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2 Deep learning-based morphology classification of activated sludge flocs in

3 wastewater treatment plants

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21 **ABSTRACT**

22 Microscopy inspection of the morphology of activated sludge (AS) flocs can provide
23 important information regarding the AS properties, which strongly affect the performance of AS
24 systems. However, the acquisition of such information from microscopy inspection results
25 requires skilled and specialized expertise. In this study, we aimed to develop two deep learning-
26 based two-label classifiers for recognizing aggregated or dispersed flocs (Classifier-1) and the
27 presence or absence of filamentous bacteria (Classifier-2). To achieve this, we used a
28 convolutional neural network (CNN)-based method and selected the pre-trained Inception v3 as
29 the CNN architecture. We developed an automatic microscopy image acquisition system,
30 enabling us to obtain 154 images for 7 min. Over 12,000 images of aggregated and dispersed
31 flocs were obtained from wastewater treatment plant (WWTP)-S and -E over 15 weeks.
32 Clasifier-1 was retrained using these images. Clasifier-1 distinguished the aggregated and
33 dispersed flocs with a training accuracy of approximately 95% and recognized a 20%
34 morphological change in the aggregated flocs. Classifier-1 also recognized the morphology of
35 AS flocs obtained from other WWTPs, the AS from which was used for retraining. Classifier-2
36 quantitatively recognized an abundance of filamentous bacteria in the AS flocs. These results
37 clearly indicated that the developed image classification model could serve as a useful warning
38 system for the settleability deterioration and abundance of filamentous bacteria in the aeration
39 tank of a full-scale AS system.

40

41

42 **1. Introduction**

43 Activated sludge (AS) systems are the most common type of biological wastewater treatment

44 technology and are applied worldwide. AS contributes to the biological conversion of

45 contaminants in AS systems. AS consists of a complex microbial ecosystem composed of a

46 variety of microorganisms (filamentous and floc-forming bacteria, protozoa, and metazoa) and

47 abiotic elements (extracellular polymers and debris) 1,2. These components form a floc, the

48 physical structure of which reflects the water quality and operating conditions of the wastewater

49 treatment system. Owing to the complex nature of microbial communities in a floc, imbalances

50 may occur among the different microorganisms, thereby leading to a change in the floc structure.

51 This causes a serious deterioration in the performance of AS systems, with profound economical

52 and environmental consequences owing to the strict regulations regarding effluent quality.

53 Therefore, the detection of disturbances is definitely an important task for the stability and

54 enhancement of process performance in wastewater treatment plants (WWTPs) 3.

55

56 A major drawback of AS systems that is relevant to the floc structure is the poor settling ability

57 of the AS for separation from the treated effluent by settling in the secondary sedimentation tank.

58 This is associated with the loose structure and lower density (that is, bulking), which frequently

59 results in sludge washout with the final effluent, consequently causing poor effluent quality as

60 well as deteriorated dewatering and thickening sludge properties 4–6. Therefore, the sludge

61 settling ability is crucial to the efficient operation of the AS system, and it is commonly

62 quantified by the sludge volume index (SVI). Various factors, such as the AS flocculation,

63 stability, aggregate size, morphology, density, and chemical composition, affect the AS floc

64 settling ability 5. Some of these factors can be visualized by microscopy inspection, which is a

65 very useful method for the rapid diagnosis of AS process malfunctions 7. The regular monitoring
66 of the microscopic parameters of AS flocs may provide important information regarding the
67 dynamic changes in the water quality and operational parameters of an AS system. Correlations
68 have been identified between the AS floc properties and operational conditions. These include
69 the biochemical to chemical oxygen demand ratio (BOD:COD), carbon to nitrogen ratio (C:N),
70 toxic compounds inhibition, hydraulic and sludge loading rate, recirculation ratio, aeration
71 equipment, substrate uptake rate, oxygen uptake rate, treatment efficiency, excess sludge
72 production, and SVI 1. Therefore, it is generally considered that the characterization of AS flocs
73 by microscopy inspection can provide important information regarding the biological processes
74 leading to the operational conditions of an AS system. However, automatic AS floc classification
75 is desirable, as the acquisition of information relating to the morphology of AS flocs from
76 microscopy inspection results requires skilled and specialized expertise.

77

78 In this sense, microscopy techniques coupled with quantitative image analysis (QIA) of AS flocs
79 may offer an advantage for the prediction purposes of an AS system. QIA applications have been
80 employed for the determination of the aggregated and filamentous bacteria contents as well as
81 the floc structure to assess the AS operating parameters 1,3,8–11. All of the studies mentioned
82 above demonstrated that image processing and analysis methodologies could provide valuable
83 morphological information (for example, the surface, area, perimeter, perimeter/area ratio,
84 circularity, minimum and maximum caliper diameter, and specific filament length 1) as well as
85 physiological information (such as the Gram-positive/Gram-negative and viable/damaged
86 bacteria ratios 3) on AS flocs, particularly in terms of the characteristics of filamentous bacteria
87 8,9. Therefore, at present, such technologies are recognized as valid monitoring tools 3. Indeed,

88 the possibility of accurately monitoring the filamentous bacteria contents, and of characterizing
89 and quantifying the aggregated biomass contents, size stratification, and structure using such
90 simple, quantitative, automated, and near-real-time methodology, overwhelms the conventional
91 monitoring methods.

92

93 Although the current QIA technologies described above are quite powerful tools for analyzing
94 the physical properties of AS flocs, these require extensive pre-processing of images, such as
95 object segmentation, background determination and removal, debris elimination, and image
96 enhancing operations 3,5,8. This process is laborious and time consuming, and may pose the risk
97 of subjective definitions (differences of appreciation between operators) of the threshold values
98 for the differentiation between the objects and background 5, which may also lead to important
99 information being discarded. Furthermore, the International Water Association Digital Water
100 Group emphasizes the importance of the development of new systems based on cyber-physical
101 systems, digitalization, and big data to enable informed decisions in increasingly changing,
102 complex, and uncertain water issues 12. In this context, the currently available QIA technologies
103 are semi-automated techniques used by human researchers, and alternative technology is
104 urgently required to extract the discriminant features from the images automatically by removing
105 subjectivity.

106

107 The logical next step is to allow computers to learn the features that optimally represent the
108 images. This concept forms the basis of many deep learning algorithms in which models
109 (networks) are composed of numerous layers that transform the input data (such as images) into
110 outputs (for example, the probability of being classified into an appropriate label) 13. Deep

111 learning is a learning approach that automatically learns the feature representations from raw
112 data without any pre-processing. The most successful type of model for image analysis to date is
113 the convolutional neural network (CNN) 14. CNNs contain many layers that transform their
114 input with convolutional filters of a small extent. Extensive work has been conducted on CNNs,
115 which have been applied, for example, to medical image analyses 13. Researchers have applied
116 CNNs to large-scale image classification tasks to address problems in civil and environmental
117 engineering 15–17. Dung and Anh developed a vision-based method for concrete crack detection
118 and density evaluation 18. Their method identified the crack path with an average precision of
119 approximately 90%. Wu et al. constructed a CNN to classify images of the surfaces of fountains,
120 lakes, oceans, and rivers into sub-categories of clean and polluted water 19. They concluded that
121 the proposed CNN could substantially decrease the manual burden of users in observing the
122 images of surveillance cameras. Yurtsever and Yurtsever achieved the automatic classification of
123 microplastics in water and wastewater based on numerous microscopic images using a CNN 20.
124 The classification revealed accuracy rates of 97% and 89% for microbeads in pure water and
125 wastewater, respectively.

