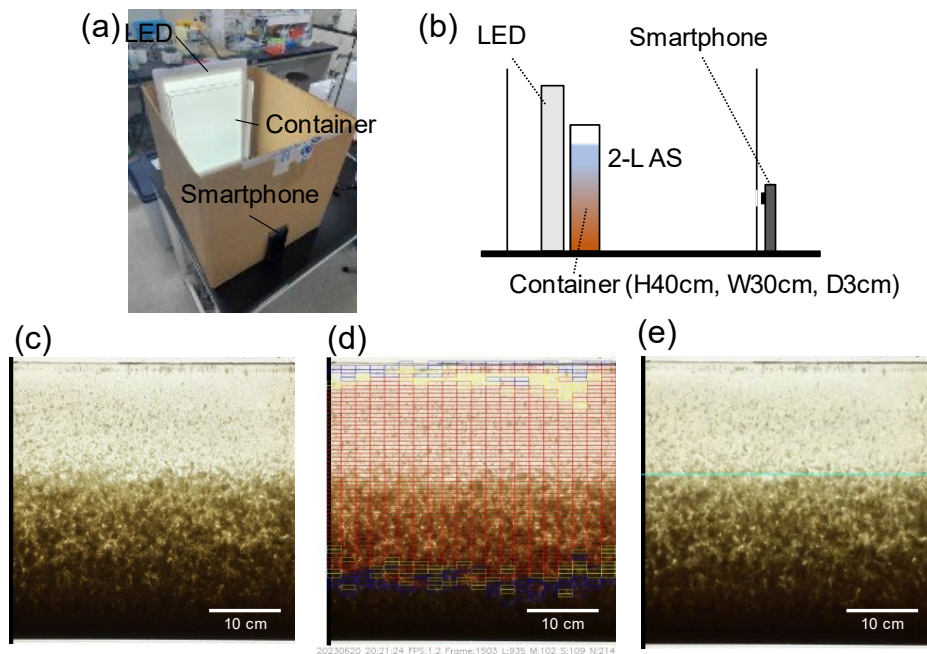


Figure 1. (a) Panoramic photograph of the moving-image capturing apparatus. (b) Schematic diagram of the apparatus as viewed from the side. (c) Representative image obtained by cutting out a certain frame of the moving image of B-WWTP. (d) Representative processed image obtained from Insect Eye at the same frame as (c). (e) An image obtained by cutting out a generated moving image by edge detection at the same frame as (c).

Results

Representative magnified images of samples from six WWTPs are shown in Figure 2. SS_{SCE} , $MLSS$, SVI_{30} , $\rho_{M/A}$, AR , RN , FD , A/F , D_{eqHD} , and P_{AF} for each sample are listed in Table 2. SS_{SCE} was lowest in F-WWTP, with G-, B-, C-, A-, and E-WWTP having higher values in increasing order (Figure 3a). This possibly indicates that the treatment performance of the settlement process in SCE was also in this order when considering particles in the supernatant after AS settling. None of them exceeded Japanese wastewater effluent standard of 40 mg/L, indicating that the treatment was good. SVI_{30} , $\rho_{M/A}$, AR , RN , FD , A/F , D_{eqHD} , and P_{AF} of all WWTPs are shown in Figure 3b–3i in the same order as SS_{SCE} . Although SVI_{30} was the largest in C-WWTP, the SS_{SCE} was smaller than that of A- and E-WWTPs (Figure 3a and 3b). In addition, although SS_{SCE} was extremely small in both F- and G-WWTP, the sedimentation performance of G-WWTP was close to that of A- and E-WWTPs, which showed the largest SS_{SCE} (Figure 3a and b).

