

Research Article

Characterization of Filamentous Flocs to Predict Sedimentation Parameters Using Image Analysis

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In wastewater treatment plants, the degradation of complex substances that contaminate water is carried out by microorganisms, which are fixed by a network formed by filamentous bacteria, creating large flocs that settle easily. However, the excessive growth of said bacteria causes a series of drawbacks such as the reduction of settling velocity, leakage of activated sludge with the effluent, and formation of supernatant, a phenomenon known as bulking. This research work seeks to develop and evaluate a procedure for the physical characterization of the flocs to determine the parameters that affect the settling velocity and thereby detect and control bulking. For this purpose, sedimentation and image analysis tests were carried out from wastewater from the Aguas Antofagasta treatment plant (Chile). The image analysis was performed with images captured from an optical microscope in two magnifications (100x and 50x), which were analyzed by marking each floc individually and characterized by an image processing software. Additionally, sedimentation tests were performed on columns (area of 74 (cm²) and height of 70 (cm)). As a result, an inversely proportional dependence was found on the settling velocity evaluated by the Vesilind equation in the zone of constant fall velocity with respect to the number of flocs connected per cluster, giving an estimate of the settling velocity depending on the number of flocs connected. This would allow predicting settling velocity with image analysis, taking into account that the problem of bulking is determined by the type of filamentous bacteria that causes it and the sedimentation process is affected in large part by local factors. It can be concluded through this study that as the number of flocs connected per cluster increases, the settling velocity decreases. This study provides wastewater treatment plants with a practical tool to determine sedimentation times and thus improve the quality of the treated water, avoiding problems of flocs leaking with the effluent. In addition, the image analysis itself allows rapid detection of the phenomenon of bulking and its severity.

1. Introduction

The basic principle of biological purification is based on a physical-biological process. On the one hand, there is the physical process of flocculation and on the other the bacterial metabolism as the biological aspect. A floc is a type of microbial aggregate that may be contrasted with biofilms and granules or else considered a specialized type of biofilm. Flocs appear as cloudy suspensions of cells floating in water, rather than attached to and growing on a surface like most biofilms. The floc typically is held together by a matrix of extracellular polymeric substances (EPS), which may contain variable

amounts of polysaccharide, protein, and other biopolymers. The formation and the properties of flocs may affect the performance of industrial water treatment bioreactors such as activated sludge systems. A floc is composed of organic matter, filamentous and floc-forming bacteria, which constitutes, in itself, the primordial unit of the purification of organic substances. The effectiveness of the active sludge process is strongly linked to a good solid-liquid separation determined by the characteristics of the sludge, which in turn depend mainly on the structural characteristics of the flocs and the microbial population. Regarding this last point, the excessive growth of filamentous bacteria constitutes one of the main

problems of the wastewater treatment process. The filamentous floc can be found in two different ways: (1) open structure or diffuse flocs. (2) Flocs are joined together with the others (cluster) forming links or bridges between the flocs. In other studies, the behavior and relevance of flocs in different processes can be seen [1–6] as well as the effect of coagulation and filtration in water treatments [7].

The factors that intervene in the proliferation of the formation of filamentous bacteria are the quality of the effluent, the operating conditions of the facility, the design of the facilities, and the climate. Image analysis is proposed as a tool to analyze and predict this phenomenon, a method that has a wide potential for the control of this type of process [8–11]. In order to compare and improve the image analysis system and find the best method of classifying data, various works carried out on water treatments are taken as references [12–16], which show the advantages of using an autonomous control system to increase water savings. The adoption of a mathematical model to predict the behavior of the settling velocity is a complex problem as already mentioned, so it is usual to resort to experimental laboratory processes where the sludge of the treatment plant is used to obtain more accurate velocity values in the plant. The mathematical model only provides an approximate attempt at the value that can be obtained experimentally and under no circumstances should be taken as an exact value of the velocity that will be obtained in a practical way.

Mineral processing, in general, has placed great efforts on developing solid-liquid separation equipment. An example of this trend is the use of high efficiency thickeners; this change has led to the development and improvement of flocculants [17]. The agglomeration of colloidal particles leads to the formation of so-called flocs. The properties of these particles (shape, size, and density) change as they settle.