126

127 Inspired by the success of CNNs for image classification tasks, in this study, we used a CNN-
128 based method to achieve the automatic classification of AS flocs in full-scale WWTPs based on
129 morphological properties. We selected Inception v3 as the CNN architecture, which is a network
130 that is pre-trained on a large-scale image classification task 21. Firstly, we developed an
131 automatic microscopy image acquisition system using only free software to obtain a sufficient
132 number of training images. Using the images, two types of two-label classifiers were developed
133 for recognizing aggregated or dispersed flocs and the presence or absence of filamentous

134 bacteria. The sensitivity of the classifier was estimated and the weekly changes in the
135 morphological characteristics of the AS flocs in WWTPs were investigated. Furthermore, the
136 versatility of the classifier was confirmed by analyzing the morphology of AS flocs obtained
137 from six different WWTPs. The evolution of filamentous bacteria in the AS was also analyzed
138 using another classifier and was correlated to the SVI values. In all of the tests, the results of the
139 classification conducted by the classifiers were compared with those obtained by humans.

140

141 **2. Materials and Methods**

142 AS samples

143 In this study, the AS samples were collected from aeration tanks in eight WWTPs,
144 denoted by S, E, N, M, Sh, H, K, and T, in five prefectures of Japan, all of which predominantly
145 treat domestic wastewater, between September 30th, 2019 and January 28th, 2020. A total of 40
146 mL of the sample was collected in a 50 mL sterile Falcon tube, transferred to our laboratory at
147 4 °C with neither feeding nor aeration, and subjected to image analysis within 2 days of the
148 sample collection. The physicochemical parameters of the AS samples and liquid in the aeration
149 tanks during a test period are described in Table S1. WWTP-S, -Sh, and -T were identical to
150 WWTP-A, -B, and -C described in a previous study 22.

151

152 Automatic acquisition of AS floc images

153 For the AS microscopic inspection, four 50 µL aliquots of mixed liquor were randomly
154 sampled from each AS sample. The sample was carefully placed on a glass slide by means of a
155 calibrated micropipette with a sectioned tip to minimize the disruption of the native floc
156 characteristics and to allow the largest AS floc to pass through. Thereafter, the sample was

157 covered with a coverslip (18×18 mm). The slide was fixed on a high-precision motorized XY
158 stage (mmu-60x; Chuo Precision Industrial Co., Ltd., Tokyo, Japan) 23. The XY stage with the
159 slide and the tabletop digital microscope (UM06; Kenis Ltd., Osaka, Japan) were covered with a
160 small darkroom. The slide was illuminated by a white LED light from a smartphone from the
161 back. We took images of floc under the same light condition to eliminate difference of color of
162 flocs among samples. The structure of the low-cost microscope and stage is presented in S1. All
163 digital images of the AS flocs on the slide were acquired with the digital microscope. The
164 tabletop digital microscope, which is commercially designed for classrooms, is manually driven
165 and lacks the ability to capture images automatically. Therefore, we installed free software
166 (UM6-CAM and Keytokey) on a computer to store images automatically every 3 s at a resolution
167 of 640×480 pixels with a corresponding area of 1.44×1.09 mm in a 24-bit JPEG image.
168 Furthermore, to capture numerous digital images of the AS flocs on the slides automatically, we
169 combined the microscope with the high-precision motorized XY stage described above, which
170 was controlled by a control driver (CAT-D 3-axis controller; Chuo Precision Industrial Co., Ltd.,
171 Tokyo, Japan) connected to a computer with free software (cat-cd3 and Keytokey) installed. The
172 field of view automatically meandered from the upper left corner to the lower right corner of the
173 object, which was adjusted stepwise to the next non-overlapped field of view every 3 s (Figure
174 S1). A total of 154 (14×11) images per slide were exhaustively acquired, which covered 73% of
175 the entire area of the object. Six slides were prepared so that a total of 924 images were acquired
176 per sample.

177

178 Devices, software, and models

179 In this study, we used an OptiPlex 7040 computer with the Windows 10 operating system
180 (version 1909). This machine has a 3.4 GHz Intel Core i7-6700 CPU processor and 8 GB 1,600
181 MHz DDR3 of RAM memory. Oracle VM VirtualBox version 6.0.14, which is a free and open-
182 source hosted hypervisor, was downloaded and installed. Thereafter, Ubuntu 16.04, which is an
183 open-source software operating system, was installed in VirtualBox. Furthermore, TensorFlow
184 1.11.0 with Python 2.7.12 bindings was installed and executed, with the following tools
185 downloaded and installed 24: (i) TensorFlow Hub was installed through the terminal with the
186 following instruction: "pip install tensorflow-hub" 24. TensorFlow Hub is a library for reusable
187 machine learning modules. (ii) The code retrain.py was downloaded 25. This code allows the
188 pre-trained Inception network to be adapted to other classification problems. (iii) The code
189 label_image.py was downloaded 26. This code uses the Inception v3 model to classify images
190 that are passed to the model (a classifier) from the command line. These instructions indicate the
191 script that carries out the retraining (retrain.py) and classification (label_image.py) on the
192 Inception model. The Inception v3 model consists of a 22-layer network with 24M parameters.
193 Moreover, it indicates the labels that are used for training: WWTP-S and WWTP-E for
194 Classifier-1 or the absence and presence of filamentous bacteria for Classifier-2. The command
195 line used to retrain the Inception v3 model and to classify the images into the two classes is
196 illustrated in Figure S2. The path where the pictures could be found was as follows: --
197 image=sample/Sample001.jpg. In this path, there were two directories: one with the images of
198 WWTP-S for Classifier-1 (the absence of filamentous bacteria for Classifier-2) and another with
199 the images of WWTP-E for Classifier-1 (the presence of filamentous bacteria for Classifier-2).
200 The AS floc image for evaluation was selected and classified into one of two classes described
201 above, and displayed quantitatively with a certain probability.

202

203 **3. Results and Discussion**

204 Characteristics of AS flocs

205 Figure 1 presents typical images of the AS flocs obtained from WWTP-S and WWTP-E (see also
206 Figure S3). The AS flocs exhibited marked morphological differences between the two WWTPs.

207 The shape, structure, and strength of the AS flocs were considered to be three important
208 morphological characteristics 5,7. According to this definition, the AS flocs from WWTP-S
209 exhibited an irregular shape, a compact structure, and a dark color. These were firm flocs and the
210 surrounding liquid was clear. In contrast, the AS flocs from WWTP-E exhibited an open
211 structure and a light color (Figure 1). These were weak flocs, which were less dense and
212 particularly highly dispersed. As these weak flocs were connected to one another, individual
213 flocs could not be identified (Figure S3). The surrounding liquid was turbid, with pinpoint flocs
214 and small particles. The results could be explained by the difference in the physicochemical
215 parameters between the WWTPs. The physicochemical parameters of the WWTPs during the test
216 period are presented in Figure 2 and listed in Table S1. All three parameters relevant to the
217 sludge properties (mixed liquor suspended solids (MLSS), the sludge volume occupied by settled
218 sludge after 30 minutes (SV30), and SVI) were higher in WWTP-E than in WWTP-S, indicating
219 that AS bulking occurred in WWTP-E. Many studies have suggested that the threshold value for
220 activated sludge bulking was an SVI value of around 150 mL/g 27. The SVI values of WWTP-S
221 were only below the threshold. In contrast, the average SVI value was highest in WWTP-E so
222 that we selected WWTP-S and WWTP-E samples for the representation of aggregated and
223 dispersed flocs.

