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Characterization of Activated Sludge by Image Analysis Methods

Charakterizace aktivovaného kalu pomocí metod obrazové analýzy

Diploma thesis

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Abstract

In the diploma thesis, an automatic image analysis procedure has been developed. It characterizes activated sludge microorganisms and aggregations which they create with the help of Image Processing Toolbox in the MATLAB environment. Activated sludge samples collected during 3 month period from wastewater treatment plant in Liberec have been processed by manual laboratory methods to find out its basic characteristic, including the most important one – sludge volume index (SVI). Digital images of activated sludge flocs have been taken by microscope acquisition system and put through the automatic image analysis procedure. Several morphological parameters characterizing flocs, like form factor, fractal dimension, aspect ration etc., have been determined by software together with flocs composition. Calculated morphological parameters and ratios of filamentous microorganisms and EPS with respect to the total activated sludge area have been compared with SVI in order to find basic relationships. Proposed image analysis method can provide support for the classification of undesirable events like sludge bulking in the wastewater treatment plant.

Keywords: image analysis, morphology, activated sludge, sludge volume index, MATLAB

Abstrakt

Diplomová práce se zabývá návrhem automatické obrazové analýzy pro hodnocení charakteristik aktivovaného kalu z čistírny odpadních vod. Za tímto účelem bylo využito programové prostředí MATLAB a jeho knihovna Image Processing Toolbox. Vzorky aktivovaného kalu byly po dobu 3 měsíců odebírány z čistírny odpadních vod v Liberci a následně zpracovány v laboratoři pomocí klasických metod. Byly u nich stanoveny základní charakteristiky společně s nejdůležitějším parametrem – objemovým indexem kalu. Fotografie vzorků pořízené za pomoci soustavy mikroskopu a digitálního fotoaparátu byly následně automaticky analyzovány navrženým softwarem. Získaná data v podobě morfologických vlastností (činitel tvaru, fraktální dimenze, poměr stran apod.) společně s informacemi o kompozici vloček (poměr vláken a EPS vůči celkovému objemu vloček) byly porovnávány s objemovým indexem kalu za účelem nalezení základních souvislostí a vazeb. Navržená metoda analýzy obrazu může poskytnout podporu při klasifikaci nežádoucích stavů, jako je bytnění kalu, v čistírně odpadních vod.

Klíčová slova: obrazová analýza, morfologie, aktivovaný kal, objemový index kalu, MATLAB

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AR	Aspect ratio
ECD	Equivalent circle diameter
EPS	Extracellular polymeric substances
FD	Fractal dimension
FF	Form factor
PA	Projected area
RN	Roundness
RG	Reduced radius of gyration
STD	Standard deviation
SVI	Sludge volume index
WWTP	Wastewater treatment plant

INTRODUCTION

Water is very important part of our environment. It is essential for all organisms on the planet including human. However, we do not care much about the water sources and consume large quantity of water everyday. Product of our working is wastewater coming from industry, agriculture, public utilities, homes etc. This wastewater has to be treated before it returns back to the natural water system, for example to a river or a sea. The treatment is based on the biological processes in the wastewater treatment plants (WWTP), in which specific microorganisms metabolize organic pollution and in this way purify the water.

Activated sludge is one of the most widely used processes in the wastewater treatment technology. In this case, a mixture of microorganisms creates structures called activated sludge flocs. These flocs and their characteristics, especially sedimentation ability, have a direct influence on the efficiency of the activated sludge process and the whole purification as well.

There are many plants that show a problem with sedimentation which can lead, in the specific conditions, to a collapse of the whole purification process. The main duty of the plant operators is to prevent this collapse using a set of common tools. One of them is a microscopic technique which allows direct optical investigation of the flocs. There are also other methods – classical laboratory ones which are used along with the optical analysis to obtain more specific results. The operators classify microorganisms by hand and control ongoing processes in the plant on the base of the classification results. As the classical laboratory methods are manual techniques, they are very time-consuming and evaluation of results is very slow. Moreover every investigator has a different approach and view in dependence on his experience. This introduces subjective criteria and interpretations which are undesirable.

Lately, one of the computer techniques has been used more and more in different kind of human activities. It is called the digital image processing. Field of wastewater treatment is not an exception. It is possible to use the methods of digital image processing to make automatic analysis of the activated sludge flocs. In general, it brings objective evaluation in connection with efficiency and high speed processing of large amount of data. It would allow quick response if any problem occurs. Unified results can be given to user without detailed knowledge of analyse procedure and it is also

significant advantage.

From this reason, the main aim of the practical part is to use the digital image processing methods in wastewater treatment process. The main idea of the work is to use this computer technique for activated sludge analysis and evaluation and verify its functionality by means of classical laboratory methods. The practical part is divided into 3 consequent parts.

In the first part, a set of classical laboratory methods, including among others sedimentation, suspended solids and sludge volume index determination, is used to obtain basic parameters of activated sludge. Also microscope slides are prepared for image acquisition. All the laboratory analyses are done manually.

In the second part, an experimental program code which uses digital image processing methods for automatic analysis of activated sludge flocs is developed and applied to the sets of photos taken by a digital camera connected to a microscope. Morphological characteristic like form factor, fractal dimension, aspect ratio etc. along with parameters describing flocs composition (filaments and ESP to total flocs area ratio) are computed.

And finally, in the third part, the results of manual investigations are taken and compared with the results from the computed image analysis to find basic relation between them. The results from the comparison could be used by plant operators for automatic efficiency analysis and prediction of activated sludge process behaviour (sludge bulking, foaming) or alternatively for its collapse. It could help them to control the treatment process better.

The theoretical part is primary focused on introduction to the activated sludge process and a set of common laboratory tools which are manually used for activated sludge characterization. This part also deals with image analysis methods and determining which morphological parameters can be determined by image analysis in the practical part.

THEORETICAL PART

The theoretical part briefly introduces the main principle of the wastewater treatment and the activated sludge process. Within this context, the techniques and methods of image processing that are used for image analysis of activated sludge are described.

1 WASTEWATER TREATMENT

How it is mentioned in the introduction, the wastewater, also called sewage, comes from various human activities starting with industry and ends with households. It is necessary to treat this kind of water before its returning to the natural water system. There are several main steps in this process which are common for all plans, see Figure 1. They are:

- Pre-treatment

- Primary treatment

- Secondary treatment

- Tertiary treatment

1.1 Pre-treatment

First, coarse particles like trash, plastic packets, tree limbs etc. are removed from the wastewater entering the WWTP using the bar screens. Then the heavy solids like sand, grit or broken glass are caught in special chambers and channels. Floating solids and fat are collected from the surface by mechanical skimmers. This step is called pre-treatment and it generally avoids pumps damage or blocking. [1]

1.2 Primary treatment

The wastewater afterwards comes to large sedimentation tanks – primary clarifiers where a large volume of suspended solids are removed, about 70 %. In this way pre-treated water enters the purification step called secondary treatment. [1]

