Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.21767

99 (2017) 117–124
December

Using laser granulometer to algae dynamic growth analysis in biological treated sewage

Tomasz Garbowski*, Krzysztof Pulikowski, Paweł Wiercik

Wrocław University of Environmental and Life Sciences, Institute of Environmental Engineering, Grunwaldzki Square 24, 50-363 Wrocław, Poland, emails: tomasz.garbowski@upwr.edu.pl (T. Garbowski), krzysztof.pulikowski@upwr.edu.pl (K. Pulikowski), pawel.wiercik@upwr.edu.pl (P. Wiercik)

Received 27 July 2017; Accepted 7 December 2017

ABSTRACT

Often within literature can be found descriptions of research using the technique of laser diffraction to characterize the composition of sewage and water, measurements of the number and volume of particle size distribution and fractal dimension of activated sludge flocs. This study focuses on the potential of the application of laser granulometric technique, which has not been widely described in other studies. It concerns the granulometric composition of sewage in which algae grow and the influence that algae has on size, properties and the stability of particles in suspension. The measurements include particle size distribution and the calculation of fractal dimension and the mean diameters of particles. In the study, an attempt was made to verify the similarity of the mechanism in which algae colonies are created to crystallization process. For this purpose, data from granulometric analysis were converted using the modified Avrami equation. The results presented indicate that the analysis of the particle size distribution of the suspension by laser diffraction generates reliable and reproducible results, and can also be successfully used to track the growth dynamics of the microbial complexes which form the suspension in a liquid medium such as sewage or water.

Keywords: Laser granulometer; Algal biomass; Particle size distribution; Fractal dimension; Mean diameter

1. Introduction

Analysis of particle size distribution using the method and application of laser diffraction is commonly used in many fields of science and branches of industry in which granulometric composition significantly affects quality of the final product. This method is used, among others, in pharmaceutical (beside microscopic analysis), powder, chemical and food industry and even in meteorology and oceanography [1–6]. Particle size distribution is of great importance in the case of granular and powdered activated carbon used in the water treatment processes, since its granulometric composition depends on the active surface of the material and thus the filtration efficiency, moisture adsorption capacity, drying efficiency and transport costs [7]. With the development of analysis methods for particle size distribution (PSD), it is now possible to conduct such investigation with the participation of simple and fast techniques, and as a result the examination of the granulometric composition of various substances has become widespread.

Current laser granulometers in use, mostly operated based on diffraction phenomenon and low angle light scattering [8]. The dynamic of the diffraction process consists of changes in the intensity of light scattering that are caused by colloidal particles movement throughout Brownian motions and/or translational motions [5,8–10]. These motions cause changes of particle sizes and their random arrangement of the light beam inducing disturbances in its cohesion [11]. The latest measuring devices for scattered light intensity adopt the Lorenz–Mie theory with its various modifications [10,12,13].

* Corresponding author.

A wider description of this theory and its other variants is given by Lock and Gouesbet [14] and Gouesbet [15]. The Mie theory in contrast to additionally used Fraunhofer theory allows the mathematical conversion of the intensity of scattered light to volume particle size distribution and then to number of particles. This conversion takes place in the case of data analysis by laser granulometric software [12]. The Mie theory describes only spherical particles, hence measurement of non-spherical particles incurs a high error of measurement in their size distribution [12,13,16].

Methods using the phenomenon of laser diffraction have substantial advantages which distinguish them from other particle size determination methods such as sedimentation and sieve analysis, microscopic images analysis or centrifugal particles size analysis [17]. The main features of methods using diffraction and light scattering phenomenon are versatility, short measurement time, wide range of particles size measurement (from 0.01 μm to few millimeters), high reproducibility, high level of process automation, ease of use and possibility of online measurements [1,3–5,17–20]. Moreover, laser diffraction allows us to obtain information not only about particles size, but also their shape, spatial structure, porosity, stability and surface [2,5,8]. In devices for particle size distribution analysis (laser granulometers) this information can be obtained directly by software (automatically). However, in the sedimentation, microscopic or centrifugal method, obtaining this data is more laborious and time-consuming since each parameter is required to be manually calculated. Particles morphology is determined by optical fractal dimension [9]. Fractal dimension is the parameter that describes the degree of three-dimensional space filled by a particle and it allows the determination of an aggregates spatial structure and its complexity. In other words, fractal dimension defines the shape and irregularity of the matter and its value varies between 1 and 3 and usually takes non-integer values [9,21–24].