224

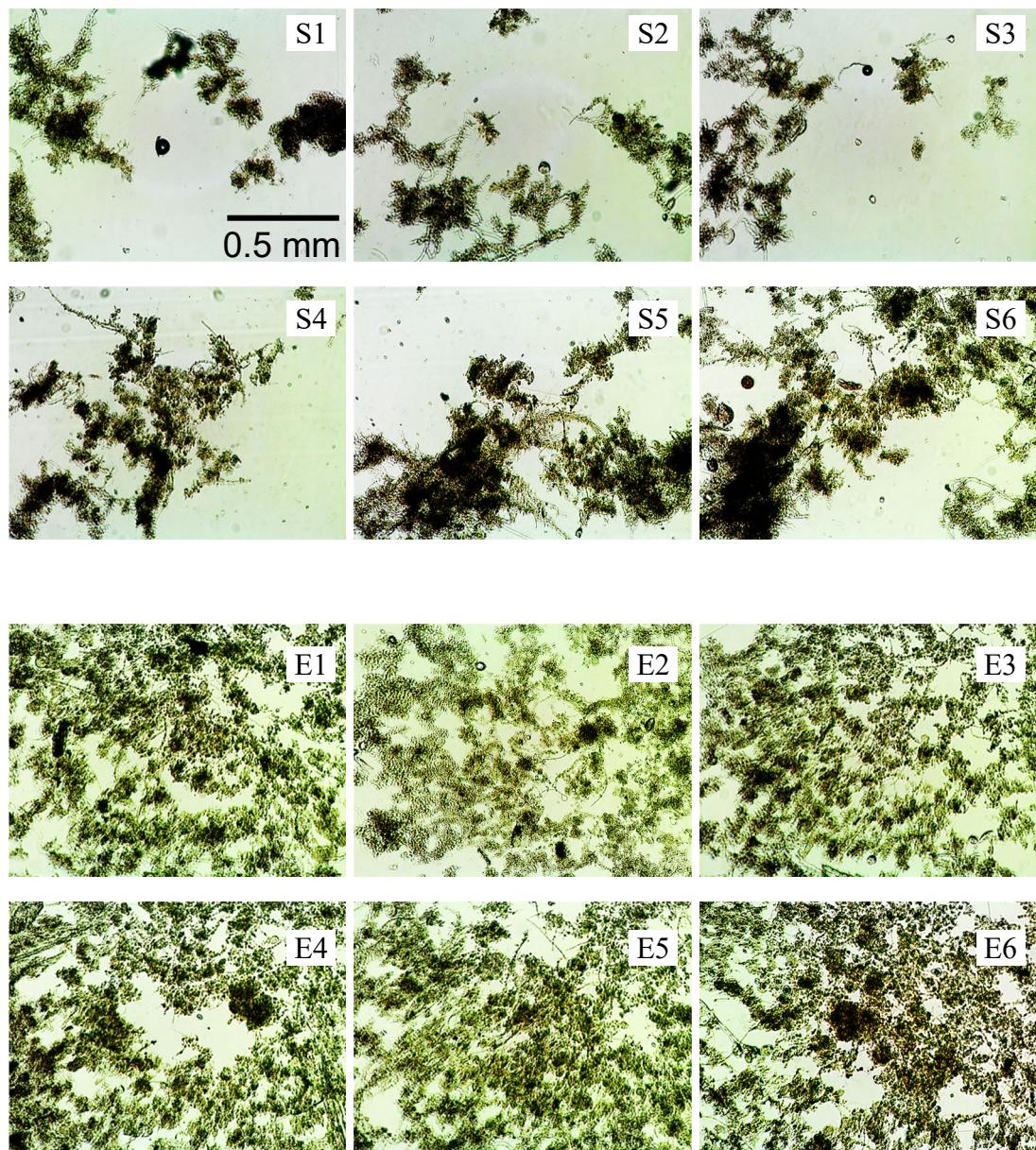


Figure 1. Typical microscopic images in bright field of AS flocs obtained from WWTP-S (S1 to S6) and WWTP-E (E1 to E6).

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226

227

228

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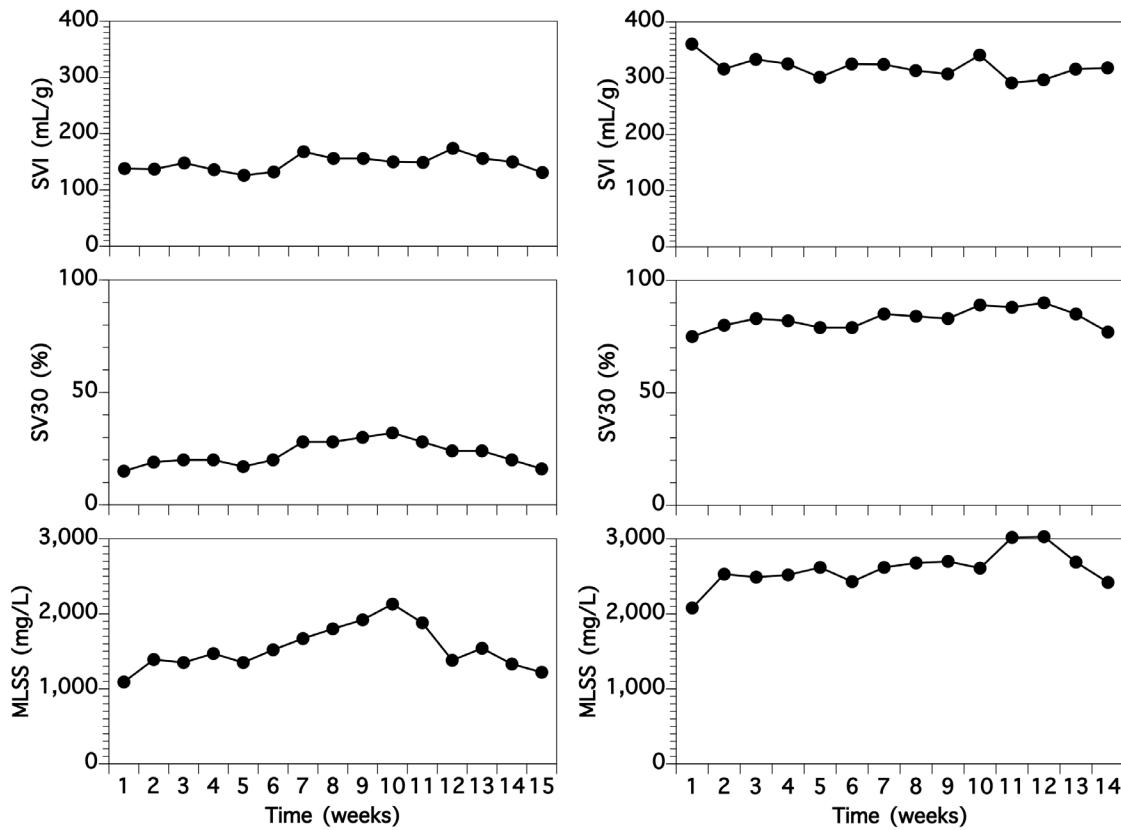


Figure 2. SVI, SV30, and MLSS of WWTP-S (left panels) and WWTP-E (right panels) during test period.