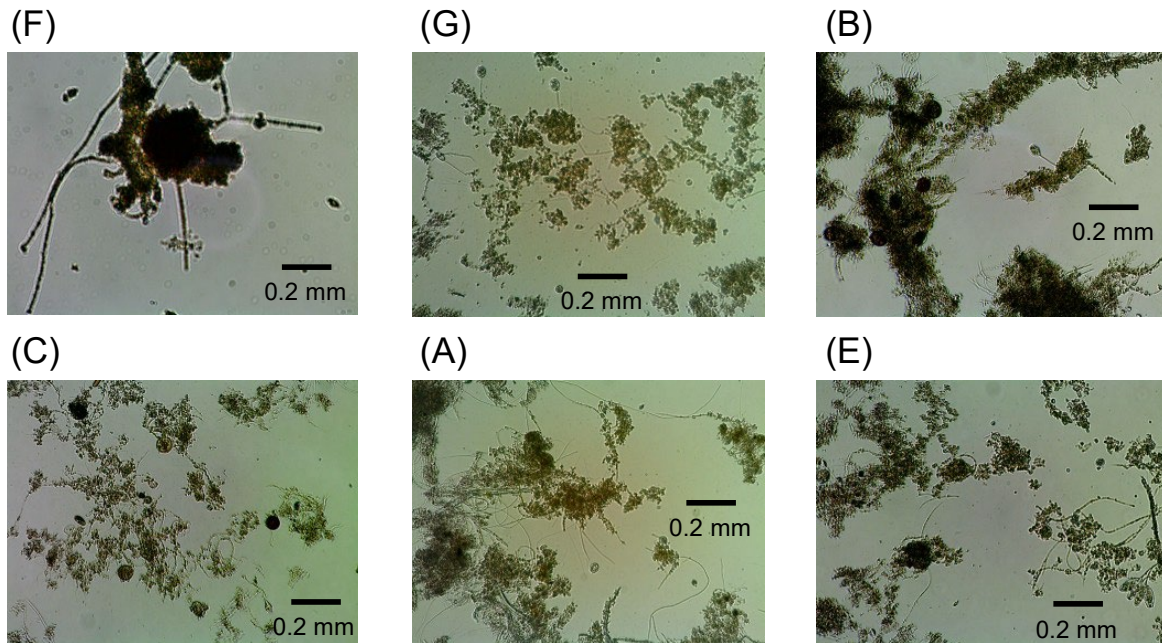


Figure 2. Representative magnified images of F-, G-, B-, C-, A-, and E-WWTPs.