The weaknesses of laser diffractometers are the significant differences between the results obtained from a single sample depending upon the configurations of the detector used and algorithms analysis diffraction patterns in different systems. Furthermore, groups of larger particles that are the part of the whole volume of the sample, and are beyond the measuring range of device are not detected which affects the reliability of the results obtained. The high affinity of measurement accuracy to sample dilution (it is important not only in diffraction methods) and to shape of single particles and their position relative to light beam (in case of non-spherical particles) are a serious disadvantage [3,4,19,25].

Often within literature can be found descriptions of research using the technique of laser diffraction to characterize composition of sewage and water, measurements of the number and volume particle size distribution and fractal dimension of activated sludge flocs [8,26]. The information regarding the particle size distribution in activated sludge is very valuable from the point of view of the selection of the appropriate method of wastewater treatment and at the mathematical modeling stage of the treatment process [27]. The dimensions and the shape of the activated sludge flocs vary according to the conditions of the wastewater treatment process and the composition of the sewage and are largely dependent on the loading rate of organic matter [28]. It means that knowing the relationship between diversity of particles size in activated sludge chambers and the conditions of the biological wastewater treatment process (especially the amount of dissolved oxygen and loading rate of organic compounds) allows us to better understand and control the efficiency of the activated sludge process. The granulometric composition of the wastewater also affects the efficiency of the common processes of the removal of the suspension (sedimentation, flocculation or filtration) [29]. Moreover, organic particle size distribution in sewage influences the biological decomposition of pollutants, among others, due to the necessity of large particle prior to their oxidization. Due to the techniques based on PSD it is possible to detect and compare the number of primary fractions (determine as COD) present in sewage and the emerging products of biological decomposition. This gives some insight into the effectiveness of the wastewater treatment process [29–31].

According to review of current literature, the range of use of optical techniques to particle size distribution measurement is very wide and differential. This study focuses on the additional prospect of the application of the laser granulometric technique, which has not been widely described within literature. It concerns the granulometric composition of sewage in which algae grows and the influence that algae has on size, properties and the stability of particles in suspension and tracking the growth dynamics of algae. For this purpose, an analysis of particle size distribution was performed, as well as a fractal dimension and the selected mean diameters of particles were determined. On the basis of the analysis of changes in the granulometric composition of suspended solids, the growth of algae was able to be observed, which together with the particles of suspension formed aggregates. It is assumed that the mechanism of the formation of these aggregates is similar to the crystallization process described by the Avrami equation. Therefore, in addition an attempt was made to verify the similarity of the mechanism of algal colonies formation to crystallization process, within the study. For this purpose, data from granulometric analysis were converted using the modified Avrami equation [26].

2. Materials and methods

Due to the short duration of individual measurement it was possible to perform numerous analyses at small intervals, thus increasing the reliability of the results obtained. In addition, the wide range of diameter detection offered by the laser granulometer enables the identification of both colloidal particles (0.02–0.1 μm) and suspensions (>0.1 μm). The data obtained from the device allows the analysis of solid particles present in wastewater on several levels, starting from the determination of the particle share of the given diameters in the solution, by the morphology of these particles (fractal dimension and mean diameters), ending with the dynamics of changes in the spatial structure of the suspension. The method of determining the mean diameters and fractal dimension has been described extensively by Kuśnierz and Wiercik [8].

Included in the measurement of particle size, sewage from wastewater treatment plant 'Janówek' in Wroclaw (Poland) was used. Artificial substrates to create a stable structure for growing algae were introduced into this sewage. Algae cultivation was conducted in order to the proliferation of biomass, which, by absorbing nutrients (mainly nitrogen and phosphorus), contributes to the purification of wastewater. Furthermore, harvested algal biomass can be used for the purposes of energy recovery, should substrates with algae be subjected to a heat treatment process. Algal cultivation was conducted in three glass aquariums in February to March 2016 and drinking water bottles made from polyethylene terephthalate (PET) and anti-erosive mats made from polymeric fibers which are used for consolidation escarpments and embankments, were among the tested substrates. These substrates were selected because of their ease of acquisition, low acquisition costs and structure that could be suitable for the development of periphyton [32]. Two aquariums contained substrates along with sewage whereas the third, which contained two types of substrates ('mixed substrates'), was further enriched by algae inoculum taken from other culture in the sewage (Fig. 1). The samples were collected every 7 d and the results show selected values with the most visible differences.