230

231 Development of image classification model and evaluation of training performance
 232 To develop the image classification model (Classifier-1), we obtained AS samples over 15
 233 weeks, and eventually accumulated 13,860 images of AS flocs for WWTP-S and WWTP-E. A
 234 split of 83%/17% was used for the training and test sets. The first step was to evaluate the
 235 training performance. In this study, we used the TensorFlow framework. TensorBoard is a
 236 TensorFlow histogram and graph visualization tool to ensure that the intended design is achieved
 237 28. Figure S4 indicates training accuracy of approximately 95% achieved in distinguishing the
 238 images of the AS flocs obtained from WWTP-S from those obtained from WWTP-E.

239

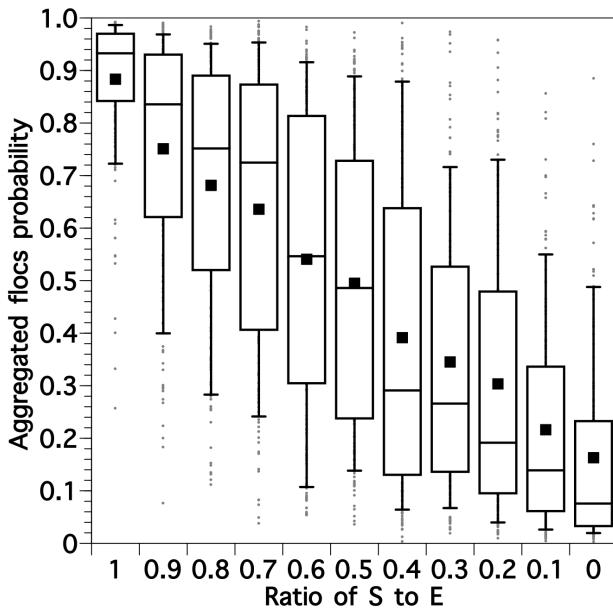


Figure 3. Boxplot of average aggregated flocs probability score of 154 images of AS flocs at various mixing ratios between AS from WWTP-S and WWTP-E. The rectangular box indicates the lower and upper quartiles and the median, the square plot indicates the average, and the whiskers indicate the 9th percentile and the 91st percentile. All other results are plotted as outliers.

240

241 Sensitivity analysis of Classifier-1

242 Prior to classifying the images of the AS flocs obtained from WWTP-S and WWTP-E, we
 243 investigated the sensitivity with which Classifier-1 could recognize the morphology (for
 244 example, the shape, structure, strength, and color) of the AS flocs. AS samples with different
 245 morphologies were prepared by mixing AS sludge samples obtained from two WWTPs at
 246 different volume ratios. The average aggregated flocs probability score of 154 images of the AS
 247 flocs from WWTP-S (that is, a ratio of 10:0) was 0.88 (Figure 3). As the volume ratio of the AS
 248 sample from WWTP-E to that from WWTP-S increased, the average probability score decreased
 249 linearly. Statistical analysis was conducted using the two-tailed student's t-test. The pair-to-pair
 250 p-values between 10% change of the volume ratio of the AS sample ranged from 0.057 to 0.51,

251 indicating that Classifier-1 could not recognize the change in morphology of the AS flocs. In
252 contrast, the pair-to-pair p-values between 20% change of the volume ratio of the AS sample
253 were <0.007, indicating that Classifier-1 could recognize the 20% change in morphology of the
254 AS flocs. The slope of the linear regression line of the average probability scores was estimated
255 using Microsoft Excel. The change rate of the average probability score per 100% change in the
256 sample volume was 0.69 with the correlation coefficient of the linear regression of average
257 probabilities of 0.996. These results indicated that the change in 0.138 of the average probability
258 score suggested the occurrence of a 20% morphological change in the original AS flocs of
259 WWTP-S.

260

261 We could also evaluate the recall based on the data at 1.0 of the S/E ration shown in Figure 3.
262 Defining that the result with >0.8 of the probability was positive, the recall was calculated to be
263 0.83.

264

265 To date, deep learning technologies have been applied successfully in various fields of civil and
266 environmental engineering with significant progress in machine learning techniques 15,17.

267 However, a critical factor that hampers the popularization of this technology in civil and
268 environmental engineering, particularly wastewater treatment systems, may be the necessity to
269 develop a program (or modify a existing program) that is adequate for the user's purpose 19,29.

270 Engineers of WWTPs are usually unfamiliar with writing a program. However, as demonstrated
271 above, the unmodified Inception v3 model could achieve classification of the morphology of AS
272 flocs with high sensitivity. This is because the two labels we adopted in this study were AS flocs
273 with a compact or dispersed structure and in the presence/absence of filamentous bacteria (see

274 below), which could easily be distinguished even by human eyes. Therefore, we are convinced
275 that the development of a novel deep learning program is not necessarily required to address
276 simple issues in AS systems. Another means of successfully achieving the classification of the
277 morphology of AS flocs could be to adopt Inception v3 as CNN architecture. Inception v3 is
278 open-source architecture developed by Google, and it is a CNN that is 48 layers deep. A pre-
279 trained version of the network trained on more than one million images from the ImageNet
280 database can be loaded. The pre-trained network can classify images into 1000 object categories,
281 such as keyboard, mouse, pencil, and many animals. As a result, the network has learned rich
282 feature representations for a wide range of images and is particularly suitable for large-scale
283 image classification tasks 21. However, these characteristics adversely become a drawback
284 because we cannot easily develop the purpose-designed model.

285

286 Classification of morphology of AS flocs with classifier-1
287 Subsequently, we attempted to recognize the weekly change in the morphological characteristics
288 of the AS flocs using the classifier. Figure 4 presents the average aggregated flocs probability
289 score of the tested AS flocs obtained from WWTP-S for 15 weeks (not sampled at the 9th week).
290 The average probability score for 15 weeks was 0.936. All of the averaged probability scores
291 were >0.798 (0.936–0.138). This indicated that the morphological characteristics of the AS flocs
292 did not change in a degree of >20% to dispersed and light-colored ones compared to the usual
293 ones. Figure 2 shows that both the SVI and SV30 of WWTP-S were stable for 15 weeks, which
294 supported the results of investigation with classifier-1.

295

296

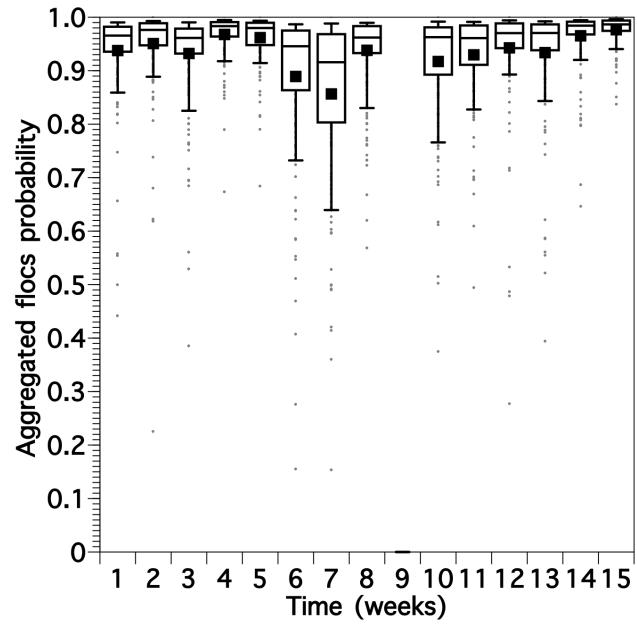


Figure 4. Boxplot of average aggregated flocs probability scores of 154 images of AS flocs obtained from WWTP-S over 15 weeks. The rectangular box indicates the lower and upper quartiles and the median, the square plot indicates the average, and the whiskers indicate the 9th and 91st percentiles. All other results are plotted as outliers.