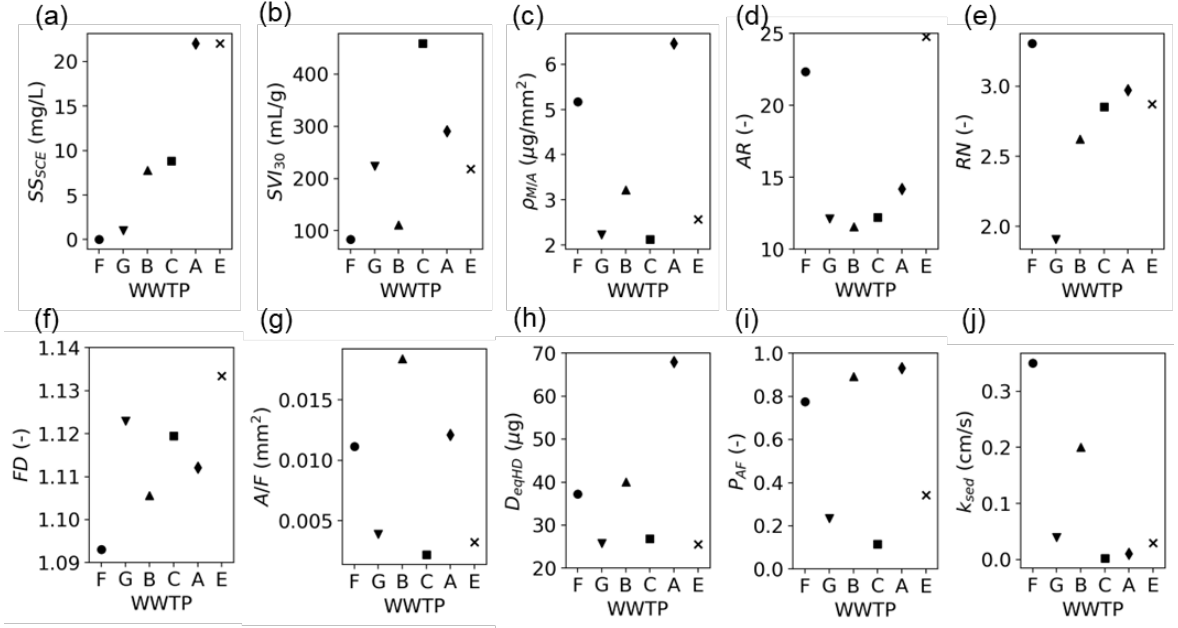


Figure 3. (a) SS_{SCE} , (b) SVI_{30} , (c) $\rho_{M/A}$, (d) AR , (e) RN , (f) FD , (g) A/F , (h) D_{eqHD} , (i) P_{AF} , and (j) k_{sed} of the AS samples collected from WWTP-F, -G, -B, -C, -A, and -E.

Table. 2. Sampling date, SS_{SCE} , $MLSS$, SVI_{30} , $\rho_{M/A}$, AR , RN , FD , A/F , D_{eqHD} , and P_{AF} of the AS samples collected from F-, G-, B-, C-, A-, and E-WWTPs, with parameters k_{sed} and b_{sed} obtained by approximating the linear function of descent of the solid–liquid interface in AS and R^2 values.

WWTP unit	SS_{SCE} mg/L	$MLSS$ mg/L	SVI_{30} mL/g	$\rho_{M/A}$ $\mu\text{g}/\text{mm}^2$	AR -	RN -	FD -	D_{eqHD} μm	A/F mm^2	P_{AF} -	k_{sed} cm/s	b_{sed} cm	R^2 -
F	0	1560	83	5.2	22	3.3	1.09	37	0.011	0.78	0.35	-2.6	0.99
G	1	1520	224	2.2	12	1.9	1.12	25	0.0039	0.23	0.04	4.2	0.99
B	7.8	1349	111	3.2	12	2.6	1.11	40	0.018	0.89	0.20	-2.6	0.99
C	8.8	2160	458	2.1	12	2.9	1.12	27	0.0022	0.12	0.002	0.3	0.99
A	22	1790	291	6.5	14	3.0	1.11	68	0.012	0.93	0.011	0.4	0.99
E	22	2564	218	2.6	25	2.9	1.13	26	0.0032	0.34	0.03	1.8	0.99

Figure 4 shows the pose images of the original (raw) moving image and the moving-image output from Insect Eye between the start of shooting to 30 minutes later ($t = 0, 30, 60, 300$, and

1200 s) for B-WWTP. The original (raw) moving images of A-, B-, C-, E-, F-, and G-WWTPs are provided at 30× speed in Video A-1, Video B-1, Video C-1, Video E-1, Video F-1, and Video G-1, respectively. The analyzed moving-image output from Insect Eye (30× speed) of A-, B-, C-, E-, F-, and G-WWTPs are provided in Video A-2, Video B-2, Video C-2, Video E-2, Video F-2, and Video G-2, respectively. Values of the difference outputted from Insect Eye (*AoC*, indicating amount of change) in the horizontal mesh was averaged at each frame interval. Through this, a one-dimensional profile of *AoC* across the depth-wise direction was obtained at each time. Since this can be obtained for every frame interval (1799 over 30 minutes) in the moving image, it can be plotted as a contour map of *AoC* as two-dimensional information in which the horizontal axis is time and the vertical axis is depth. This is called a time-series heat map in the depth-wise direction of AS settlement, and it is shown in Figure 5 for each WWTP sample. The first 30 seconds were excluded from analysis because of the influence of turbulence when AS was poured. The movement of the AS/supernatant interface²⁸ was clearly recognized by the peak of *AoC* caused by the rapid replacement of the AS with the background when the interface passed through the mesh. In Figure 5F (F-WWTP), a consolidated region with little change in the elements of the moving image was generated in the first 120 seconds, and sediment surface²⁸ rose quickly but gradually descended. It was also observed that a relatively large *AoC* occurred above the interface because of the settling of the AS left in the supernatant. It is suggested that the AS in the supernatant settled and was taken in below the interface within about 500 seconds. In Figure 5G (G-WWTP), the difference in the supernatant was not as remarkable as that in F-WWTP. However, in the first 450 seconds, a region with large *AoC* occurred below the interface. This corresponds to the generation of gaps where the background became temporarily visible because of the aggregation of AS. The aggregated AS gradually settled, and the consolidated part of the AS (the