A laser diffraction technique was used in order to determine the degree of cultivated biomass succession (variation in time) and this was conducted with the use of 'Mastersizer 2000', a laser granulometer produced by 'Malvern Instruments Ltd'. The measuring range of this device is 0.02–2,000 µm. Through the analysis of the results obtained, it was possible to determine the stability and structure of the aggregates precipitated during the cultivation period. It is crucial from a standpoint of the separation of suspension during the sedimentation process. In addition, this analysis allows us to see whether the algae growth in sewage can form larger clusters, which will be able to attach to the immersed substrates or can be removed from wastewater by other means of separation.

The basic elements of a laser granulometer include:

- A red light source (helium–neon laser) and a blue light source.
- An optical system that directs a laser beam to a sample cell.
- A sample cell containing a mixture of particles in a liquid or gas medium that absorbs and scatters the supplied light beam.
- A set of detectors for the capture of scattered light. The set consists of a central detector that measures small

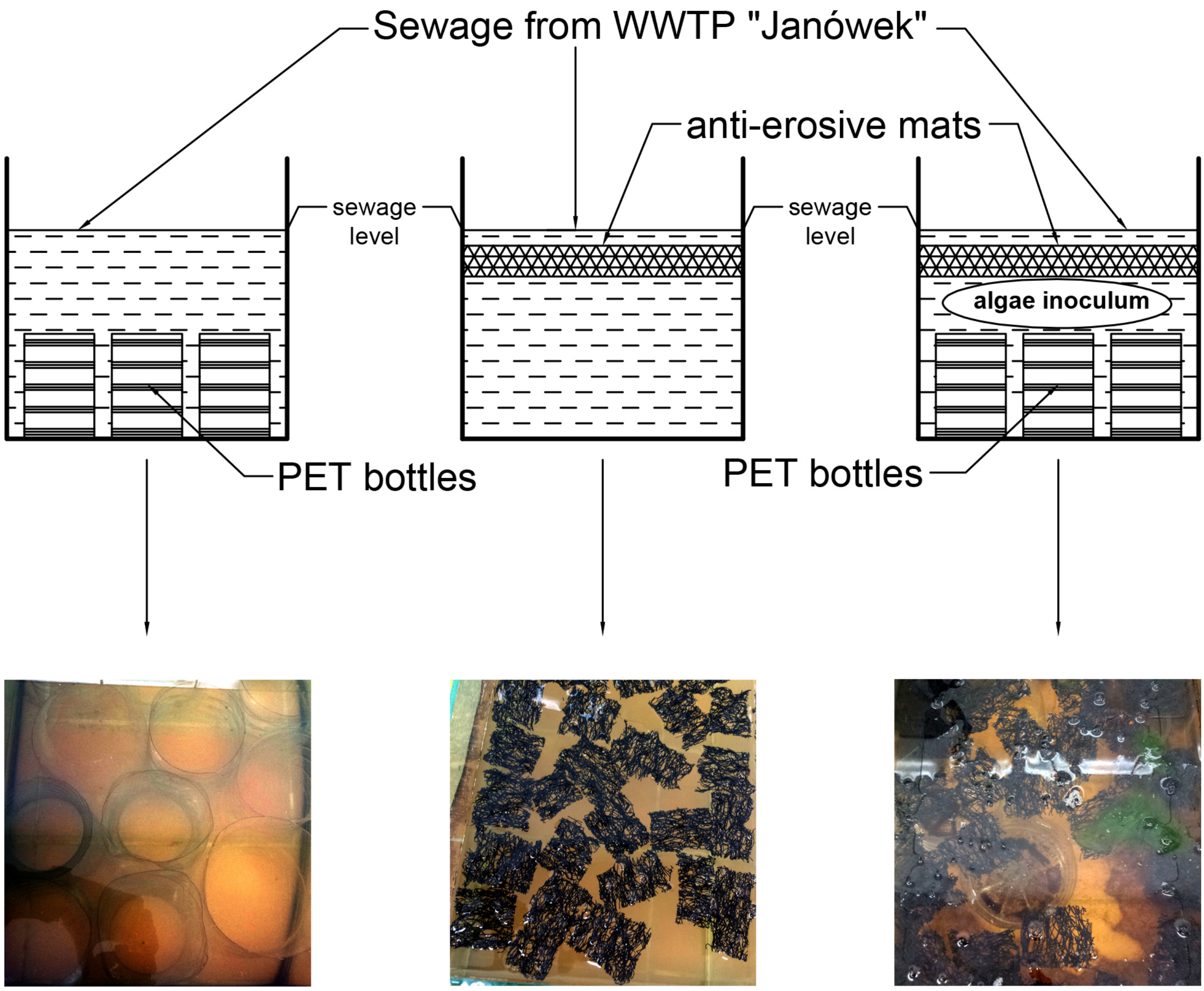

Fig. 1. Plan of the experimental setup. Tanks with sewage and types of artificial substrates for the immobilization of algae.

angles of scattered light caused by the presence of large particles, side detectors for microscale detection and back scatter detectors for colloidal particles.