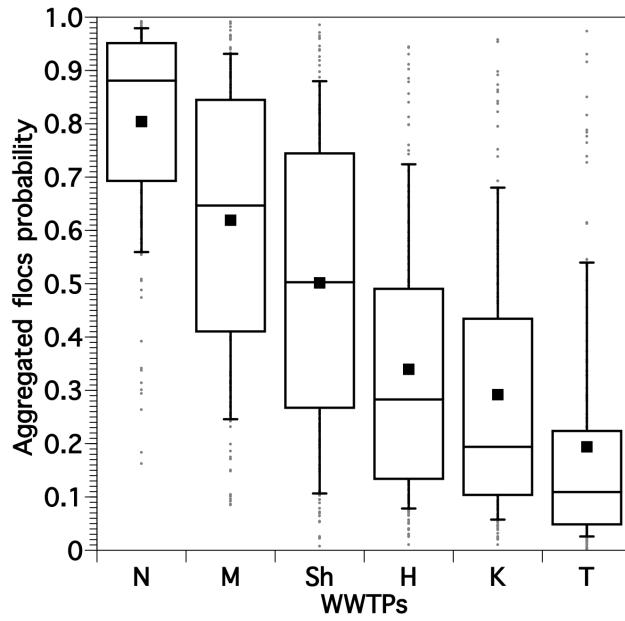


Figure 5. Boxplot of average aggregated flocs probability scores of 154 images of AS flocs obtained from six different WWTPs. The rectangular box indicates the lower and upper quartiles and the median, the square plot indicates the average, and the whiskers indicate the 9th and 91st percentiles. All other results are plotted as outliers.

299 Furthermore, we attempted to recognize the distinct morphological changes in the flocs from
300 WWTP-S. As we could not obtain flocs with clearly different morphological characteristics (for
301 example, open, light-colored, less dense or dispersed flocs) from the full-scale WWTP-S, the AS
302 was cultivated in our laboratory with vigorous aeration to disperse the floc structure
303 intentionally. The SVI of the flocs prior to the cultivation was 78 mL/g. The SVI increased to
304 128 mL/g after 1 day and to 157 mL/g after 2 days of cultivation, indicating poor settleability.
305 The microscopy inspection demonstrated that the flocs were dispersed and their size decreased
306 (Figure S5). The averaged probability scores decreased significantly from 0.94 to 0.38 during 2
307 days of cultivation (Figure S6), indicating that Classifier-1 could recognize the morphological
308 changes (mainly dispersion) in the flocs from WWTP-S even though the images originated from
309 WWTP-E were used for the retraining of Classifier-1.

310

311 To investigate the versatility of the classifier developed in this study, we applied Classifier-1 to
312 recognizing the morphology of AS flocs obtained from WWTPs other than WWTP-S and -E.
313 The order of the averaged aggregated flocs probability scores from highest (similar to WWTP-S)
314 to lowest (similar to WWTP-E) was WWTP-N, M, Sh, H, K, and T (Figure 5). Images of the
315 flocs obtained from these WWTPs were shown in Table S1. The flocs from WWTP-N exhibited
316 a compact structure and dark color. These were firm flocs and the surrounding liquid was clear,
317 and these characteristics were quite similar to those of the flocs from WWTP-S. In contrast, the
318 flocs from WWTP-T exhibited an open structure and a light color. These were weak flocs, which
319 were less dense and highly dispersed, and the surrounding liquid was turbid. Their characteristics
320 were quite similar to those of the flocs from WWTP-E. The flocs from WWTP-H and WWTP-K
321 exhibited similar morphologies to those of the flocs from WWTP-E and WWTP-T but the

322 density of the flocs from WWTP-H and WWTP-K appeared to be lower than that of the flocs
323 from WWTP-E and WWTP-T. In particular, the flocs from WWTP-K were connected to one
324 another with filamentous bacteria so that individual flocs could not be identified. The flocs from
325 WWTP-M and WWTP-Sh exhibited similar morphologies to those of the flocs from WWTP-S
326 and WWTP-N, but the color of the flocs from WWTP-M and WWTP-Sh appeared to be lighter
327 than that of the flocs from WWTP-S and WWTP-N. A comparison of the results obtained from
328 the analysis by Classifier-1 and the microscopy inspection revealed that the estimation of the
329 averaged probability scores by Classifier-1 evidently enabled the classification of the
330 morphological similarity of the flocs from each WWTP into two morphologically distinct labels.

331

332 However, the order of the density of the AS flocs described above (Figure 5) was not completely
333 identical to the order of SVI: S < Sh = T < M < E (Table S1). This was also probably because
334 parameters other than the morphology of the flocs could influence the SVI. An improvement in
335 the correlation between the results from a classifier and conventional water quality monitoring
336 could be achieved by selecting a training dataset that is more appropriate for the purpose.

337

338 Classification of filamentous bulking occurrence with classifier-2

339 Furthermore, we attempted to develop an early warning system for the occurrence of filamentous
340 bulking in an AS system. To achieve this, we constructed a new classifier (Classifier-2). AS
341 samples were obtained from December 10th, 2019 to January 28th, 2020 at WWTP-M, where
342 filamentous bulking has occurred every winter. In this case, 924 images were acquired per
343 sample, as described above. We gathered AS samples at 6 weeks. A split of 75%/25% of images
344 at the 1st (December 10th) and 4th (January 14th) weeks was used for the training and test sets,

345 respectively, because the abundance of filamentous bacteria was the lowest at the 1st week and
346 additional filamentous bacteria were found at the 4th week (see Figure S8) and the SVI of the AS
347 were 191 mL/g and 352 mL/g, respectively. Therefore, the images at the 1st and 4th weeks were
348 used to retrain Classifier-2 and were used as the two respective classification labels (the absence
349 or presence of filamentous bacteria).

350

351 The SVI of the AS of WWTP-M fluctuated from 120 mL/g to 270 mL/g by the middle of
352 December, whereas it increased gradually thereafter (Figure S7). It reached the peak at the
353 middle of January and then decreased slightly. Figure S8 presents images of the flocs obtained
354 from WWTP-M during the test period. The abundance of filamentous bacteria increased
355 obviously over time from December 10th to January 14th, and it was comparable thereafter. The
356 averaged bulking probability score was 0.85 on December 10th, with a sudden decrease to 0.47
357 on December 24, and it was comparable thereafter (Figure 6). The correlation coefficient
358 between the averaged bulking probability score and the SVI over the operational period was
359 0.67. Thus, Classifier-2 developed in this study could detect the abundance of filamentous
360 bacteria in AS flocs, which was roughly correlated with the SVI.