sediment surface) rose, causing the interface to fall and fill the space created by the aggregation. The heat map of B-WWTP (Figure 5B) was similar to that of F-WWTP, but the AS settling in the supernatant was faster than that in F-WWTP. In Figure 5C (C-WWTP), the sedimentation of the interface occurred extremely slowly and at a short distance, with almost no solid–liquid separation being observed in 30 minutes. A small *AoC*, which seemed to be due to the aggregation of AS, was observed below the interface, but the growth of the consolidated region and the sediment surface were not clearly observed. In the AS of A-WWTP, the interface settled earlier than in C-WWTP, and AS aggregation under the interface was more active. It was observed that the consolidation region extended from the bottom at around 600 seconds. The settling of the AS in E-WWTP was similar to that in G-WWTP. However, AS settlement above the interface (in the supernatant) was not clearly observed.

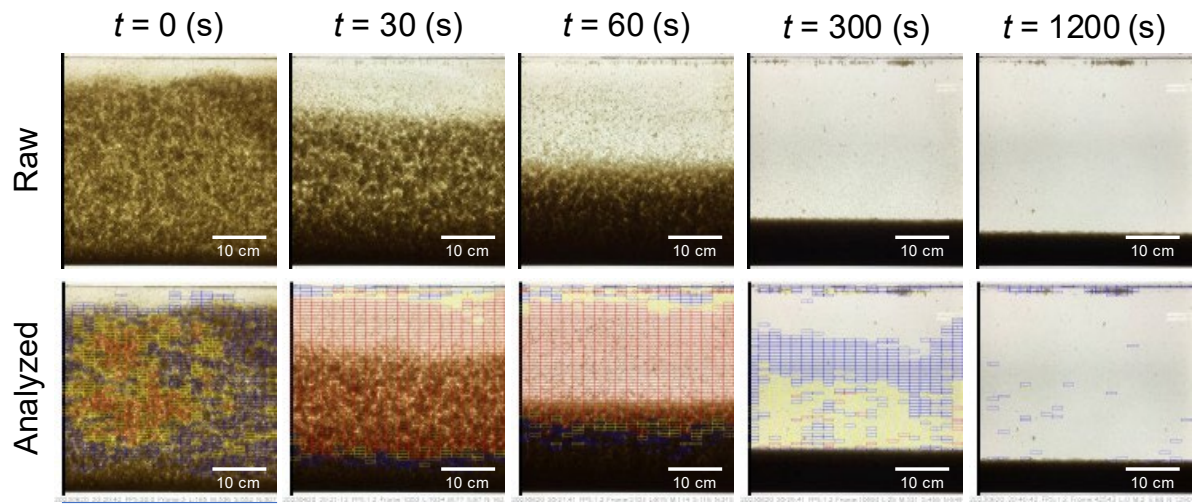


Figure 4. Pose images of the original (raw) moving image (top 5 images) and the analyzed moving-image output from Insect Eye (bottom 5 images) between the start of shooting and 30 minutes later ($t = 0, 30, 60, 300, \text{ and } 1200 \text{ s}$) for B-WWTP.