1.3 Secondary treatment

Secondary treatment is based on the biological processes in which specific microorganisms (also called biomass) metabolize suspended and soluble organic matter

in the presence of oxygen. Part of the organic content is synthesized into new cells and part is oxidized to carbon dioxide and water to derive energy for cell growing and division. [3]
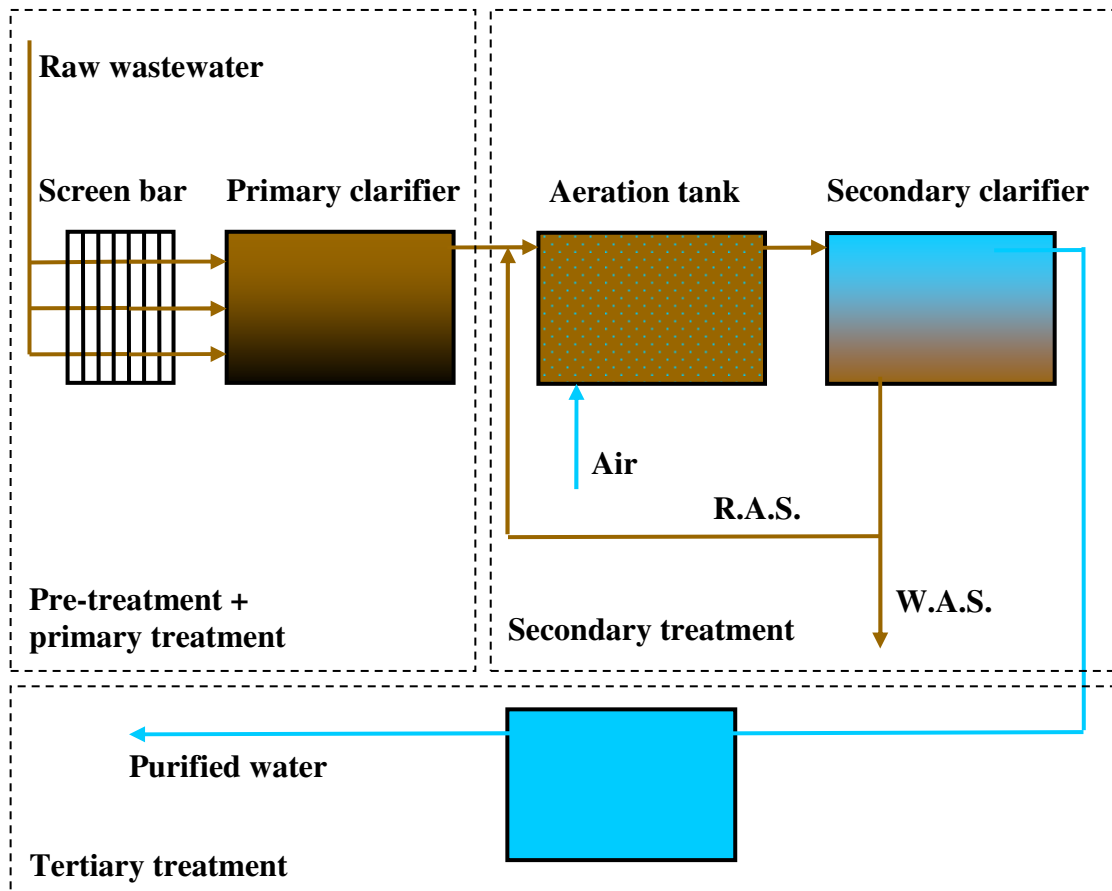
Figure 1 - Activated sludge process [4]

There are two main philosophies used in the biological treatment which differ from each other in principle of biomass growth. They are:

- Attached growth (fixed-film) systems

- Suspended growth systems

In the attached growth systems, the biomass is fixed and grows on suitable media called carrier. The media can be natural materials such as carbonized coal or limestone chips, or specially fabricated plastic media. The wastewater is in contact with a biomass film on its surface and in this way the microorganisms remove organic pollution from the water. [1] [2]

On the other hand, in the suspended growth systems, there is a free-floating biomass in contrast to fixed-film. The activated sludge process is a part of these systems and it is introduced in the next chapter.

At the end of this step the suspended biomass is separated from the water in the sedimentation tank – secondary clarifier and the purified water leaves the plant or alternatively goes to the tertiary treatment.

1.4 Tertiary treatment

The purpose of this stage is to raise the effluent quality before the water enters the receiving environment. It is usually consists of several techniques to decrease chemical or microbiological pollution – sand filtration, active carbon absorption, disinfection, stabilization etc. For example, residual nitrogen and phosphor cause excessive growth of cyanobacteria that produce toxins in static waters so it is desired to remove these nutrients from the water first. [1]

1.5 Activated sludge process

In a huge tank, called aeration tank, see Figure 1, the wastewater free from coarse particles is aerated by bubbles so that a mixture of microorganisms in an aquatic environment is created. Microorganisms produce extracellular polymeric substances (EPS) reducing an electric charge on their surfaces which allows them easily to adhere together and develop aggregations, biological flocs, in the process called bio-flocculation. [6] More information about the flocs structure and composition is in next chapter.

These aggregations are in contact with pollutants and reduce the biological content of the wastewater. Beyond a certain time period spent in the aeration tank, the mixed liquor (water and microorganisms) enters secondary clarifier also called sedimentation tank, see Figure 1. Here the biomass settles by gravity to the bottom while the clean water overflows the weir. Part of the settled material is return back to the aeration tank to mix with the new wastewater entering the plant. This fraction of the sludge is called Return Activated Sludge (RAS). [5]

Excess sludge is called Waste Activated Sludge (WAS) and it is removed from the process to keep the ratio of biomass and suspended/soluble nutrients in balance. This waste is stored in sludge tanks and stabilized by aerobic or anaerobic digestion. It can be

used as a soil fertilizer. [5]

Simply said, this is the base of the activated sludge process. There are many other processes in parallel to the main one but they are not so important for understanding the context.

1.5.1   Activated sludge floc

An activated sludge floc composition is shown on Figure 2. Whole aggregation sticks together thanks to EPS which are high molecular weight compounds secreted by floc-forming *zoogloea* bacteria. EPS protect them from a hostile environment – against cold, toxins etc. Floc-forming bacteria create colonies which are the bases of the floc [7], see Figure 2.
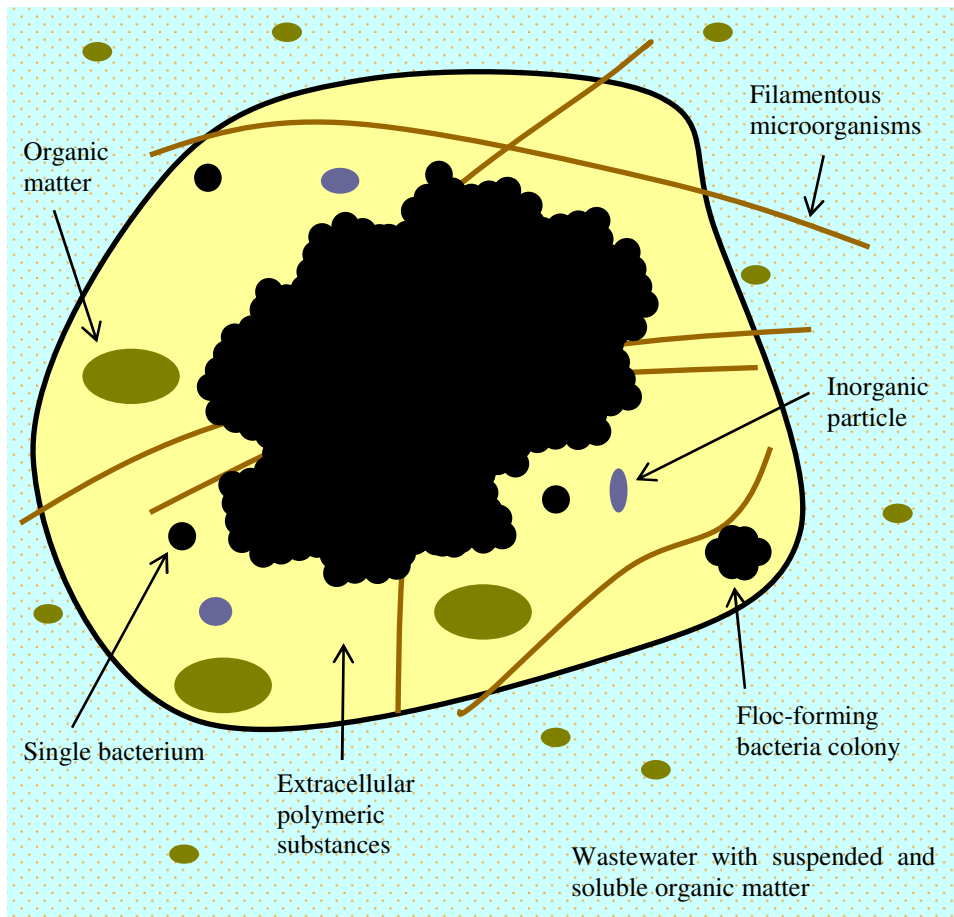
Figure 2 - Activated sludge floc