1.1. Other Forms of Sedimentation. *Free settling:* sedimentation due to free settling occurs when there is a low concentration of fluid particles and these settle without obstructing each other.

Hindered settling: for high concentrations of flocculent particles (higher than 500 (mg/l)), these are at such small distances that they adhere to each other and settle massively creating a clear separation surface between the flocs and the liquid above, giving rise to the phenomenon known as hindered or zone settling.

The hydraulic forces create bridges between particles by the drag or suction that the larger particles exert on the smaller ones and by the effect generated by the particles that have greater velocity over the others, which produces changes in the trajectory of the particles [18]. On the other hand, the electrochemical forces between the particles that are generated on their surfaces as electrostatic charge make the particles repel each other. These forces are relevant in the case of microparticles, since the surfaces exposed to the electrolyte, which in this case is the fluid, are very important given the large exposed surface. Finally, the osmotic forces arise from the Brownian movement of the microparticles, which tend to try to balance their concentration between different zones of the suspension.

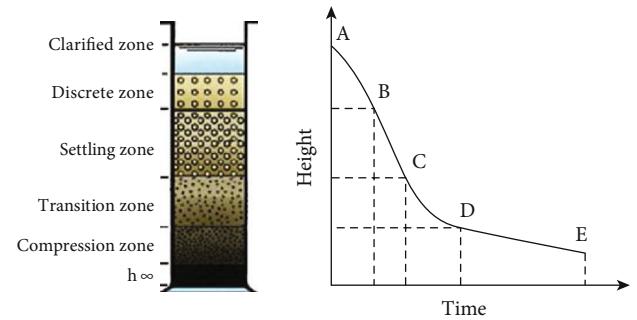


FIGURE 1: Sedimentation by hindered settling (without bulking).

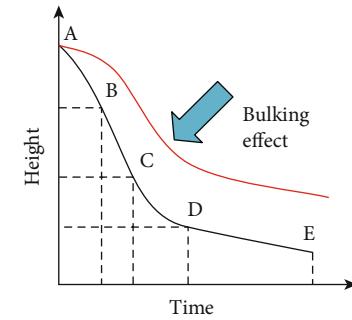


FIGURE 2: Sedimentation by hindered settling (with bulking).

The equation of Vesilind (1), which was developed by Mauden and Whitmore, is commonly used for the relationship between particle size and terminal velocity:

$$V_t = V_{to}(1 - C)^n, \quad (1)$$

where V_t is the terminal settling velocity, V_{to} is the terminal velocity of a single sphere, C is the volume fraction of the solid in suspension, and n is a function of the value of the Reynolds number.

For fluids with $Re < 0.3$, n equals 4.65; for fluids with $Re > 1000$, n equals 2.33.

To have a complete view of the sedimentation process that occurs in the treatment plants, it is convenient to divide this process into stages or zones through which the fluid passes as it sediments, which can be seen in Figure 1.

In contrast, the effect of bulking can be seen in Figure 2.

The adoption of a mathematical model that predicts the behavior of the settling velocity is a complex problem, so it is usual to resort to experimental laboratory processes where the sludge from the treatment plant is used in order to obtain more exact velocity in the plant. The mathematical model only provides an approximate attempt at the value that can be obtained experimentally and under no circumstances should be taken as an exact value of the velocity that will be obtained in a practical way.

2. Materials and Methodology

2.1. Image Analysis Procedure. The sample was taken at the Aguas Antofagasta wastewater treatment plant. This was transferred to the biotechnology laboratory of the

Universidad Católica del Norte where the image analysis was carried out. The software utilized for the analysis was MATLAB®, a multi-paradigm numerical computing environment and proprietary programming language developed by MathWorks. MATLAB allows matrix manipulations, plotting of functions and data, implementation of algorithms, creation of user interfaces, and interfacing with programs written in other languages. This software also provides a comprehensive set of reference-standard algorithms and workflow apps for image processing, analysis, visualization, and algorithm development. With this, you can use image segmentation, image enhancement, noise reduction, geometric transformations, image registration, and 3D image processing, resulting in a very effective tool in the image analysis needed for this work. The time elapsed between taking the sample and the image analysis is approximately one hour and the volume of the sample is 4.5 l. The sludge was stirred gently to obtain a homogeneous composition, preventing it from beginning to sediment and being careful not to damage its structure. For the analysis of the sample, a Carl Zeiss Axiostar microscope and a Sony digital camera model MPEG moviEX cyber-shot 3.3 megapixel are used. The magnification used was of 50 and 100 times the actual size of 1280 pixels wide by 960 pixels high and an image resolution of 28,346 (pixels/cm), taking photographs at random in the sample under a microscope.