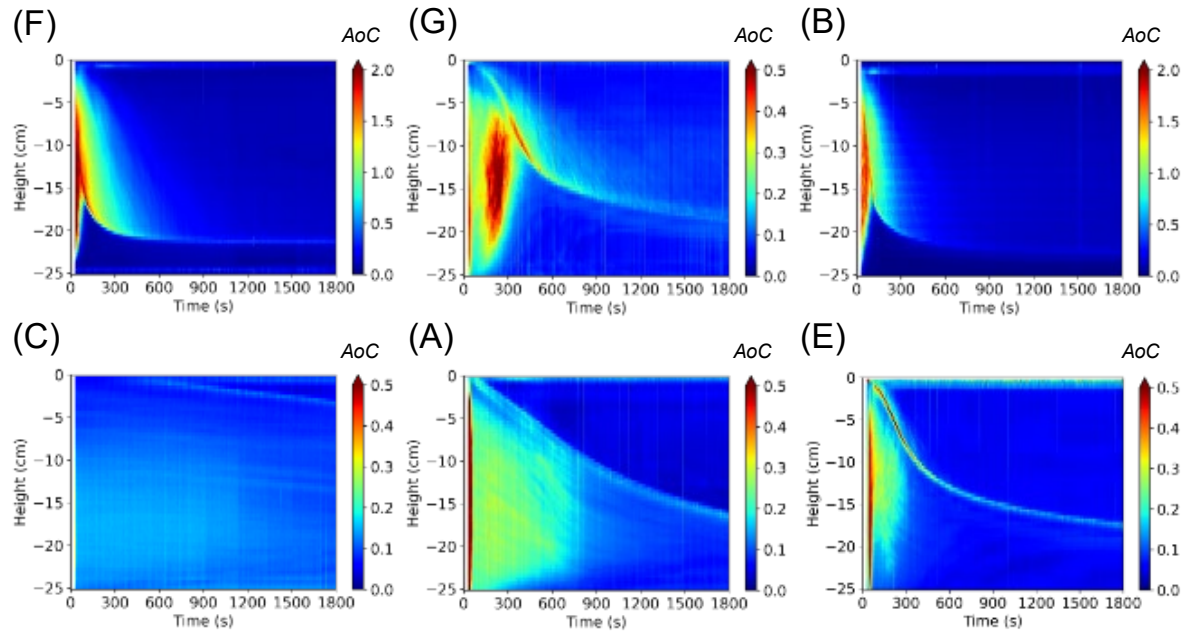


Figure 5. Time-series heat maps of *AoC* (the difference value outputted from Insect Eye) in F-, G-, B-, C-, A-, and E-WWTPs.

Figure 6a shows changes in the interface position over time resulting from analysis by edge detection of the moving images of AS settling. The moving images of A-, B-, C-, E-, F-, and G-WWTPs in which the interface was highlighted (30× speed) are provided in Video A-3, Video B-3, Video C-3, Video E-3, Video F-3, and Video G-3, respectively. The interface recognized by edge detection for all WWTPs (Figure 6a) exhibited almost the same trends as the interface recognized in Figure 5. In the AS of F- and B-WWTPs, the interface rapidly settled, and then the interface sedimentation velocity gradually slowed, and the interface hardly moved after 15 minutes (Figures 5F, 5B, and 6a). In the AS of G- and E-WWTPs, it took about 3 minutes for interface descent to be recognized, after which it settled at a constant speed for about 3 minutes until the sedimentation velocity gradually decreased (Figure 5G, 5E, and 6a). In the AS of C- and A-WWTPs, interface sedimentation was relatively slow, and the change in interface sedimentation

velocity was not clearly observed, but the sludge seemed to settle at a constant sedimentation velocity (Figure 5C, 5A, and 6a). In samples from G-, C-, A-, and E-WWTPs, the interface seemed to completely settle after the end of the experiment, but it was difficult to analyze settling for more than 30 minutes as the interface could rise due to foaming caused by denitrification. The apparent interface sedimentation velocity (k_{sed}) was obtained by fitting the slope in the region in which the interface position settled at a constant speed with a linear function (Figure 6b), and it was compared in Figure 3j for each WWTP. The slope (k_{sed}), intercept (b_{sed}), and R^2 values of each linear fit are shown in Table 2.