Beside floc-forming bacteria, there are also filamentous microorganisms (filamentous bacteria, actinomycetes, fungi) and protozoa. There are also presented organic or inorganic particles in various proportions. [7]

A good balance between filamentous organisms and floc-forming bacteria favours formation of strong, large and dense flocs which are very compact and settle well. On the other hand, an imbalance causes bad settleability, washing out of the sludge and a contamination of the receiving water system. Filaments presented in the system often create a backbone inside of the floc and add a rigid structure which helps to settle faster. This type of internal filaments is advantageous. [8]

Several studies have verified this assumption and shown that flocs morphology and balance between both types of microorganisms are directly related to settling and compaction properties of the activated sludge. [8]

In special cases, it can be very difficult to sediment the sludge as there are several problems explained in the next chapter.

1.5.2   Activated sludge separation problems

The quality of the final effluent and resulting efficiency of whole plant as well is in relation with behaviour of the secondary clarifier where the biomass sediments. Primary conditions for successful operation are good separation and thickening properties of activated sludge. [9]

Problems with activated sludge separation are the major problems in WWTP which can cause serious operational issue. There are the most common malfunctions found in activated sludge process that cause bad sedimentation: [11]

- Pinpoint flocs

- Dispersed growth

- Filamentous bulking

- Viscous (zoogleal) bulking

- Rising sludge

- Foaming

1.5.2.1   Pinpoint flocs

This malfunction leads to the breakage of large flocs and formation of small unsettleable micro-flocs with size about 50 – 100 μm, see Figure 3. These flocs are compact and roughly spherical but structurally weak due to low filaments concentration.

During the settling test, the activated sludge rapidly separates into two fractions – large and small flocs. Although, large flocs settle well the small ones settle very bad or not at all. A final effluent is turbid and has high matter content. The reasons for the floc disintegration are high sludge age, total absence of filamentous organisms or cutting effect of high speed mechanical aerators. [10] [11]
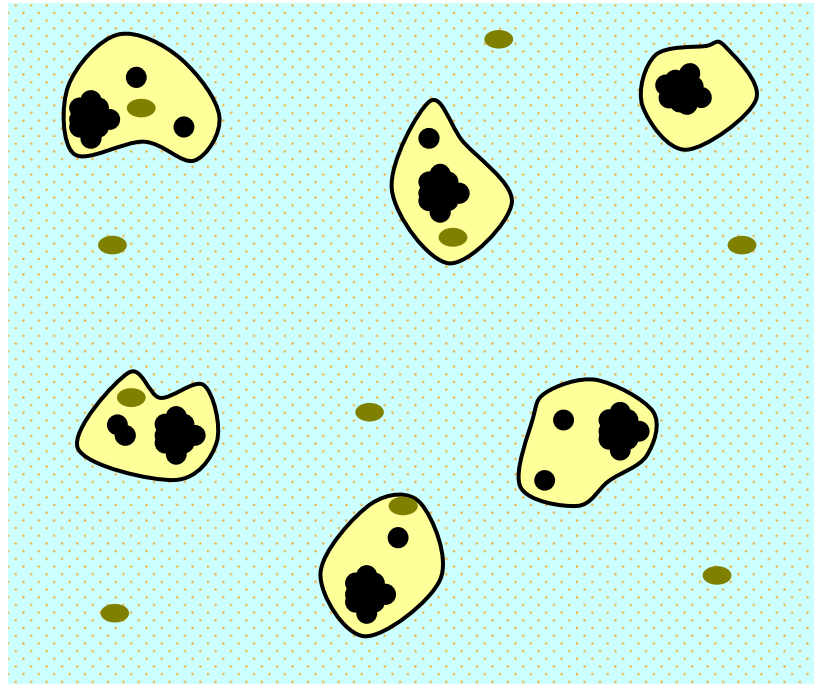
Figure 3 - Pinpoint flocs

1.5.2.2   Dispersed growth

In this case, individual cells or their small aggregations up to $10 - 20$ μm are freely dispersed in the liquid, see Figure 4. Dispersed growth is caused by poor bio-flocculation – low production of EPS. Floc-forming bacteria do not need to produce EPS in the environment with high organic loading (with high food to microorganism ratio – F/M). Ability to sediment of these dispersed cells is very low so it is almost unable to remove them by gravity sedimentation in the secondary clarifier. The final effluent is turbid like in the previous case and a significant amount of biomass escapes from the system. This problem usually appears during the start up phase of the activated sludge process. [10] [11]
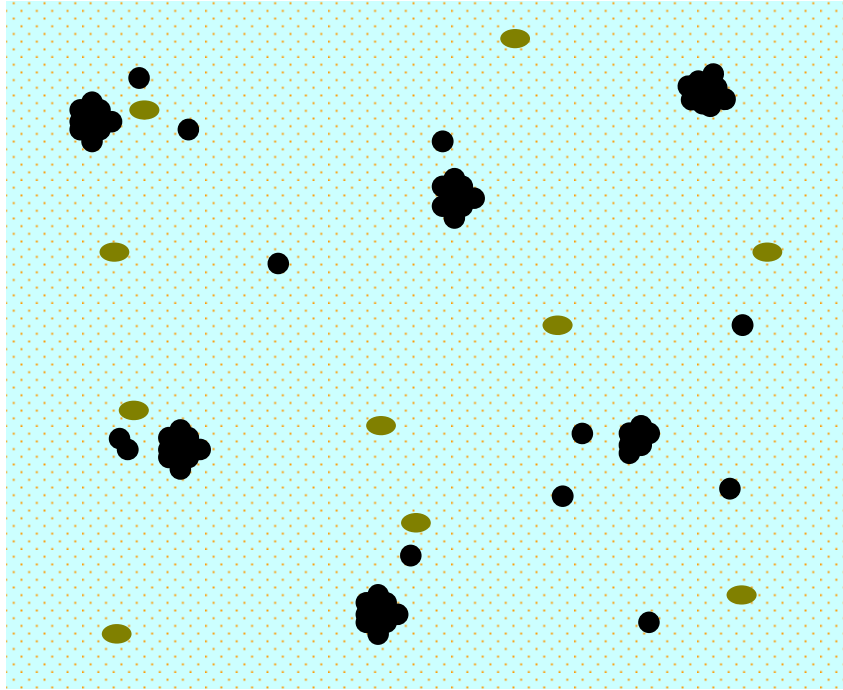
Figure 4 - Dispersed growth