2.2. Floc-Marking Technique. A photograph is made up of pixels; each pixel represents a different hue or color that results from the combination of the colors red, green, and blue (RGB) using a *k*-means algorithm. The intensity level of each of these generates a different color; for example, the combination of red and green results in yellow and the variation in the intensity of either of these two will result in a change in the hue of the color. If the green color is predominant, the shade of yellow will turn light green. However, if red is the predominant color in the mixture, yellow will turn with an orange tint and if combined properly they can give brown tones. The resulting color is obtained by arranging a vector of three coordinates in which each one represents a color (red, green, and blue) and its intensity value; this ranges from 255 which is the maximum intensity of the color to 0 which represents the total absence of this. This means that for each color, there are a total of 256 degrees of intensity; this will help the program to identify each of the marked elements (flocs). To measure the size and four connectivity characteristics, these are marked using a color from the palette, redrawing the floc of the original photo, as shown in Figure 3.

To measure the size and connectivity characteristics of the flocs, they are marked using different colors by redrawing the floc of the original photo. To obtain the geometric properties, the program decomposes the image in three parts, one for each base color that composes the image and evaluates the tonality of each of these by comparing it with the database of colors that it has been asked to analyze; any color combination that is not in this database is rejected. Every pixel that fulfills the color vector and all the adjacent pixels that also comply with it are considered an object. The above explained can be seen in Figure 3.

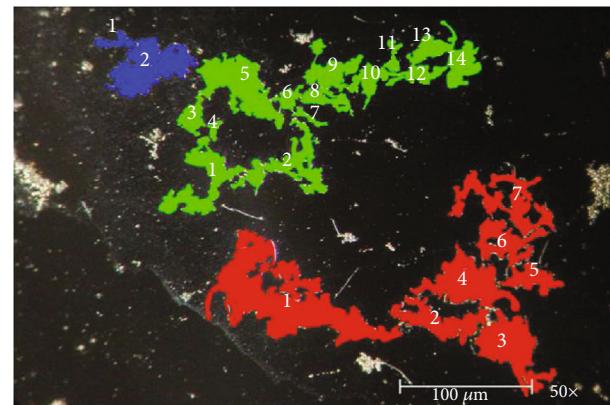


FIGURE 3: Example of the use of color to determine floc clusters.

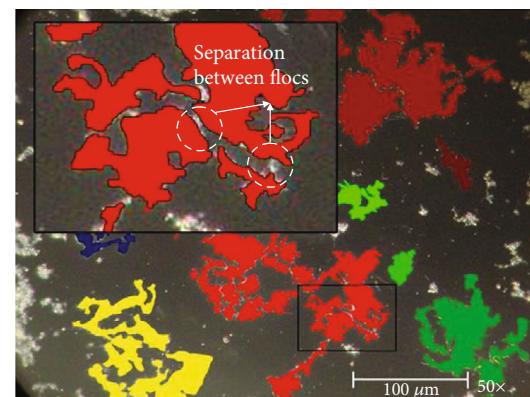


FIGURE 4: Separation of flocs in the same cluster.

In Figure 3, it can be seen that there are two blue elements in the upper left part of the image, which are connected to each other and with no other, and for this reason, they share the same color. In the same way, the green flocs that are a little lower are connected with one or more flocs of this color and only with them; a grouping of flocs or clusters share the same color because in this way the software can differentiate a grouping from another and each floc is slightly separated from the others to distinguish them from the others in the same cluster, as shown in Figure 4.

Once the software identifies the flocs and their characteristics are obtained (shape, size, and density), the distance in pixels (dimensionless unit) is transformed into units of length (mm). This is achieved by using a size pattern, in this case a sphere of 1 mm in diameter, which establishes the scale to measure all the samples analyzed.