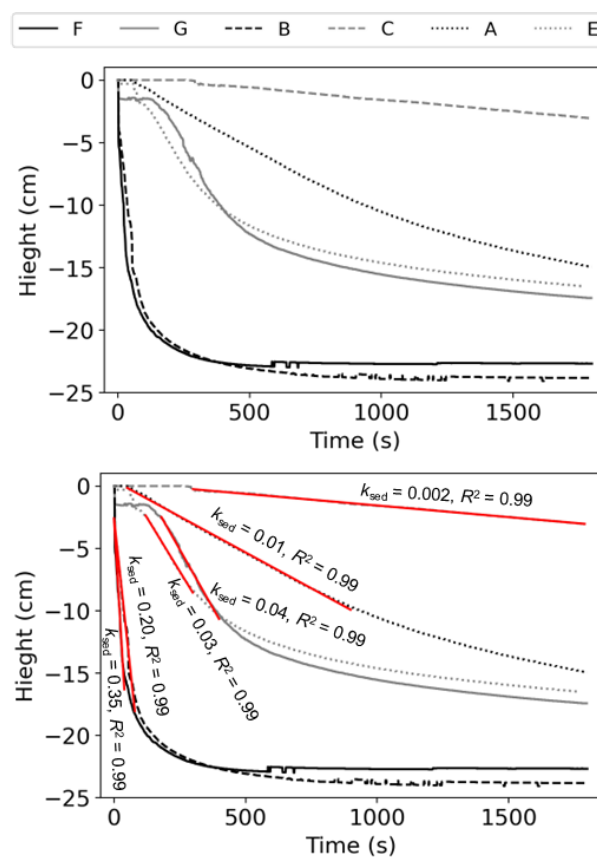


Figure 6. (a) Change over time in the interface position of the moving images of AS settling, and (b) plot with lines (red) fitted by a linear function.

Discussion

The sediment surface is supposedly more difficult to track by visual observation than the AS/supernatant interface²⁸. However, it was possible to visualize the sediment surface of F-, G-, B-, A-, and E-WWTPs in the cases by setting an appropriate threshold during video analysis. In the previous study's model²⁸, the sediment surface was assumed to be a curve (L curve) in which the rise gradually becomes gentle, but such behavior could be recognized only in A-WWTP (Figure 5A). In F-, G-, B-, and E-WWTPs, the rise of the sediment surface gradually accelerated, and when it merged with the AS/supernatant interface, the growth of the consolidation region stopped and the interface transitioned into a gentle state. Such lowering of the interface is often quantified as the zone settling velocity by fitting with an exponential curve²⁹. The change of AS/supernatant interface position over time visualized by the heat map agreed with the trace from edge detection, and both of these methods can be used to quantitatively analyze sedimentation velocity.

From the magnified images, it can be considered that AS in F-, B-, and A-WWTPs was well-aggregated sludge, while the others (G-, C-, and E-WWTPs) were dispersed (Figure 2). This can be quantitatively explained from the relatively large values of $\rho_{M/A}$, A/F , D_{eqHD} , and P_{AF} (Figure 3c, 3g, 3h, and 3i). In addition, from the values of FD , it can be understood that AS in F-, B-, and A-WWTPs had a more complicated shape than that in G-, C-, and E-WWTPs (Figure 3f). According to the moving-image analysis and SVI_{30} of F- and B-WWTPs, the interface rapidly settled, and the sludge above the interface settled sufficiently in 30 minutes (Figure 3b, 3j, 5F, and 5B). However, SS_{SCE} was 0 mg/L in F-WWTP and 7.8 mg/L in B-WWTP. There is a possibility that a large number of small solid particles, which cannot be seen in the video, were left in the supernatant in the settling of B-WWTP. Recent research²⁵ reported that such minute solid in the supernatant can

be captured when AS of a visible size settles. According to this report, it has been proposed that AS with a large amount of filamentous substances may have a higher capacity for capturing solid particles. Indeed, there were more filamentous substances in the AS of F-WWTP than in the AS of B-WWTP, which agrees with the result in the previous study by Kaushalya and the authors. In addition, the fact that the *AR* of F-WWTP was larger than that of B-WWTP may indicate that more elongated AS was present in F-WWTP. This suggests that it is possible to reduce SS_{SCE} through a mop-like capturing effect by filamentous substances and/or elongated sludge.