The optical device is correlated with the computer and Malvern Instruments Ltd. software for analyzing the signals received by the detectors set. The wet sample dispersion unit 'Hydro MU' was used to obtain a homogenous wastewater sample. The unit is equipped with a stirrer and a pump that pumps a liquid sample into a sample cell. In the 'Hydro MU' unit inlet to the sample cell is located relatively high in relation to the bottom of the vessel. In the vessel, the sample is homogenized. Due to the raised inlet location to the sample cell, stirring action can be insufficient to bring heavier particles, such as sand, from the bottom of vessel to the sample cell [33]. Sample homogeneity was obtained at 1,500 rpm mixing speed. After each sample of sewage, the sample cell was rinsed several times (2–3) using tap water at 2,000 rpm. Prior to the introduction of the sample into the device, the background measurements for tap water were performed to create a point of reference for the software that analyses signals received by detectors. All samples were characterized by a low concentration of suspensions, as a result the obscuration values were low, in the order of 2%–5% for undiluted samples. The study was conducted in 800 mL beakers, a single measurement lasted 15 s and 25 measurements were made for each sample. The great advantage of using the granulometer in this study was the lack of special preparation of the wastewater samples which significantly reduced the analysis time.

The results obtained in the program showed the volume distribution of particle size in suspension. Based on this, it was possible to read the percentage share of particles of different diameters in relation to the total volume of particles in the sample [8,26]. The results after a whole series of measurement were averaged. Records with the highest degree of alignment were selected for the average value. For better visualization of variations in particle size distribution, the data for each type of substrate were compiled on cumulative charts that were then used for non-linear estimation using the modified Avrami equation.

2.1. *Using the Avrami equation to determine crystallization process*

According to Kuśnierz and Łomotowski [26], the formation of algal agglomerates can take place as a crystallization mechanism. The basis of this process is the formation of crystallization nuclei around which, crystals of solutes precipitate from solution. In this case 'crystallization nuclei' can be single algal particles producing a mucus and merging into larger colonies.

The general form of Avrami equation describing the kinetics of crystallization process for substances precipitated from water solutions is as follows:

$$V(t) = 1 - \exp(-k \times t^n) \tag{1}$$

where $V(t)$ is a volume share of crystallized fraction in solution after time t, k is a constant of rate of transformations depending on nucleation and growth rate, n exponent associated with geometry of growth and nucleation mechanism, it assumes values 1–4 [26].

In the analysis of the formation process of algae aggregates, a modified general form of the Avrami equation for particles precipitate from water solutions was utilized. This modified form of the equation using the dependence of substitution diameter of the growing crystals (d) from the linear growth rate of the crystals (G) in time (t) [27]:

$$d = G \times t \tag{2}$$

A final modification of the general Avrami equation that was applied for calculation was as follows:

$$V(d) = 1 - \exp(-k \times d^n) \tag{3}$$

Eq. (3) describes curve of cumulative volume frequency of particles of a given diameter including those of smaller diameters in relation to total volume of all particles present in sample [26,34]. The empirical coefficients k and n in Eq. (3) were determined on the basis of the results of the granulometric composition of wastewater using the 'Nonlinear Estimation' function in the STATISTICA 12PL program.

3. Results

The curves shown in Figs. 2–4 relate to cumulative volumetric distribution of particles of different diameters.

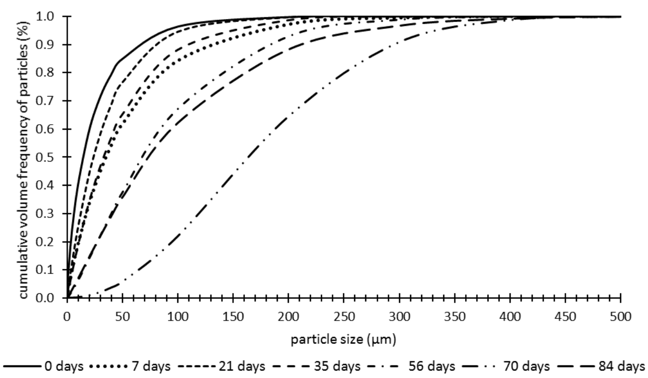

Fig. 2. Cumulative volume frequency of particles diameter in the tank with PET bottles.