2.3. Procedure for the Sedimentation Column. A total of 9 sedimentation experiences are carried out during a period of 3 weeks, each one with a different concentration. The concentrations used in each column are the following: 5.25, 4.35, 4.26, 3.13, 3.05, 2.52, 2.17, 2.10, and 1.68 (g/l). The sedimentations are carried out in a cylindrical vessel, with a basal area of 74 cm² and a height of 70 cm with a sludge volume of 4.5 l, equipped with a slow stirrer to avoid damaging the flocs, for which a magnetic stirrer is used

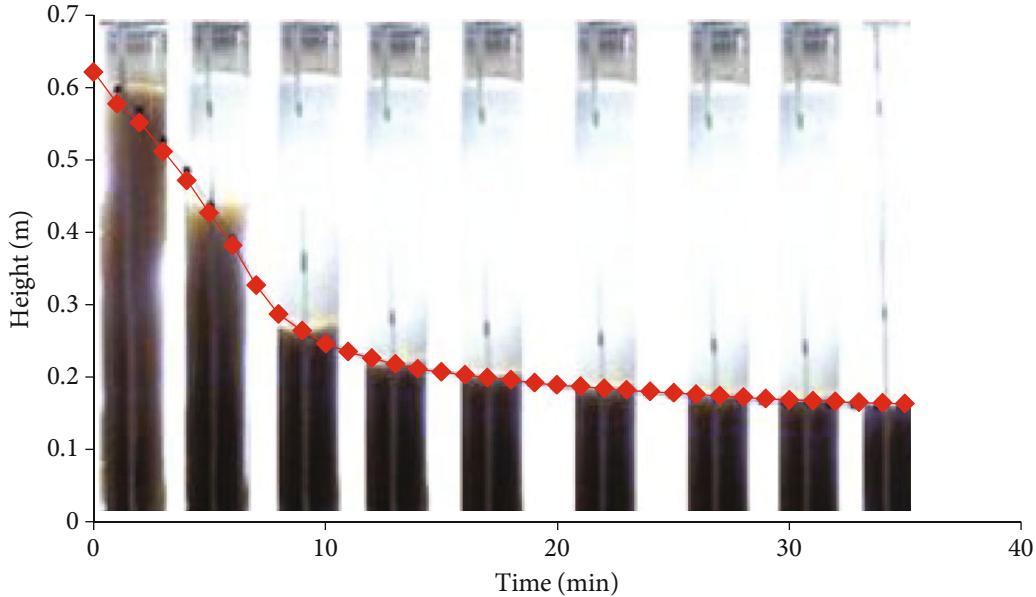


FIGURE 5: Experimental process of sedimentation (initial concentration 2.52 (g/l)).

(Spin Master model 4803-02). Once the sludge is homogenized (5 (min)), the magnetic stirring is stopped and the timer starts running. Height measurements are made for a period of 35 minutes each minute. The purpose of this is to determine the settling velocity of the sludge at different concentrations.

Once the sedimentation process was started, it was observed that there is a delay for the system to stabilize. The speed at the beginning increases rapidly (free settling) until the system stabilizes and then it begins to decrease. For this reason, the data that are considered to calculate the sedimentation velocity are those that are found after this zone, where the slope is already linear. The velocity is given by the slope of the equation (2) of the line formed by the data where the velocity is linear:

$$h = -Vs * t + C, \quad (2)$$

where h is the height in meters (m), t is the time in hours (h), and the slope Vs turns out to be the zone velocity (m/h).

To determine the parameter of the settling velocity, the Vesilind model is used, which represents the sedimentation of flocculent particles. The data to obtain it will be provided by the sedimentation experiments carried out by measuring the zone settling velocity at different concentrations. The formula that defines the Vesilind model is given by

$$Vs = Vo e^{-nx}, \quad (3)$$

where Vs is the zone settling velocity (m/h), Vo is the maximum settling velocity, n is the Vesilind parameter (m^3/kg), and x is the concentration in (kg/m^3) [19].