1.5.2.3   Filamentous bulking

This is the most common malfunction in the activated sludge. In situation of specific nutrients deficiency, presence of toxic substances and poor oxygenation, the filamentous organisms start to grow abnormally high along with lower pH. Depending on the type of concerned filaments, two forms of interference in sludge settling occur.
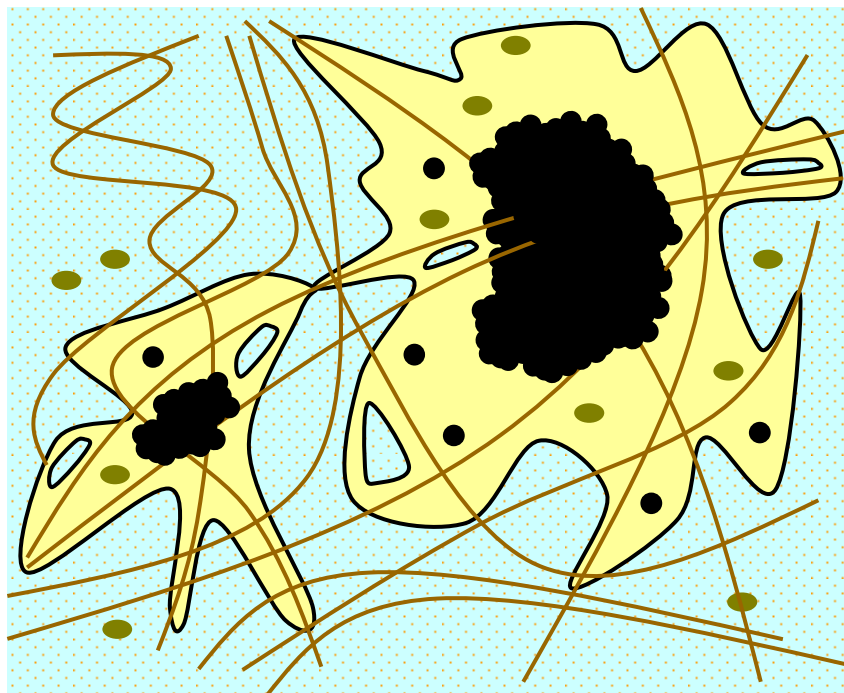
Figure 5 - Filamentous bulking

Inter-floc bridging is the first one where the overgrown filaments among flocs hold them apart. The second one is called open-floc structure where the filaments grow mostly within the floc and create floc internal voids, see Figure 5. Highly irregular and large flocs are formed. Therefore settling velocity is strongly decreased as well as sludge compaction. It can result in the sludge loss to the effluent, decreasing of the plant capacity and failure of the process. [10] [11]

There are approximately 24 recognized filaments or groups of filaments that cause activated sludge bulking and/or foaming. These filaments are isolated and named but in some cases their generic names were replaced by four digit number code. [12]

There are some ways how to control the filamentous bulking (dominant growth of filaments) including the addition of chemicals such as chlorine or hydrogen peroxide, a change of the dissolved oxygen concentration in the aeration tank or addition of nutrients (nitrogen, phosphor) into the wastewater. [10]

1.5.2.4   Viscous bulking

This malfunction is caused by overproduction of EPS. The activated sludge becomes highly water retentive because of the hydrophilic property of the extracellular biopolymers. It has a viscous, jelly-like consistence which can foam when it is aerated intensively.
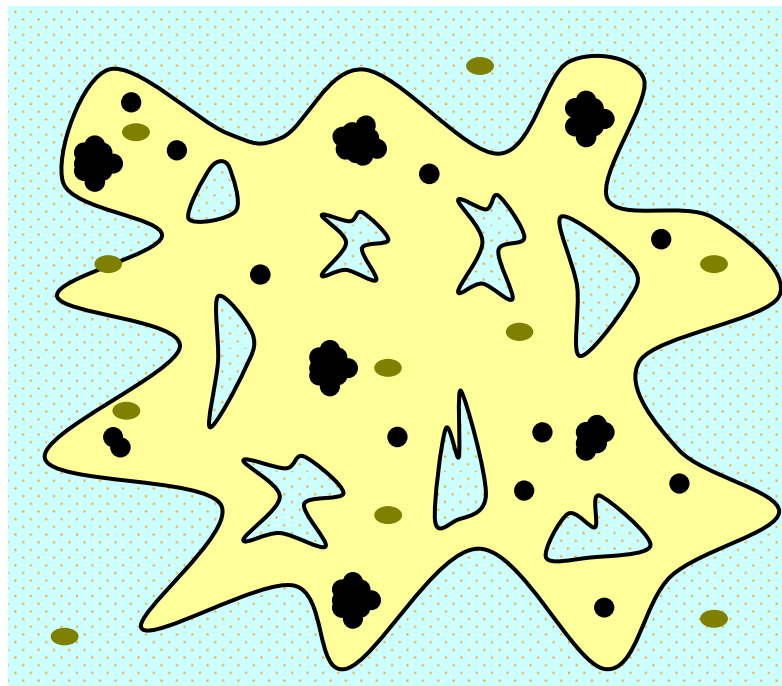
Figure 6 - Viscous bulking

As the result of that, the flocs have poor settling ability and compaction. It leads to overflow of the sludge. Under normal condition, the amount of biopolymer is generated just enough for right flocs forming. In the presence of toxic compounds or bad nutrients balance, it is produced in higher amount. [10] [11]

1.5.2.5 Rising sludge

In this case, the water surface of the secondary clarifier is covered by patches of floating activated sludge. Settled and thickened sludge starts to floating and move up to the water surface after a certain period of time. It is full of gas bubbles coming from the denitrification process which takes place in the settled layer. It is usually caused by poor performance of the removing system in the secondary clarifier that allows the sludge to stay too long in the tank. [10] [11]

1.5.2.6 Foaming

This malfunction is caused by hydrophobic filamentous bacteria overgrowth such as *Nocardia sp.* and sometimes *Microthrix parvicella* or non-degradable surfactants presence. Small air bubbles are entrapped in the flocs and make their density lower. It leads to the floating and biomass loss over the weir. [10] [11]

1.5.2.7 Summary

Six problems with activated sludge separation have been presented. It is necessary to solve and rectify these problems when occur to avoid the activated sludge process collapse. The first step is identification of the problem usually using the microscopic examination together with standard laboratory methods that are done manually. On the base of the result, the plant operators make an intervention. [10]

1.5.3 Activated sludge characterization

Characterization of the activated sludge and determination of the right microorganisms balance is a task for a specialist in water treatment process and always depends on the particular situation in a plant and what kind of wastewater is treated.

There are several laboratory methods which are commonly used by plant operators for activated sludge characterization. These methods follow the European Standards and the basic ones used in this work are introduced in chapter Laboratory methods.

2   IMAGE PROCESSING

Image processing is a type of signal processing where the input is an image. Nowadays in the Computer Age, it usually relates to digital image processing but analogue image processing can be also used. In this work, term image processing always means the digital approach.

Image processing in digital domain uses computers as executive tools and that is why it has many advantages resulting from this relation. It allows high efficiency, high speed processing of large quantity of data with unified results, avoidance of noise increasing etc. Image processing provides wide set of tools and methods that are applied to the input data usually in chronological steps. The methods used in this work are introduced in this chapter. They can be classified into these categories:

- Image acquisition

- Image pre-processing

- Image enhancement

- Image segmentation

- Morphological image processing

- Region identification

2.1   Image acquisition

Before the processing itself, it is necessary to perform an image acquisition first. Digital CCD camera or any other input device, for example X-ray sensors in digital radiology, can be used. Obtained image enters the first step of image processing.