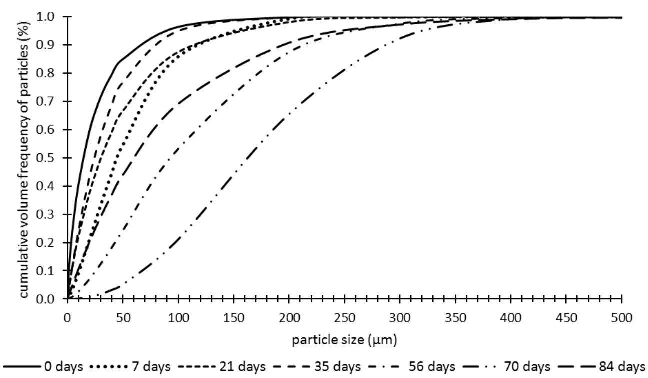

Fig. 3. Cumulative volume frequency of particles diameter in the tank with anti-erosive mats.

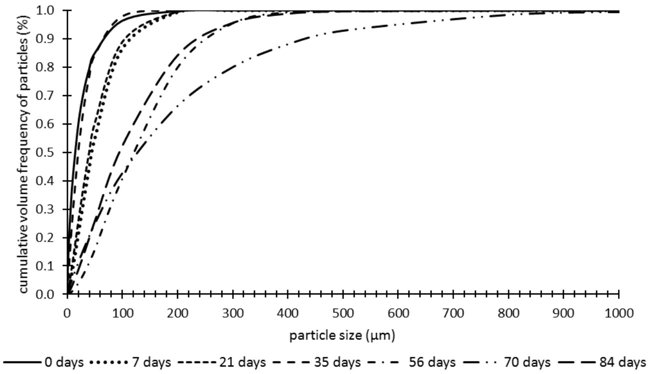

Fig. 4. Cumulative volume frequency of particles diameter in the tank with mixed substrates.

These charts were obtained after the Avrami equation solution for the values *k* and *n* and range of diameters from 0.02 to 2,000 µm. The coefficients *k* and *n* were calculated in statistical software by non-linear estimation. Then, the values for the coefficients were introduced into Eq. (3) and the volume of particles of a given diameter in the total volume of the sample was calculated.

As shown in the charts (Figs. 2–4), the particle size of the suspensions increased after 7 d of the experiment in each of the three aquariums. Following this time, the maximum particle diameters increased twice as much, compared with the first day of the experiment (increase from 100 to 200 µm). The dynamics of the particle size increase varied, as evidenced by changes in the percentage share of particles of the given diameters in the individual days as shown in the charts. In the case of aquarium with PET bottles, the particle size drop lasted from 7 to 21 d and in the other two aquariums to 35 d. After this time, in all tanks a gradual increase in the size of the particles was observed. In this instance, the particles forming the suspension were larger than the first week of the experiment. The maximum increase of the size of suspensions occurred after 70 d in each aquarium. With the exception that in the aquariums not inoculated with algae, the maximum particle diameters were up to 500 µm, while in the tank enriched with these microorganisms, the particles were twice as large (1,000 µm). After 84 d, the particle diameter decreased again in each tank, which confirms the occurrence of fluctuations in the particle size distribution in sewage.

Changes in the fractal dimension in the course of the experiment are shown in Figs. 5–7.

Fig. 5 shows that the fractal dimension increased after 7 and 45 d and reached the highest value (Df = 2.5) following 70 d of the experiment, when the particles of suspension had the largest diameters, whereas in the further step particles sizes constantly decreased until the 84th day, the end of experiment. It can be found that the change in the structure and size of particles are to a certain extent dependent on each other. Similar dependencies as in the case of the tank with bottles can be seen in Fig. 6, it shows the fractal dimension of the particles in the aquarium with anti-erosive mats. In this case, a sharp increase and subsequent decline in the fractal dimension is also observed. A sharp increase occurred after 45 d from the beginning of the experiment, reaching about Df = 2.2 similar to the one reached on the 70th day of the experiment.