To obtain the Vesilind parameters, the linearized equation (4) must be used:

$$\ln (Vs) = \ln (Vo) - nx. \quad (4)$$

The values Vo of n and Vo are obtained by plotting the data of $\ln (Vs)$ vs. concentration. Once the values Vs of the slope and the intercept of the curve are known, it is possible to determine the variables Vo and n , all with a small mathematical adjustment.

Figure 5 shows on a large scale the experimental process of sedimentation in chronological order. It can be seen how the sedimentation rate decreases over time, being considerably faster in the first 10 minutes compared to the interval between 20 and 40 minutes, where a steady state is reached. The speed that is sought to be calculated through image analysis is in the most stable area, where a more linear sedimentation rate can be perceived (between 10 and 40 minutes).

2.4. Calculation of Sludge Volume Index (SVI). The sludge volume index is obtained from the ratio of the volume of settled sludge in 30 minutes of a 1000 ml sample and the sludge concentration (mg/l) multiplied by 1000.

This was carried out in a graduated cylinder (1000:10) in order to characterize the sedimentation from a structural point of view as well as the level of dispersion of the flocs and to obtain an estimate of the settling velocity. The steps to perform this analysis are the following:

- (1) Homogenize the sludge. Once homogenized, a known volume of it is removed and placed in a previously weighed container, allowing it to dry at 105°C for 24 h. Once the sample has dried, it is removed from the oven and weighed. The weight of the solid is given by the difference between the weight of the container and the weight of the sample after drying. The quotient between the weight of solid and the volume of the sample provides its concentration

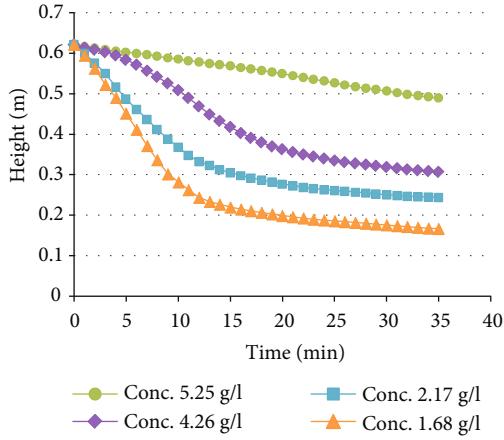


FIGURE 6: Height vs. time graph, first experience (sed. 5.4 (m/h)).

- (2) Homogenize the sample, take 1 liter, place it in a graduated cylinder, and allow to settle for 30 min. After this, measure the volume (in ml) occupied by the settled material. The SVI is expressed according to

$$\text{IVL} = \frac{V_{30}}{x}, \quad (5)$$

where SVI is the sludge volume index, V_{30} is the volume of settled sludge after 30 minutes (ml/l), and x is the initial concentration of suspended solids of the sludge (g/l).

2.5. Calculation of the Sludge Volumen Index (SVI). The calculation of total solids is determined by drying at 105°C for 24 h of a sample of known volume (approximately 10 ml), which is put in a container of known weight. After drying the sample in the oven (Binder model APT line), it is weighed (Boeco model BBL scale). The weight difference between the container with and without the sample gives the weight of the solid. The ratio between the weight of the solid and the volume of the sample before being dried provides the concentration of the sludge (g/mg).

3. Results and Discussions

A total of 9 different sedimentation experiences were carried out, each with a different concentration. These different experiences are classified according to their velocity in the following way: sed. 5.4 (m/h), sed. 8.7 (m/h), and 41.8 (m/h). These groups will be called the first, second, and third experiences from now on. The results that show the sedimentation speed of each experience and with which they were classified are shown in Figures 6–8.

From these data, it appears that the highest settling velocity occurred in the third experience followed, in descending order, by the second and the first experience.