361

362

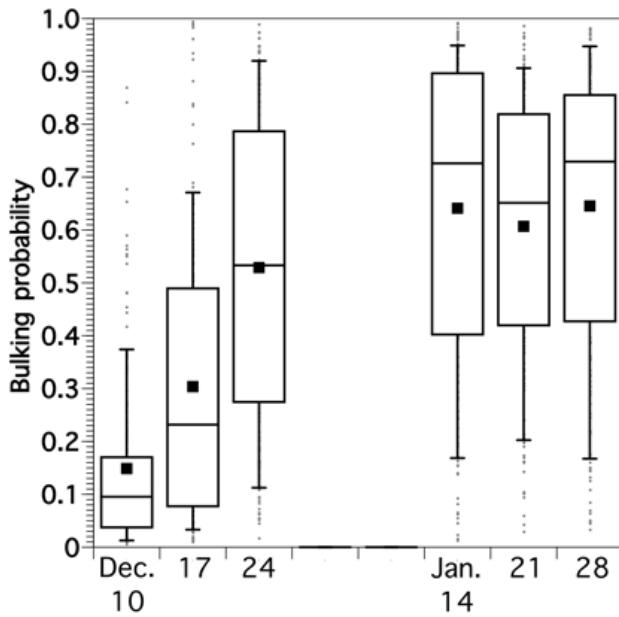


Figure 6. Boxplot of average bulking probability scores of 154 images of AS flocs obtained from WWTP-M. The rectangular box indicates the lower and upper quartiles and the median, the square plot indicates the average, and the whiskers indicate the 9th and 91st percentiles. All other results are plotted as outliers.

363

364 **4. Conclusions**

365 We developed an image classification model (classifiers) using a CNN-based method.
 366 Clasifier-1 distinguished the aggregated flocs from the dispersed ones with high training
 367 accuracy (approximately 95%) and could recognize 20% morphological changes in the
 368 aggregated flocs to dispersed ones. We selected the pre-trained Inception v3 as the CNN
 369 architecture. Inception v3 is open-source architecture developed by Google and is a widely used
 370 image recognition model. As it has been trained on more than one million images from the
 371 ImageNet database, we could use it without any modification or adaptation for the recognition of
 372 AS flocs. These findings imply that the features of Inception v3 can accelerate the popularization
 373 of deep learning-based image classification technology to analyze digital images obtained in an
 374 AS system automatically. We could develop an automatic microscopy image acquisition system

375 with an inexpensive microscope, and are currently developing an auto-sampler of mixed liquor in
376 an aeration tank and a microfluidic device for automatic image acquisition. In the future study,
377 we will develop a single simple coherent system. Eventually we will develop a useful warning
378 system for the settleability deterioration and abundance of filamentous bacteria in the aeration
379 tank of an AS system in the near future, which will contribute to the stability and even
380 improvement in the performance of wastewater treatment processes.

381

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385 in the public, commercial, or not-for-profit sectors.

386

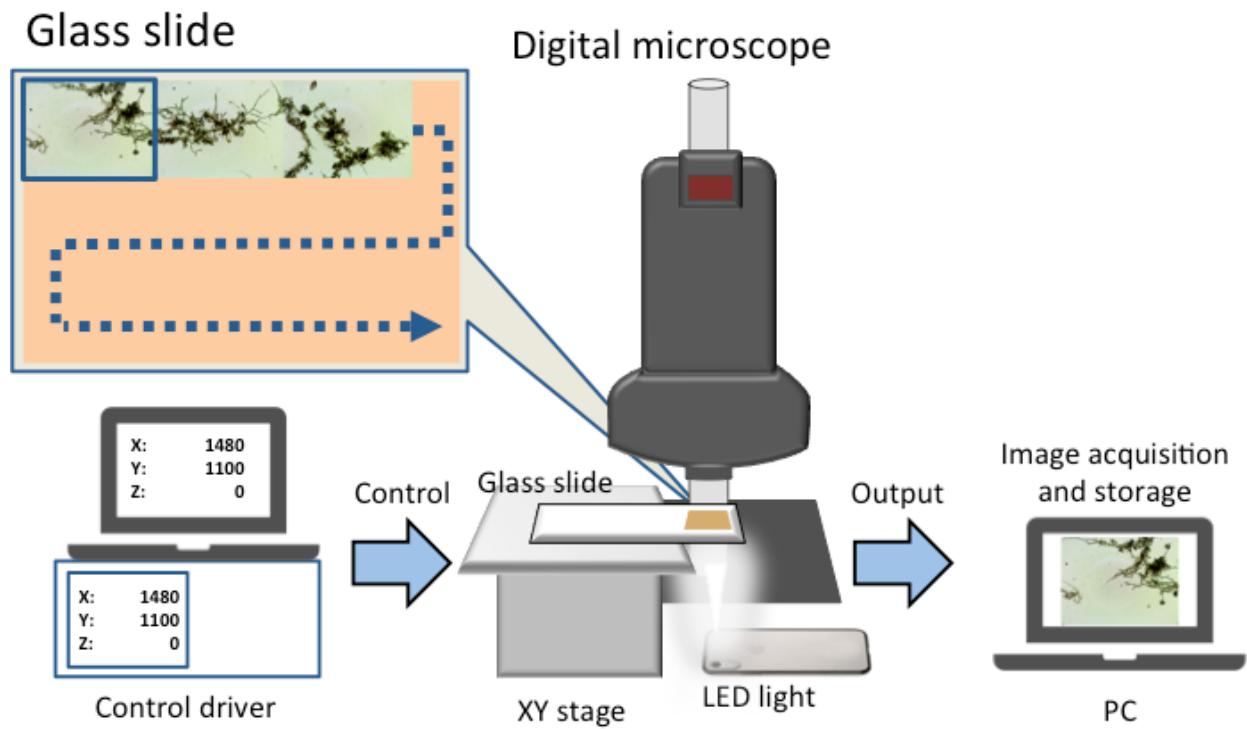
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443 Figure S1. Schematic representation of automatic microscopy image acquisition system.

444

```
# call retrain.py

source ~/tensorflow/venv/bin/activate

cd ~/tensorflow/retrain

python retrain.py \
--bottleneck_dir=tmp/bottlenecks \
--how_many_training_steps=100 \
--model_dir=tmp/inception \
--summaries_dir=tmp/training_summaries/basic \
--output_graph=output/retrained_graph.pb \
--output_labels=output/retrained_labels.txt \
--image_dir=test_data

# classify images

source ~/tensorflow/venv/bin/activate

cd ~/tensorflow/retrain

python label_image.py \
--graph=output/retrained_graph.pb \
--labels=output/retrained_labels.txt \
--input_layer=Placeholder \
--output_layer=final_result \
--image=sample/Sample001.jpg
```