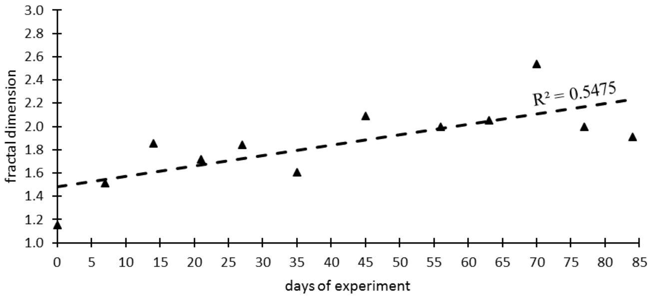

Fig. 5. Fractal dimension of particles in the tank with PET bottles.

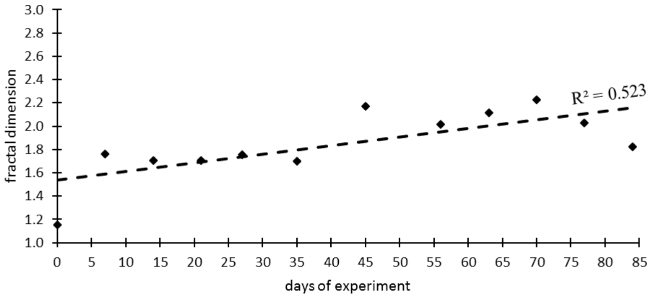

Fig. 6. Fractal dimension of particles in the tank with anti-erosive mats.

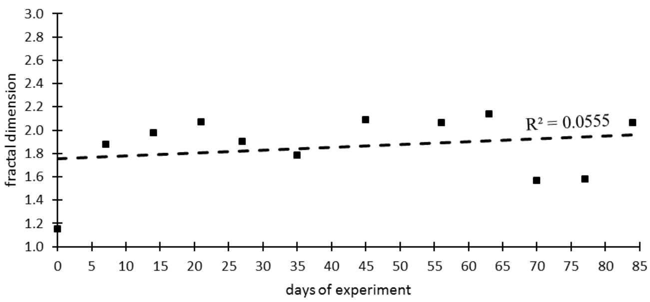

Fig. 7. Fractal dimension of particles in the tank with mixed substrates.

A slightly different situation is observed in Fig. 7, as between the 70th and 77th day of the experiment, there was a significant decrease in the fractal dimension in the aquarium containing both substrates from 2.1 to 1.6, whereas the results of the Avrami equation indicate that in these days the particles had the largest diameters. In addition, in the earlier days of the experiment, the dynamics of fractal changes are similar to those in the other two tanks and reflect the changes in particle size distribution in Fig. 4.

The results from granulometric analysis were used to calculate mean diameters, which are the way of expressing many properties of particles in suspension. Sauter mean diameter D(3.2) is calculated as the ratio of the sum of the volume of particles to the sum of their surface [35,36]. The increase in its value indicates the reduction of the active surface and decrease in the degree of fragmentation of particles in solution [8]. Based on Figs. 8–10, the increase in this parameter has been observed, though, from the first day of the experiment, the mean diameter D(3.2) showed on upward trend, with the highest values on the 45th and 70th day for the tank with only bottles and the tank with only mats. In the case of

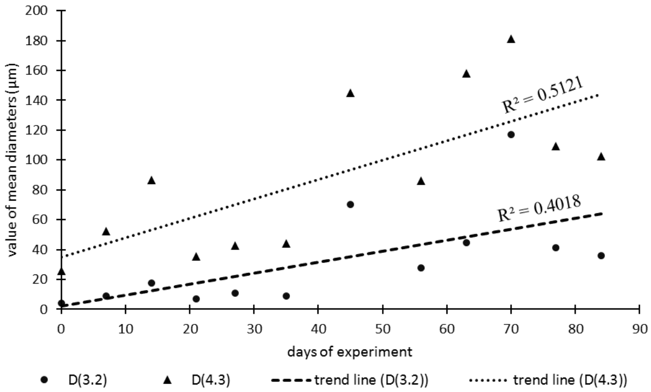

Fig. 8. Mean diameters D(3.2) and D(4.3) determined for particle size distribution of suspension in the tank with PET bottles.