Having the height and the time, it is possible to determine the point velocity of the bed sediment while with the height and the SVI the point concentration for each recorded height

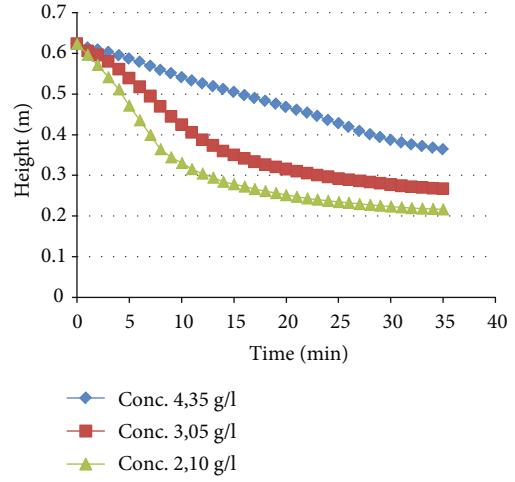


FIGURE 7: Height vs. time graph, second experience (sed. 8.7 (m/h)).

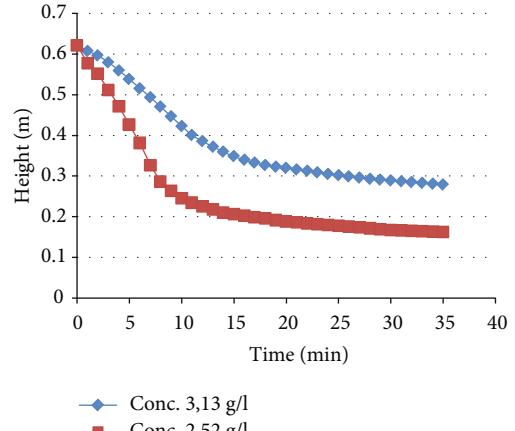


FIGURE 8: Height vs. time graph, third experience (sed. 41.8 (m/h)).

is determined. Figure 9 compares the velocity vs. similar concentration.

As shown in Figure 9, there is a clear difference between the settling velocities for the different experiences carried out despite the fact that the concentrations are similar; this is due to the floc size.

With the data obtained from experimental velocity, the different parameters of the Vesilind equation can be determined to find the theoretical velocity. From these parameters, equations (6)–(8) are obtained, which represent the Vesilind equation for each experience.

$$Vs = 5.347e^{-0.553x}, \quad (6)$$

$$Vs = 8.721e^{-0.669x}, \quad (7)$$

$$Vs = 41.788e^{-1.133x}. \quad (8)$$

Equations (6)–(8) give the theoretical velocity values (Vs) at different concentrations of sludge (x) for each experience, being valid where the slope of the graph concentration vs.

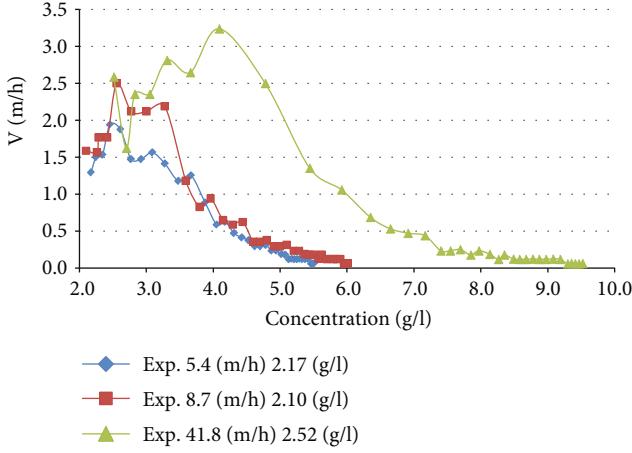


FIGURE 9: Graph comparing velocity vs. similar concentrations.

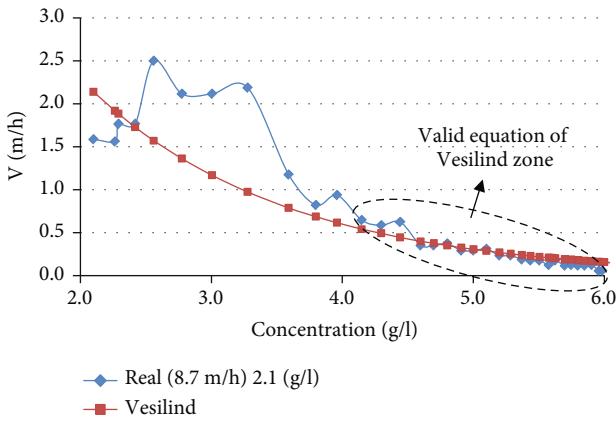


FIGURE 10: Comparison of real velocity and Vesilind sed. 8.7 (m/h) (2.10 (g/l)).