2.2   Pre-processing

Prior to data analysis, initial processing also called pre-processing is usually performs to correct any distortion in image due to physical characteristics of the acquisition system or conditions during which the image was taken. One of the often problems is a non-homogenous light that can significantly affect resulting image quality. Another one is for example vignetting reducing an image brightness or saturation at the periphery compared to the image centre. It is caused by camera settings

or lens limitations. All of these influences should be compensated in pre-processing to avoid problems in next steps.

2.3 Image enhancement

Considering the limited capacity of optical imaging instruments and the noise inherent in optical imaging, image enhancement is desirable for many reasons. Image enhancement is the process of enhancing the appearance of an image for better contrast or visualization of certain features and to facilitate subsequently more accurate image analysis. [16]

Various methods and algorithms for image enhancement have already been developed and verified. They can be classified into two categories: spatial domain and transform domain methods. Spatial domain methods manipulate the image in the common image form and they are applied on a whole image area or selected regions of the interest. Techniques that belong to this category include contrast stretching, histogram equalization, image averaging, sharpening of important features such as edges or contour and noise removal. [16]

On the other hand, the transform domain methods manipulate the image in modified form for example in frequency domain obtained by Fourier transform. In many cases, important features cannot be extracted from the image in the spatial domain so that the transform domain methods have to be used. [16]

For purpose of this work, only contrast stretching and noise removal by Median filtering are discussed below.

2.3.1 Contrast stretching

Contrast stretching is a simple method which can enhance an image contrast. It is often called normalization or, in more general fields of data processing, dynamic range expansion. It is a linear process that maps intensities of all the pixels in the image to desired range of values, usually to maximal possible range. For example for 8-bit grayscale image the lowest and highest limits are 0 and 255 respectively. [16]

$$O_{(x,y)} = \frac{I_{(x,y)} - I_{\min}}{I_{\max} - I_{\min}} \left(I_2 - I_1 \right) + I_1 \tag{1}$$

$I_{(x,y)}$… Input original image

$O_{(x,y)}$… Output image with stretched contrast

I_{min}… The lowest intensity value in the input image

I_{max}… The highest intensity value in the original image

I_1… The lower limit of desired range (usually 0)

I_2…The upper limit of desired range (usually 255)

2.3.2 Median filtering

The median filter is commonly used operator that can effectively reduce salt and pepper noise which randomly occurs as white and black pixels in the image. It replaces the original intensity of the pixel by the median of the pixel intensities in the specific neighbourhood, see Figure 7. This filter is very useful because it can reduce noise without blurring edges in the image. [16]
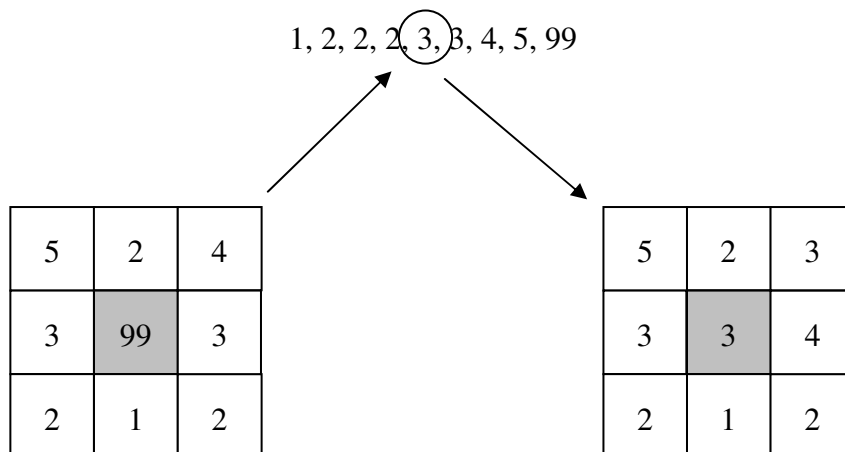
Figure 7 - Median filter

2.4 Image segmentation

Image segmentation is a fundamental task in image processing and it is very important for subsequent image analysis. It is a process that divides an image into disjoint regions typically corresponding to particular objects. Once these objects are isolated, they can be measured, classified and analysed. [16]

Image segmentation is usually approached from one of two different perspectives – seek either the regions or boundaries of objects in the image. A region is a connected set of adjacent pixels with the same properties – colour, intensity, texture etc. In the

region-based approach, we consider each pixel in the image and assign it to a particular region or object. In the boundary-based approach, either we attempt to locate directly the boundaries existing among the regions or we try to find edge pixels and link them together to establish required boundaries. Segmentations resulting from these two approaches may not be exactly the same but both are useful for solving image segmentation problems. [16]

2.4.1 Thresholding

Thresholding is an essential region-based segmentation technique that is particularly useful for scenes with solid objects located on uniform contrasting background. This technique tries to distinguish the objects of interest (foreground) from background on which they lay. The input is a grayscale image and the output is a binary image containing only two values (logical 0 and 1) which represent background and foreground class respectively. To which class the pixels are assigned, depends on their intensity. All pixels at or above the threshold value are assigned to background class and the rest of the pixels to foreground class. This approach assumes that objects of interest have low level intensity while the background is high level. [16] In general, the operation can be described as

$$O_{(x,y)} = \begin{cases} 0 & if\,(I_{(x,y)} \ge T) \\ 1 & if\,(I_{(x,y)} < T) \end{cases} \tag{2}$$

$I_{(x,y)}$… Input grayscale image

$O_{(x,y)}$… Output segmented binary image

T… Threshold value

The threshold value can be fixed or can be estimated from the image before the segmentation itself. For the optimal threshold value detection, several techniques can be used. One of them is the Otsu's method. It is an automatic nonparametric method that tries to find by iteration the value of intensity by which the foreground and background classes are divided in the image with bimodal histogram distribution. For more information, see [17].

2.5 Morphological image processing

Morphological processing is a broad set of image processing operations based on shapes which apply a structuring element (simple pre-defined shape) to an input image. We can say that the main idea is to probe an image with the structuring element and to quantify the manner in which the element fits or does not fit within the image. Simply said, the value of each pixel in the output image is based on a comparison of the corresponding pixel in the input image with its neighbours. The pixel's neighbourhood is determined by the shape and size of the structuring element. By modifying of these two parameters, it is possible to construct a morphological operation which is sensitive to specific shapes in the input image. [16] [18]

As the morphological operations can be applied to a grayscale image, it this work, only binary morphological operations are introduced and discussed but only in very brief way. For more information, see [16]. Basic morphological operations are erosion and dilation.

2.5.1 Binary erosion and dilation

How it was mentioned in the previous chapter, binary image contains only two values – logical 0s and 1s representing background and foreground of the segmented image. Generally, erosion shrinks the image objects – removes pixels on their boundaries while dilation expands the image objects – adds pixels to their boundaries. The number of pixels which are removed or added depends on the shape and size of the structuring element.