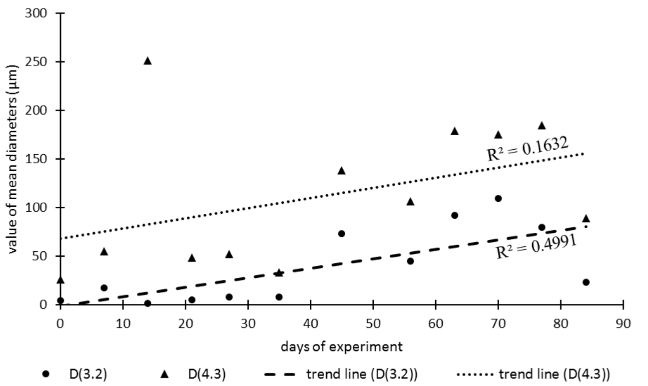

Fig. 9. Mean diameters D(3.2) and D(4.3) determined for particle size distribution of suspension in the tank with anti-erosive mats.

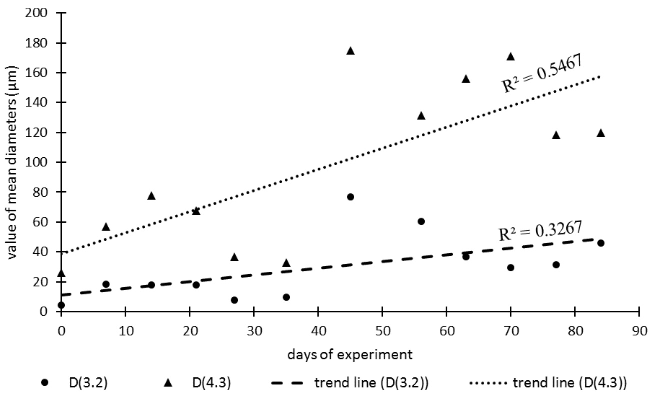

Fig. 10. Mean diameters D(3.2) and D(4.3) determined for particle size distribution of suspension in the tank with mixed substrates.

mixed substrates, a decrease in Sauter's mean diameter was observed on the 70th day of the experiment.

Figs. 8–10 also illustrate the dynamics of the mean diameter D(4.3) changes, which determines the mass of the particles in suspension in relation to their volume [8]. The increase in the proportion of large particles in solution, causes an increase value of mean diameter D(4.3). It is positive to observe that on the 7th, 45th and 70th day of the experiment, as the size of the particles increased, the mean diameter D(4.3) in each aquarium also increased. On the basis of the results, it can be concluded that the type of substrate did not have an influence on the size of particles which altered their diameters along with the growth of algae in wastewater. It should be noted that in each of aquariums, the diameter of the particles increased in comparison with the day of the start of the experiment. This is confirmed by mean diameters D(4.3) and D(3.2), which, despite the fluctuations during the experiment, indicates an upward trend (with coefficient of determination 0.1632–0.5467 and 0.3267–0.4991, respectively).