TABLE 1: Number of photos of image analysis in each experience.

Experience	Number of photos
First experience 50x	8
First experience 100x	7
Second experience 50x	8
Second experience 100x	5
Third experience 50x	8
Third experience 100x	8

velocity is linear. It is taken into account that sedimentation is a process that is subject to multiple local factors both of the sludge (bacteria that make it up, the age of the sludge) and of the surrounding environment (temperature, possible vibrations) so that the theoretical velocity obtained will not always represent with accuracy the values obtained experimentally. By way of example, Figure 10 compares the actual velocity with the theoretical velocity obtained.

A total of 44 photos were taken with the microscope for later analysis with the software. The number of images analyzed by each experience is shown in Table 1.

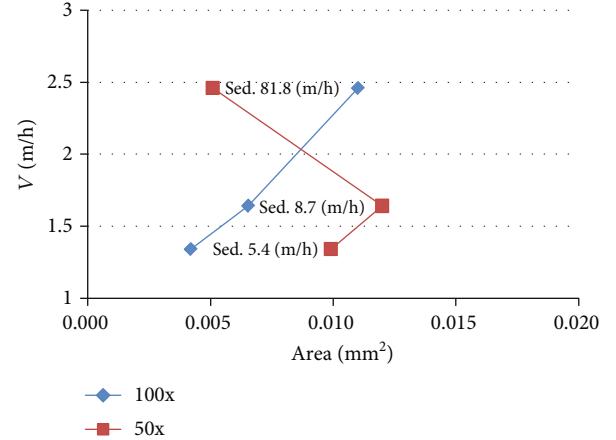


FIGURE 11: Comparison of velocity (m/h) and area of the flocs (mm^2).

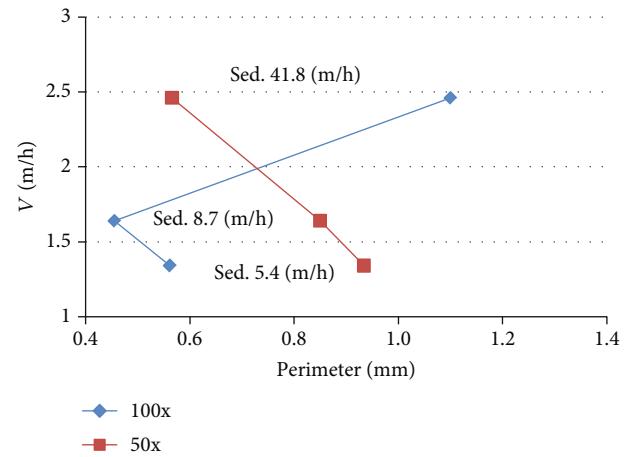


FIGURE 12: Comparison of velocity (m/h) and perimeter of the flocs (mm).

To have a common point of comparison, the theoretical sedimentation velocity according to Vesilind (equations (6)–(8)) at a concentration of 2.5 (g/l) will be taken and compared with the physical properties obtained by image analysis using the median of the data. The comparisons are made using the values obtained from the area and perimeter of the flocs, measured by the image analysis of the software. The comparison of velocity vs. area is shown in Figure 11.

Figure 12 shows the comparison of velocity vs. perimeter of the flocs.

The image analysis regarding the area and perimeter of the flocs shows that no dependence can be established, which can be seen in Figures 11 and 12. Although in some cases there seems to be a pattern, this is not always true. This non-dependence can be explained because the analysis performed was in 2D; a 3D technique should be applied in future works to relate the sedimentation velocity with the floc volume.

Table 2 shows the laboratory analysis results regarding the sludge volume index:

TABLE 2: Comparison of the values of SVI for different tests.