445

446

447 Figure S2. Command line used to retrain Inception v3 model and to classify images into one of
448 two classes.

449

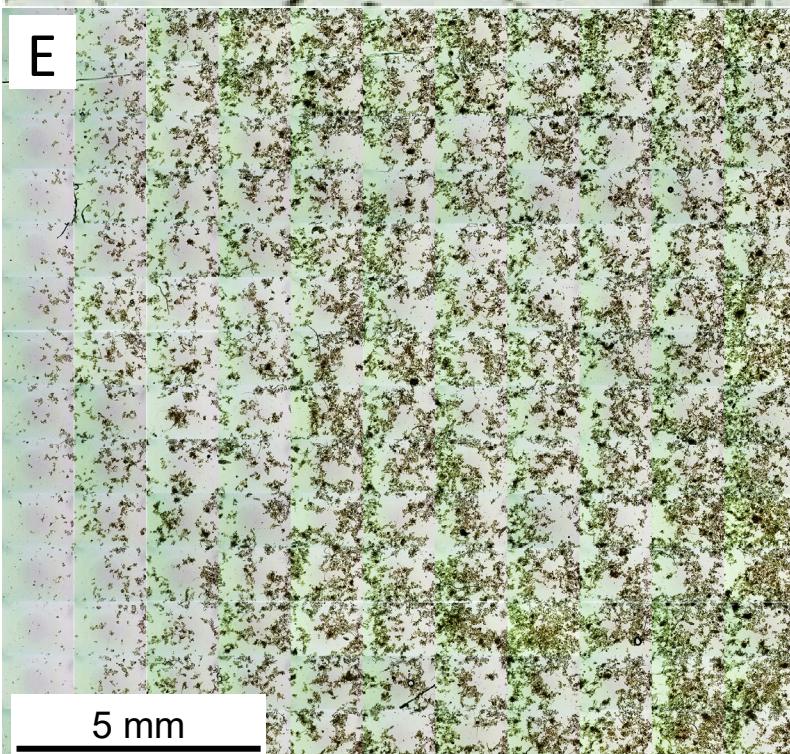
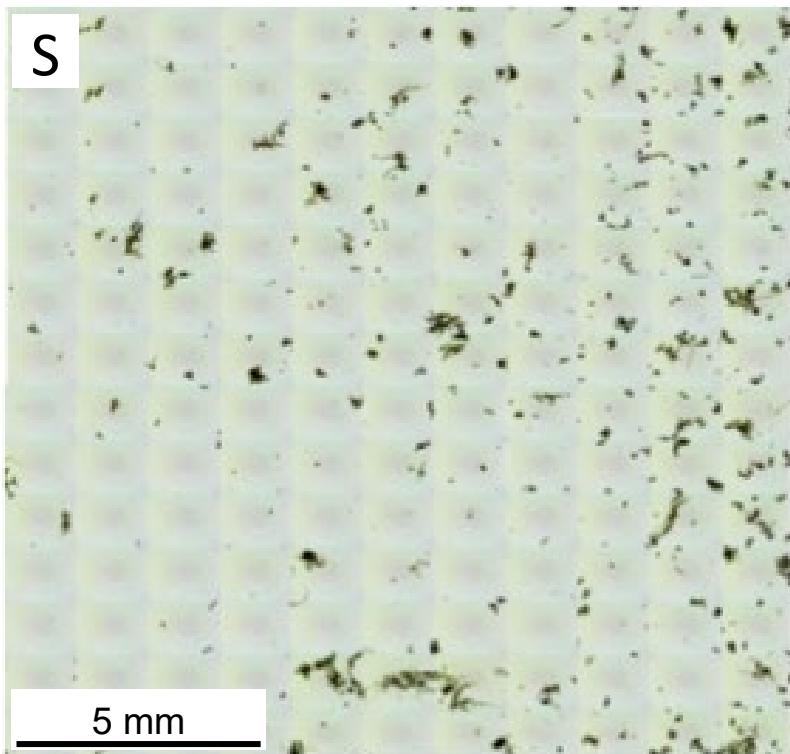
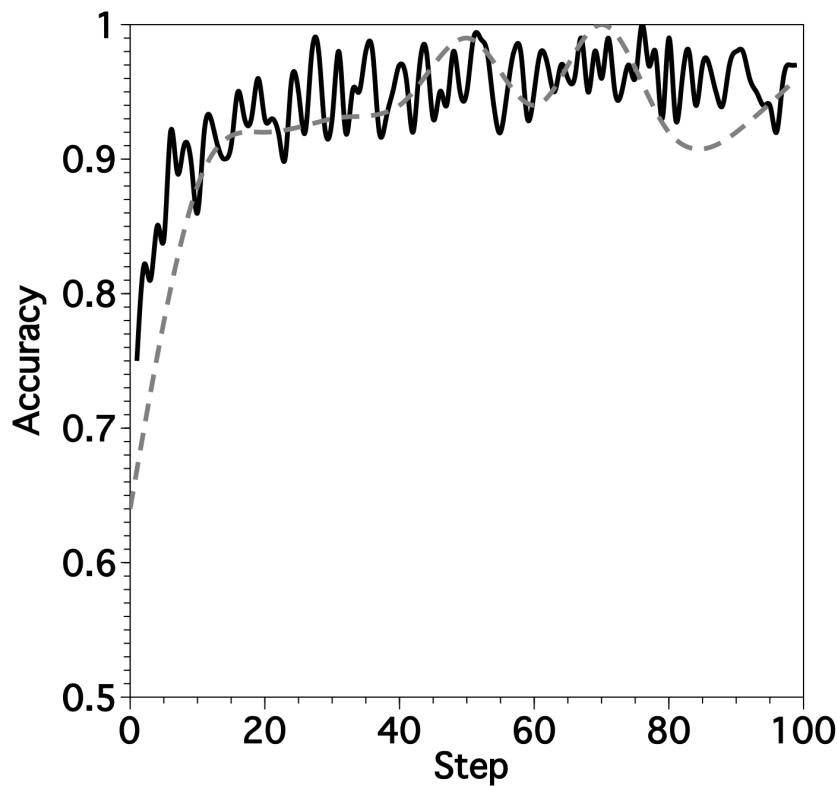
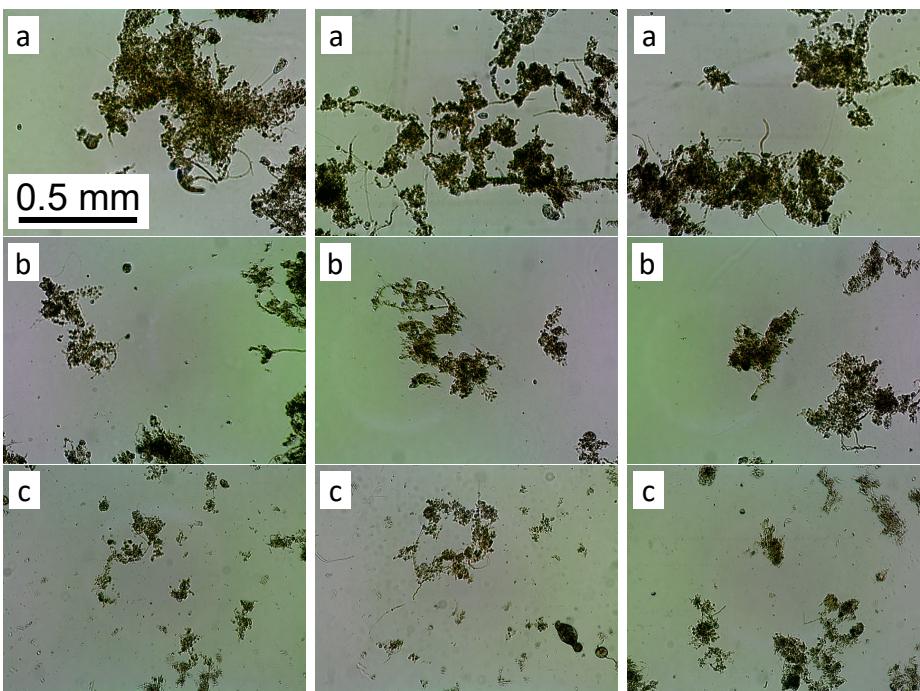


Figure S3. Typical microscopic images in bright field of AS flocs obtained from WWTP-S (S) and WWTP-E (E). A total of 154 were exhaustively acquired in a single slide and assembled. Small gaps between images were closed.