On the other hand, the SS_{SCE} of A-WWTP was the largest among WWTPs with aggregated AS (F-, B-, and A-WWTP) (Figure 3a). The interface was sufficiently lowered in 30 minutes, but unlike F- and B-WWTPs, there were no sludge particles that were observable by moving-image analysis in the region above the interface (Figure 5A). The shape of solids above the interface was too small to be recognized in the moving image, but it could still be present in the supernatant, escaping the trapping and coprecipitation phenomena in the supernatant. In addition, the rate of aggregation under the interface could be slower in A-WWTP than in B-WWTP (Figure 5). AS in A-WWTP is relatively rich in filamentous substances (Figure 2A), and it has the highest *FD* (Figure 3f), suggesting complicated irregularities in its shape and potentially preventing aggregation. This is generally called filamentous bulking³⁰.

Similar to A-WWTP, AS of F-WWTP contained filamentous substances (Figure 2). Nevertheless, a region with a large *AoC* under the interface rapidly disappeared because of the lowering of the interface (Figure 5F). This means that the amount of filamentous substances in the sludge of F-WWTP was not so large as to prevent the flocculation of the sludge, and the heavy flocs rapidly settled. In the case of F-WWTP, it was observed that some sludge remained in the supernatant and gradually settled. The reason why SS_{SCE} was small in F-WWTP is likely due to

the high capacity of the sludge remaining above the interface, which acts like a mop that captures minute particles. From the viewpoint of reducing the SS in the SCE, an ideal sludge should have heavy parts settling quickly, and it must contain a moderate amount of filamentous and elongated parts such that mop-like properties that capture small solids are preserved but aggregation is not hindered. In the future, it is necessary to analyze in detail the behavior of minute solids, as well as capture and coprecipitation phenomena.

AS in the G-, C-, and E-WWTPs was small and dispersed (Figure 2), with relatively large FD values, indicating complicated irregularities on the surface (Figure 3f). It was proposed that the proximity of the sludge is inhibited by such features³⁰ and FD is correlated with large SVI_{30} values (i.e., difficulty to sink) (Figure 3b). In particular, AS from C-WWTP had little aggregation below the interface compared to other WWTPs (Figure 5C), confirming that small dispersed sludge has a tendency to cause interfaces to rise, as suggested by previous papers^{25,26}. However, SS_{SCE} of C-WWTP was lower than that of A- and E-WWTPs, which displayed better sedimentation than C-WWTP (Figure 3a and 3b). Since sedimentation in C-WWTP was extremely slow (Figure 3j), the minute solids might be steadily attached to the AS, resulting in low supernatant SS.

In both G- and E-WWTPs, interface sedimentation and aggregation of AS below the interface occurred on similar time scales and had similar SVI_{30} values (Figures 3b, 3j, 5G, 5E, and 6). However, SS_{SCE} of E-WWTP was extremely large compared to that of G-WWTP (Figure 3a). Compared to AS from E-WWTP, RN was very low in G-WWTP, suggesting that the AS was round-shaped (Figure 3e), which was also reflected in AR (Figure 3d). Such round-shaped particles of G-WWTP would be able to settle gradually and could explain low SS_{SCE} while solids remained in the supernatant of E-WWTP. It is suggested that the shape (roundness) of minute particles may become an important parameter in future studies on the behavior of solids left in the supernatant.