In erosion, the output pixel is set to logical 0 if any of the input pixel's neighbours, lying under the structuring element, is set to logical 0. The erosion is denoted as: [18]

$$O_{(x,y)} = I_{(x,y)} \ominus S \qquad\qquad (3)$$

$I_{(x,y)}$… Input binary image

$O_{(x,y)}$… Output eroded binary image

S… Structuring element

On the other hand, in dilation, the output pixel is set to logical 1 if any of the corresponding pixels in the input binary image is set to logical 1. The erosion is denoted as: [18]

$$O_{(x,y)} = I_{(x,y)} \oplus S \tag{4}$$

$I_{(x,y)}$… Input binary image

$O_{(x,y)}$… Output dilated binary image

S… Structuring element

2.5.2 Binary opening and closing

Besides the basic operations already mentioned, there are two more important operations playing key role in the morphological image processing. They are binary opening and closing and they are derived from fundamental operations erosion and dilation.

The binary opening of an image is defined as erosion followed by dilation using the same structuring element for both operations. It is possible to remove isolated regions or subregions on the object boundaries that are smaller than the structuring elements while preserving the shape and size of the larger objects in the image. The opening is denoted as follows: [16] [18]

$$O_{(x,y)} = (I_{(x,y)} \ominus S) \oplus S \tag{5}$$

$I_{(x,y)}$… Input binary image

$O_{(x,y)}$… Output opened binary image

S… Structuring element

The binary closing is a dual operator for binary opening and it is defined as dilation followed by erosion using the same structuring element for both operations like in the previous case. By binary closing, we can fill gaps, holes and concavities in the image objects that are smaller than the structuring element. The closing is denoted as:

$$O_{(x,y)} = (I_{(x,y)} \oplus S) \ominus S \qquad\qquad (6)$$

$I_{(x,y)}$… Input binary image

$O_{(x,y)}$… Output closed binary image

S… Structuring element

2.6 Region identification

Region identification is a process which allows identification of the particular regions (objects) in an image. It is necessary for region description and it can be done by connected component labeling method.

2.6.1 Connected components

A set of pixels (region) is said to be connected if any two pixels from this set can be linked by sequence of neighbour pixels also included in this set. The region can be either 4-connected or 8-connected in 2D image depending on adjacency of the neighbour pixels. In the first case, only vertically and horizontally adjacent pixels are taken into account (4 pixels). In the second case, diagonally adjacent pixels are involved as well (8 pixels). If a region is not a proper subset of a larger connected region in the image, it is maximally connected. All regions with this property are called the connected components of the image. [16]

2.6.2 Connected components labeling

Connected component labeling is a method which transforms a binary image into a symbolic one in order to identify each connected component in an image. Unique label (integer number that can represent a colour for example) is assigned to each connected component, see Figure 8. There are several algorithms solving this problem but only one – the classical algorithm, which makes two passes through an image, is very briefly introduced. The first pass performs label propagation and creates an equivalence table, if different labels propagate to the same pixel. The second pass then performs translation – assigning to each pixel the final label, taking into account the equivalence table obtained from the first pass. For more information, see [19].
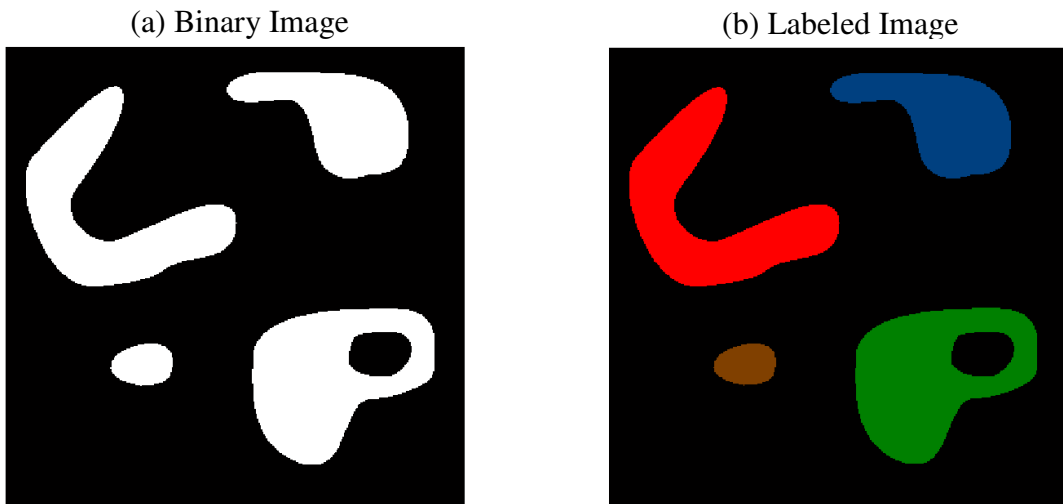
(a) Binary Image | (b) Labeled Image

Figure 8 - Connected component labeling

3   IMAGE ANALYSIS

Image analysis is a process of extraction meaningful information from an image by means of the image processing techniques. Which information is important depends on the particular task or situation. It can be simply colour, texture, size or shape of the investigated objects or more complex parameter. Image analysis strength is in ability to remove human subjectivity during evaluation of data and to avoid very time-consuming tasks for human researchers. Image analysis is used in many fields of human activities, for example in medical applications (Computed Tomography, Magnetic resonance, Ultrasound, X-ray and Microscopy), science, industry, person identification etc.

It this work, image analysis is used for characterization of the activated sludge – morphology and composition of the flocs. This fact defines the main task and to fulfil it, it is necessary first to specify which parameters can express the flocs morphology and composition as well as can be. Several techniques have been proposed in literature in order to describe the complex structure of the activated sludge. In [20], morphological parameters such as equivalent circle diameter, form factor, roundness as well as fractal dimension have been used and correlated with sludge volume index. In another work [9], reduced radius of gyration has been introduced in order to identify filaments in the activated sludge and correlate their length with SVI. In [21], various morphological parameters including among others reduced radius of gyration have been evaluated to find the one which best distinguishes between flocs and filaments. Based on these facts, following morphological parameters based on Euclidian and fractal geometry have been chosen to characterize activated sludge shape in this work:

- Projected area

- Equivalent circle diameter

- Aspect ratio

- 2D-porosity

- Form factor

- Roundness

- Reduced radius of gyration

- Fractal dimension

3.1 Morphological parameters

3.1.1 Length and breadth

There is no unique definition for the length of an object. For this work, the length is defined as the major axis length which it the longest axis of an ellipse with the same second order moment as the object itself. By analogy with the length, the breadth of an object is defined as the minor axis length, the shortest axis of an ellipse with the same second order moment as the object itself. [21]

3.1.2 Perimeter

The perimeter of an object is the length of the pixel line which forms the boundary between the object and the background. It can be 4-connected or 8-connected depending on the approach.

3.1.3 Projected area

The projected area (PA) is simply an area of an object. It can be estimated by number of pixels which the object consists of and it shows how large the object is.