4. Discussion

The results presented from the studies suggest that the growth of algae within 84 d of the experiment fluctuated substantially, which probably was caused by the cycle of growth and atrophy of living suspension components, resulting in a decrease (at the time of atrophy) and growth (during proliferation) of particle sizes. Particle size distribution, fractal dimension and mean diameter D(4.3) imply that after 7, 45 and 70 d from the beginning of the experiment biomass growth occurred [29]. As predicted by the Avrami equation, the biomass accumulated in solution as grains with large diameters. In these periods, the structure of the particles was more spatially developed and compacted so as to increase their density. In the time between the development of algal biomass, the atrophy or destruction of the structure of suspensions occurred. The increase in the diameter of the suspended particles after 7 d of the experiment may be associated with the formation of a large amount of dark brown flocculent suspension, which proved to be very unstable and under the influence of water movements quickly settled down and formed a deposit at the bottom of the tank. Presumably, the flocculent suspension was formed by the precipitation of colloidal fraction of humic substances, which had been coagulated under the influence of the light. Wang et al. [37] showed that effectiveness of humic acid removal is significantly improved by UV radiation in the coagulation process at pH from 6.0 to 9.0. On the day of the appearance of the flocs, the pH in each of the aquariums was about 8.5 which furthermore advantageously affected the agglomeration of humic substances. The largest particles appeared in aquarium where anti-erosive mats were located. This is evidenced by the sharp increase in mean diameter D(4.3) at the 14th day of the experiment to 250 µm and the significant displacement of the cumulative volume chart to larger particles diameters. The fractal dimension of the particles in suspension also increased in comparison with the first day of the experiment, however, it was not too high (1.5–1.9). It was caused by loose and flake-like structure of flocs, ipso facto easily disintegrated into smaller fragments [24]. On the basis of the charts, it can be stated that the increase in the size of the particles occurred at intervals of approximately a month. As a result of the growth of algal biomass, the diameter of the suspension particles increased [25]. In the case of two aquariums with one type of substrates, particles also had a more elaborate structure, as evidenced by higher values of fractal dimension. According to the flocculation mechanism, the agglomeration of the particles in suspension occurs until the aggregates reach a balance between the aggregation and disintegration index. Subsequently, the particle enters a stable phase of its growth [21,25,38]. Upon reaching this phase, further increases in aggregate diameter results in imbalance and disintegration of the particles. It is presumed that during the experiment the particles in tanks underwent aggregation and disintegration phases due to changes in the stage of algal biomass growth. Fig. 4 shows that in the case of the tank with mixed substrates, particles reached a larger size before the disintegration process occurred. Possibly this phenomenon resulted from the introduction of algae from other cultivation, which increased the durability of the aggregates. In this tank, along with the maximum increase in particle size there was a simultaneous decrease in fractal dimension, which did not occur in the other two tanks, where the fractal dimension increased along with particles diameter. According to the data given by Jarvis et al. [38], as the particles size increases, their durability decreases due to weakening the bonding force between the aggregates. This leads to the disintegration of the particles and loosening of their structure. Hence, as a result of the large increase in particle diameter in the aquarium with two types of substrates, there was a disintegration of the aggregates structure and thereby a decrease of the fractal dimension. Jarvis et al. [38] describe that along with an increase in the fractal dimension, the structure of the particles is more compact. Such a structure is characterized by small particles of greater durability, though, with the increase in flocs size, the fractal dimension is reduced for aggregates with weak bonding structure. A decrease in the mean diameter D(3.2) on the 70th day of the experiment in the tank with mixed substrate suggests that smaller, more compact aggregates with a larger active surface formed from the breakdown of large diameter particles. In the latter days (77–84), the mean diameter D(3.2) decreased along with a lowered particle size distribution. The decrease in this mean diameter indicates the increase in the active surface of the particles [8], which is associated with a decrease in the bonding force between the components in suspension. It should be noted that the trend lines shown in the charts of the fractal dimension and mean diameter change, show an upward trend. This is due to the increasing particle size of suspension in wastewater caused by the growth of algal biomass.

5. Conclusions

The study shows that the type of substrate used in the experiment, did not have an influence on the growth of algal biomass within the wastewater. During the experiment, alternating increase and decrease phases in particle size can be distinguished, which is probably related to the cycle of growth and atrophy of algal colonies. In aquariums with PET bottles and anti-erosive mats, the increase in the particle diameter was accompanied by an increase in the fractal dimension and mean diameter D(3.2) and D(4.3) throughout the entire duration of the experiment. Nevertheless, in the aquarium containing mixed substrate and the addition of algae from another culture, what was observed was that the value of fractal dimension and mean diameter D(3.2) decreased for the largest particles in wastewater on the final day. This may be due to the not so compact structure of the aggregates formed, which easily undergoes spatial dispersion, thereby increasing their active surface. The results presented indicate that the analysis of the particle size distribution of the suspension by laser diffraction provides reliable and reproducible results, and can also be successfully used to track the growth dynamics of the microbial complexes forming the suspension in a liquid medium such as sewage or water. The ability to process data obtained with the 'Mastersizer 2000' software produced by 'Malvern Instruments Ltd.' allows simultaneous comparison of numerous parameters characterizing the particle size distribution. With short intervals between measurements and no additional preparation of sewage samples, it was possible to conduct multiple measurements which significantly influenced the reliability of the results. It is noteworthy that the features of particles in suspension analyzed in this study do not exhaust the scope of software correlated with laser particle size distribution devices. Perhaps in the course of subsequent experiments it will be possible to fully exploit the analytical capabilities of the laser granulometer.

Symbols

$V(t)$ — A volume share of crystallized fraction in solution after time
$V(d)$ — A volume share of particles of given diameter
t — Duration time
k — A constant of rate of transformations depending on nucleation and growth rate
n — Exponent associated with geometry of growth and nucleation mechanism, it assumes values 1–4
d — Substitution diameter of the growing crystals
G — Linear growth rate of the crystals
Df — Optical fractal dimension
R^2 — Coefficient of determination
PET — Polyethylene terephthalate

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