457
458 Figure S4. Training (solid line) and validation (dashed line)
459 accuracy for Classifier-1 during 100
460
461



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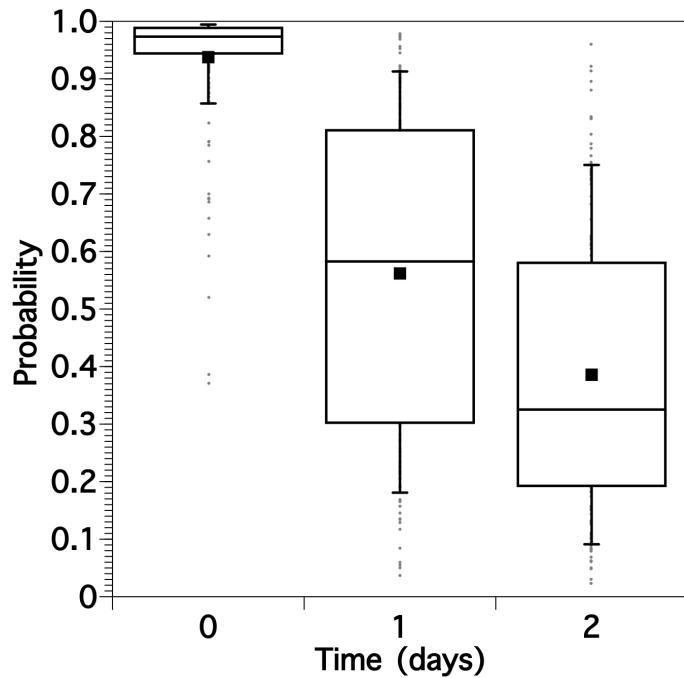
463

464

465 Figure S5 Typical microscopic images in bright field of AS flocs obtained from WWTP-S at (a)
466 day 0, (b) day 1, and (c) day 2.

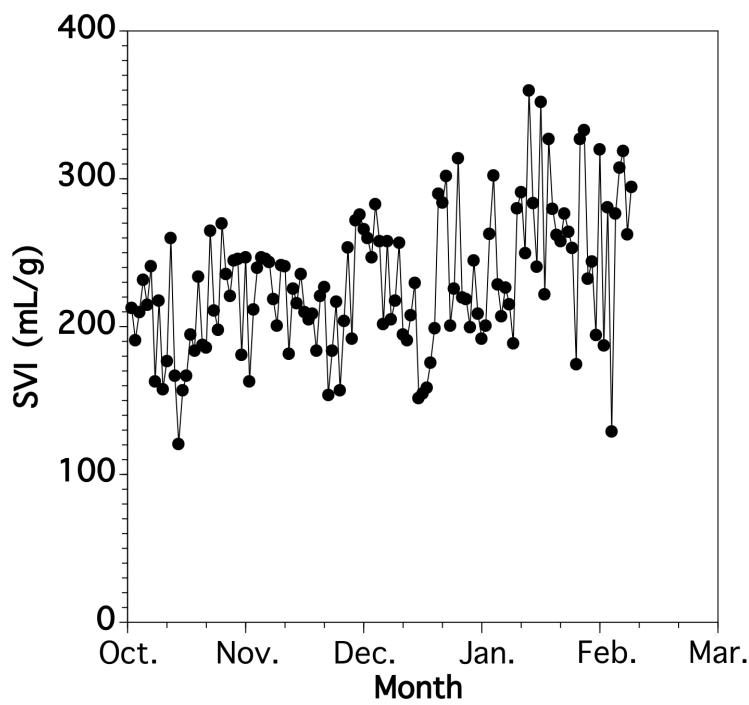
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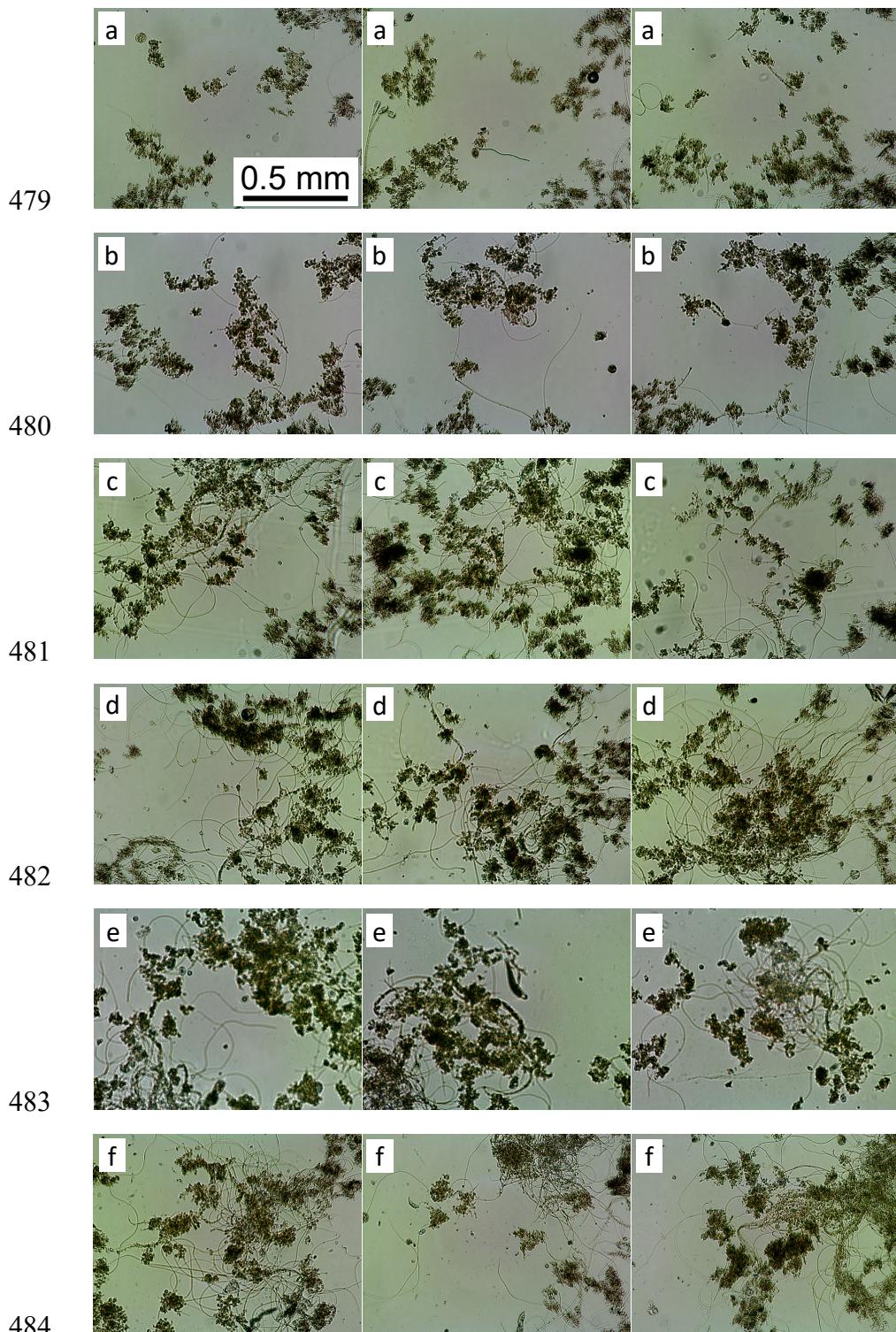
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Figure S6. Boxplot of average probability scores of 154 images of AS flocs obtained from WWTP-S and cultivated in our laboratory with vigorous aeration. The rectangular box indicates the lower and upper quartiles and the median, the square plot indicates the average, and the whiskers indicate the 9th and 91st percentiles. All other results are plotted as outliers.



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478

Figure S7. Time-course changes in SVI of AS of WWTP-M.



485 Figure S8. Typical microscopic images in bright field of AS flocs obtained from WWTP-M on
486 (a) December 10th, (b) December 17th, (c) December 24th, (d) January 14th, (e) January 21st,
487 and (f) January 28th.

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