Potential limitations

In the acquisition of microscope images and moving images of AS, if the MLSS is too large, it is considered that floc overlaps with each other. In this study and previous studies^{20,25,26}, the upper limit of the MLSS that can be analyzed without being affected by the overlap and the lower limit of the MLSS that can detect a statistically significant number of flocs have not been discussed. These are potential limitations of the proposed methods and need to be studied in the future. In this study, it was succeeded to illustrate that the behavior of AS settling differs from plant to plant as shown in Figure 5. Regarding the bias of the analysis by Insect Eye, however, it is considered necessary to confirm the reproducibility of result acquired at the same WWTP on the same sampling day. In addition, it should also be noted that only microscopic images of aggregated or dispersed AS from two specific WWTPs were used as training data for the classifier used in the acquisition of the P_{AF} . It is considered that the visual discrimination of microscopic images of AS captures not only whether it is aggregated or dispersed but also the color and the presence or absence of filamentous bacteria. Therefore, it is necessary to use training data that is appropriately classified and labeled.

Future perspective

Although it is not yet known whether the behavior of AS and supernatant solids in the container actually occurs in the secondary clarifier due to the difference in system size, routine AS analysis based on still and moving images may be used for objective AS characterization without relying on skilled staffs. In addition, a smartphone was used for video recording, and analysis was performed only with light processing using frame subtraction. This approach proposes a method

that is advantageous for low-cost deployment of technology in small-scale treatment plants and developing countries. Still-image analysis was conducted by AI-supported technology, which enables objective analysis that eliminates arbitrariness by observers. These approaches are considered to be advantageous for low-cost deployment of technology to small-scale treatment plants and developing countries. It is not yet clear whether the method proposed in this study can be applied to other WWTPs or different types of sludge. Attention should be paid to the shape and behavior of minute solid components that may not have been recognized in the moving image but exist in the supernatant. By promoting their sedimentation, as well as capturing and/or coprecipitating with coarse AS, the reduction of SS concentration in treated water can be examined.

Conclusion

To improve the sedimentation of AS and SS concentration in treated water, morphological and physical factors relating to AS settling behavior were examined. AS from six municipal WWTPs were analyzed on the basis of moving images and still images and then compared with SVI and the actual SS concentration in SCE. The AS samples could be characterized as rapidly settling and leaving coarse solids in the supernatant, slowly coagulating and settling, and extremely slow settling, and it was shown that the speed of aggregation, the speed of settling of particles left in the supernatant, and adhesion of minute particles on coarse solids were possibly related to the shape of the AS. The specific characteristics of the WWTPs targeted by this study are summarized below. In our results, there were several WWTPs (F, G, and B) in which AS of a size recognizable in the moving image remained above the interface when the interface settled, and other WWTPs in which minute particles that could not be recognized in the moving image remained (C, A, and E). Large AS left above the interface gradually settled, and it was found that there were cases in which SS

concentration in the supernatant was reduced by settling while supplementing minute particles depending on the shape of AS (F-WWTP). There were also cases where solid particles in the supernatant were left without being captured (B-WWTP). It is proposed that this capturing effect may be improved by the existence of filamentous materials that increase the surface area of AS and/or flocs with geometric characteristics such as high FD. In addition, when there is less large AS with capturing abilities left on the interface, minute particles above the interface is only removable by its own sedimentation, and supernatant solids with small RN and AR (close to spherical shape) may settle more easily (G-WWTP). In the case of C-WWTP, the descent of the interface was extremely slow and SVI was large, but the SS concentration in the SCE was small, contrary to expectations. It is assumed that the cause was that the supernatant region was small and that there were many contact opportunities between minute particles and coarse AS solids.

Supporting Information

Files of 18 moving images converted to .mp4 composed of six original moving images (Video #-1_#-WWTP_OriginalMovingImage; # is name of WWTP), six video files outputted from Insect Eye (Video #-2_#-WWTP_InsectEyeOutput), and six video files outputted from edge detection (Video #-3_#-WWTP_EdgeDetectionOutput) can be found online at <http://hdl.handle.net/2115/95742>.

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Notes

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