3.1.4 Equivalent circle diameter

The equivalent circle diameter (ECD) represents a diameter of the circle that would have the equivalent area as this object. It is the most suitable parameter for object size evaluation. [21]

$$ECD = 2 \cdot \sqrt{\frac{PA}{\pi}}$$

(7)

3.1.5 Aspect ratio

The aspect ratio (AR) is the ratio of the length to the breadth of an object. This parameter provides a measure of how elongated the object is. A circle has an equal sized length and breadth and consequently an aspect ratio is 1. The more elongated object, the larger its aspect ratio. [21]

$$AR = \frac{Length}{Breadth}$$

(8)

3.1.6   2D-porosity

It is based on the hole-ratio parameter which is described as the ratio of an object area excluding holes to the total area of the object with all holes filled. This parameter shows how the object is porous. It is equal to 0 if no holes are presented and greater than 0 if there are pores inside the object. [8]

$$2Dporosity = 100 \cdot (1 - HoleRatio)$$

(9)

3.1.7   Form factor

The form factor (FF) is the most widely used shape parameter and it is defined as the ratio of an object area to the area of a circle with the same perimeter as the object. An object having ragged contour has a smaller form factor than the smooth one because of its larger perimeter. So it can be used to determine how regular the floc is. Form factor of a circle is equal to 1. [21]

$$FF = \frac{4 \cdot \pi \cdot PA}{Perimeter^2}$$

(10)

3.1.8   Roundness

The roundness (RN) is also a measure of how elongated an object is. It is defined as the ratio of the object area to the area of a circle with a diameter equals to the length of the object. The roundness of a circle is equal to 1 and it decreases for objects deviating from a circle. The roundness is similar to the form factor but instead of the perimeter, it uses the length of an object which makes it more sensitive to how elongated object is rather than how irregular its outline is. [21]

$$RN = \frac{4 \cdot PA}{\pi \cdot Length^2}$$

(11)

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3.1.9 Reduced radius of gyration

The reduced radius of gyration (RG) is a parameter based on the moment of an object and it shows how the pixels are dispersed form object's centre of gravity. For example for a regular disk, it is equal to 0,707. How the pixels are more dispersed, the value increases. This parameter seems to be the best guide how to determine floc or filament. [21]

$$RG = \frac{\sqrt{M_{2x} + M_{2y}}}{D_{eq}/2} \qquad (12)$$

M_{2x}, M_{2y}... The second order moments, defined below

$$M_{2x} = \frac{1}{N}\sum_{N}(x_i - M_{1x})^2 \qquad M_{2y} = \frac{1}{N}\sum_{N}(y_i - M_{1y})^2$$

N... Number of pixels that the object is composed of

x_i, y_i... Coordinates of pixels

M_{1x}, M_{1y}... The first order moments (the objects centre of gravity), defined below

$$M_{1x} = \frac{1}{N}\sum_{N}x_i \qquad M_{1y} = \frac{1}{N}\sum_{N}y_i$$

3.1.10 Fractal dimension

The fractal dimension (FD) of the perimeter of an object is used to characterize the contour irregularity of the object. The value is between 1 and 2. Higher value means higher irregularity. Fractal dimension is calculated by Minkowski Sausage Method using dilation of the object perimeter. For more information, see [22].

3.1.11 Summary

There are 8 morphological parameters mentioned above. Two of them (projected area and equivalent circle diameter) are expressed in pixels and can be used for object size representation. The others are dimensionless. All of the parameters are independent from the orientation of the object in 2D space.

LABORATORY METHODS

There are basic laboratory methods for activated sludge characterization introduced in this chapter. These methods are standards and follow the Czech versions of the European Standards and they are:

- Determination of suspended solids

- Determination of settleability

- Determination of dry residue and water content

- Determination of the loss on ignition of dry mass

- Determination of pH-value

4   ACTIVATED SLUDGE CHARACTERIZATION

4.1   Determination of suspended solids

Full designation of the standard is ČSN EN 872 – Water quality – Determination of suspended solids – Method by filtration through glass fibre filters. This standard describes a method for determination of suspended solids (SS) in raw water, wastewater and effluent from WWTP through glass fibre filters. [23]

In brief, a sample of the activated sludge is taken and weighed as well as the glass fibre filter. Then the sample is placed into the filtration system and filtered with the help of a vacuum air pump. The filter with solids is put into the laboratory dryer for at least 1 hour at 105 °C. After the time period, the filter is weighed again. Weight concentration of the suspended solids is calculated by: [23]

$$\rho = \frac{1000 \cdot (b - a)}{V} \qquad (13)$$

ρ… Weight concentration of suspended solids [mg.l^{-1}]

b… Weight of the filter after filtration [mg]

a… Weight of the filter before filtration [mg]

V…Volume of the sample [ml], if weighed 1 g = 1 ml

In the Czech Republic, the filter with medium size pores (0,7 μm – 1,3 μm) is recommended. For more information see [23].

4.2 Determination of settleability

Full designation of the standard is ČSN EN 14702-1 – Characterisation of sludges – Settling properties – Part 1: Determination of settleability (Determination of the proportion of sludge volume and sludge volume index). This standard specifies a method for determination of sludge suspension settleability which can be expressed by a parameter called sludge volume index (SVI). It is the most important parameter which characterizes quality of sedimentation of the sludge in the final clarifier. It is influenced by the flocs size and the presence of filamentous organisms. It is an empirical measurement determined by standard laboratory methods. [14]

In brief, a one-litre sample of activated sludge is filled in a vessel and left to settle for 30 minutes. After this time period, settled volume of the sludge is read. If the volume is greater than 250 ml.l^{-1}, it is necessary to repeat the process again with new sample which is diluted by sludge water in ratio of 1:1, 1:2 or 1:3. The read settled sludge volume is then multiplied by dilution ration 2, 3 or 4. [13]

For SVI calculation, the weight concentration of SS is required as its determination is introduced in chapter 4.1. SVI is expressed in ml per gram so it represents the volume occupied by a gram of sludge. SVI can be calculated by given formula: [13]

$$SVI = \frac{SV}{\rho}$$

(14)

SVI… Sludge volume index [ml.g^{-1}]

SV… Settled sludge volume [ml.l^{-1}]

ρ… Weight concentration of SS [g.l^{-1}]

For more information see [13].

A high SVI (> 150 ml.g^{-1}) indicates bulking conditions, on the other hand low SVI (< 70 ml.g^{-1}) indicates the predominance of small flocs (pinpoint flocs). SVI between these values usually means normal conditions – good settling flocs. The limits are not

strictly given. They depend on the particular situation in the plant and can be shifted. [15]

4.3 Determination of dry residue and water content

Full designation of the standard is ČSN EN 12880 – Characterisation of sludges – Determination of dry residue and water content. This standard is used for determination of total solids (TS) which are suspended and dissolved in the activated sludge.

A laboratory crucible is placed into a laboratory dryer for at least 30 minutes at 105 °C, cooled down in desiccator and weighed. A sample of activated sludge is put into the crucible, weighed and placed again into the dryer at 105 °C. After a certain time period, when the sample is dry, the crucible is cooled in desiccator and weighed again. The dry residue or TS concentration is given by formula: [24]

$$w_{dr} = \frac{m_c - m_a}{m_b - m_a} \cdot 1000$$
(15)

w_{dr}... Concentration of TS [g.kg^{-1}]

m_a... Weight of empty crucible [g]

m_b... Weight of crucible with activated sludge sample [g]

m_c... Weight of crucible with dry mass [g]

For more information see [24].

4.4 Determination of the loss on ignition of dry mass

Full designation of the standard is ČSN EN 12879 – Characterisation of sludges – Determination of the loss on ignition of dry mass. This standard specifies a method for rough determination of the organic content in the activated sludge.

The loss on ignition is usually determined after dry mass calculation. The crucible with dry mass is put into the laboratory furnace for at least 60 minutes at 550 °C ± 25 °C. After the time period, it is cooled down in desiccator and weighed. The loss on ignition is calculated by formula:

$$w_v = \frac{m_b - m_c}{m_b - m_a} \cdot 100$$
(16)

w_v… The loss on ignition [%]

m_a… weight of empty crucible [g]

m_b… weight of crucible with dry mass [g]

m_c… weight of crucible with dry mass after ignition [g]

For more information see [25].