Experience	SVI (ml/g)
Sed. 5.4 (m/h)	136.15
Sed. 8.7 (m/h)	134.42
Sed. 41.8 (m/h)	146.96

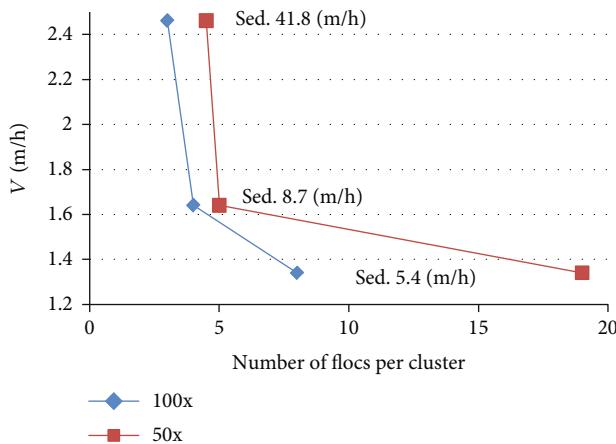


FIGURE 13: Graph of comparison of settling velocity and number of flocs per cluster.

To corroborate the relationship between the bulking phenomenon and the settling velocity, the data obtained in the image analysis are utilized, using the median of the data and comparing it with the velocity at 2.5 (g/l) obtained according to the equation of Vesilind. The results are shown in Figure 13.

From what can be seen in Figure 13, there is a clear relationship between the number of flocs per cluster. In the first experience, a velocity of 1.34 (m/h) and 8 flocs per cluster was recorded, this being the lowest recorded velocity. The second experience shows a velocity of 1.64 (m/h) and 4 flocs connected per cluster, while in the third experience, the highest velocity of all is recorded, 2.64 (m/h), and the number of flocs per cluster of 3 is the lowest for images with magnification of 100 times the actual size, while for images magnified 50 times, the same happens. From the above, it follows that an increase in the number of flocs connected per cluster leads to a decrease in settling velocity.

4. Concluding Remark and Future Work

A floc characterization technique was developed by means of image analysis through which the settling velocity can be determined depending on the number of flocs per cluster.

It can be appreciated that for the experience Sed. 5.4 (m/h), the lowest settling velocity was obtained 1.34 (m/h) (velocity obtained according to the Vesilind equation for each experience at a concentration of 2.5 (g/l)) and a number of 8 flocs per cluster for an increase of 50 and 19 for a magnification of 100 times the actual size, this being the highest number of flocs per cluster recorded. For the experience Sed. 8.7 (m/h), the middle values of both settling velocity

and flocs connected per cluster are recorded, 1.64 (m/h) velocity and 4 and 5 flocs for a magnification of 100 and 50 times, respectively. Finally, the experience Sed. 41.8 records the highest velocity, 2.46, and the lowest number of connected flocs, 3 and 4.5 with magnification of 100 and 50 times, respectively. Thus, it can be concluded through this study that as the number of flocs connected per cluster increases, the settling velocity decreases.

With the results obtained, the settling velocity can be predicted depending on the number of flocs connected, taking into account that the problem of bulking is determined by the type of filamentous bacteria that causes it and the sedimentation process is affected in large part by local factors, whereby each sedimentation itself is a different process and the velocity obtained will only be applicable to the sludge that was analyzed.

There is no evident dependence between the evaluated physical properties (area and perimeter) and the settling velocity. The result of this experience may have been altered due to three factors. The first factor is that the flocs are not totally inside the image, which means that the actual area of the flocs and the tested area are not the same, very similar to the iceberg phenomenon, in which only a portion of these is seen and it is not possible to determine their true dimension. Another factor is the marking which is done manually, thus conditioned by the eye of the observer. This mainly affects when deciding which structure is a floc and which is not and where a floc ends and another begins. The last factor is that the connectivity between flocs is such that the relationship that could exist between the physical properties (area and perimeter) and the settling velocity is not relevant enough to be appreciated; this is also applicable to the sludge volume index analysis.

This study provides wastewater treatment plants with a practical tool to determine sedimentation times and thus improve the quality of the treated water, avoiding problems of flocs leaking with the effluent. In addition, the image analysis itself allows rapid detection of the phenomenon of bulking and its severity.

Data Availability

All data supporting this study is provided as supplementary information accompanying this paper.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

M.A.M. and C.A.A. conceived, designed, and performed the experiments; all the authors analyzed the data; C.A.L. wrote the paper.

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Supplementary Materials

The supplementary material corresponds to 44 photos that were analyzed. (*Supplementary Materials*)

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