4.5 Determination of pH-value

Full designation of the standard is ČSN EN 12176 – Characterisation of sludges – Determination of pH-value. This standard describes how to prepare and measure pH-value of the activated sludge. For more information see [26].

5 GRAM STAIN OF MICROSCOPIC SAMPLES

Gram stain is a method used for differencing between two classes of bacteria – gram-positive and gram-negative. It is based on the chemical and physical properties of their cell walls. It is consist of several steps described below: [27]

1. Flood the microscopic slide with crystal violet solution for up to a minute. Wash it off with distilled water and drain it.

2. Flood the slide with a mordant (Gram's iodine solution or Lugol's iodine solution) for up to a minute. Wash it off with distilled water and drain it.

3. Flood the slide with ethyl alcohol, which works as a decolourant, for 10 seconds. Wash it off with distilled water and drain it.

4. Flood the slide with safranin solution and allow counter stain for 30 seconds. Wash it off with distilled water and drain it.

After the procedure, the gram-positive bacteria retain the initial violet stain while gram-negative bacteria are decolourized by the organic solvent and coloured by pink counter stain. [27]

In activated sludge microscope investigation, gram stain is primary used for classification of the filamentous organisms, in other words it is used for identification of the right group which they belong to. While the automatic image analysis is applied, gram stain brings better contrast of the images that can make the characterization easier.

PRACTICAL PART

The practical part can be divided into 3 consequent parts. In the first one, the activated sludge has been manually processed in laboratory to obtain its characteristics and to prepare microscope samples. In the second one, a code for image analysis has been developed in MATLAB environment. Output data of the analysis has been afterwards processed and compared in the last part with the characteristics obtained by the laboratory investigation.

6 LABORATORY WORK

During 3 month time period, 8 samples (approximate 10 litres each) of activated sludge have been taken from the WWTP in Liberec. Each sample has been manually processed in laboratory to obtain its characteristics and to prepare a microscopic slide for the image analysis. Particular samples have been designated as A – H.

6.1 Activated sludge characterization

Each sample of the activated sludge has been characterized with the help of the methods and equations introduced in the chapter 4. There are particular processing steps for a sample shown below. Each step has been repeated 3 times together with mean calculation to minimize errors.

First, a 5ml sample of the activated sludge has been filtered through a glass microfiber filter Whatman GF/C 47 mm with 1,2μm pores, more information [28], which satisfy the condition given by the Standard ČSN EN 872. Filter with suspended solids has been put into a dryer for approximate 2 hours at temperature 105 ˚C. In terms of weights and equation (14), the concentration of suspended solids has been calculated.

Once it is know it is possible to step to settleability determination. First of all an experimental sedimentation has been passed to be able to choose the right dilution ratio as the settled sludge volume should not exceed 250 ml.l^{-1}. Diluted sludge has been slightly homogenized and left to settle for 30 minutes. Found volume has been divided by the weight concentration of suspended solids according with equation (15) to determine the SVI.

As a next step, a laboratory crucible with the activated sludge sample (50 ml) has been dried for approximately 4 hours at 105 ˚C and afterwards put into a furnace for

2 hours at 550 °C. Dry residue and loss on ignition have been determined in terms of the weight differences after particular steps and equations (16) and (17).

As the last step, pH-value has been determined by the portable laboratory pH meter WTW Multi 350i. Sensor has been put into a sample for approximately 5 minutes until the pH value has stopped changing.

There are determined parameters of all samples, taken during 3 month time period from the WWTP in Liberec, in Table 1. The most important parameter SVI, showing quality of sedimentation in the final clarifier, ranges from 78 to 92 ml/g. It is the normal operating condition but it can probably show beginning of predominance of small flocs.

Sample designation	A	B	C	D	E	F	G	H
ρ [mg/l]	3191	9950	8654	9904	10173	9848	10640	4150
SVI [ml/g]	86	78	83	79	79	82	88	92
wdr [g/kg]	3,52	10,82	9,18	10,23	10,26	9,89	11,54	5,03
wv [%]	73,36	68,68	69,17	62,52	63,68	64,51	63,47	60,87
pH [-]	6,86	6,60	6,84	6,65	6,90	6,75	7,07	6,82

Table 1 - Characteristics of sludge samples

6.2   Microscope samples preparation

Approximately 2 drops of well homogenized activated sludge have been spread by plastic spreader on glass microscope slides and left to get dry. Afterwards it has been stained by Gram according to the procedure introduced in chapter 5. There are 2 images on Figure 9 that show the difference between raw sample (left) and stained one (right). The contrast of the stained image compared to the raw one is evident.
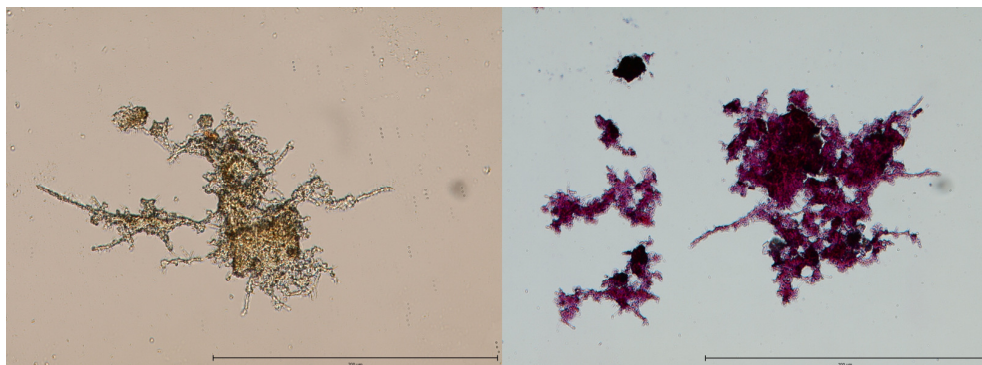

Figure 9 - Raw and stained microscope sample (500x)

Two microscope slides have been prepared for each sample of the activated sludge – it means 16 slides in total prepared for the image acquisition.

7   IMAGE ANALYSIS OF THE ACTIVATED SLUDE

7.1   Image acquisition

Prior to image analysis, it has been necessary first to acquire the digital images of the activated sludge samples. All images have been taken by digital acquisition system composed of microscope Olympus BX51, digital single-lens reflex camera Olympus E-510 in automatic mode and computer software QuickPHOTO MICRO 2.3. Image resolution has been set the highest possible one – 3648 x 2736 pixels (9,98 megapixels) and data has been saved in JFIF/JPEG format.

Optical magnification 200x and 500x have been chosen to be able to extract all the desired information from the samples. In 200x magnification a pixel represents 0,2381 μm while in 500x it is 0,0952 μm. From this facts, real size of the object can be determined.

For each sample of activated sludge, a data set of 100 images has been acquired in double optical magnification. Particular sets have been designated like sludge samples which they correspond to (A – H).

7.2   Development of image analysis code

The code for image analysis has been developed all in the MATLAB environment 7.3.0.267 (R2006b) by means of Image Processing Toolbox. The code can be divided into 2 parts depending on the fact which meaningful information has to be extracted from the image. They are:

- Flocs morphological characterization

- Floc composition characterization

This classification closely relates to the optical magnification quantity. Higher magnification allows extraction of detailed information describing particular floc composition and presence of the filamentous organisms in the aggregation. On the other hand, lower magnification allows investigation of the larger area of the sample. It brings possibility to see entire flocs and determine their morphological characteristics and the shape.

Each part is demonstrated step by step on the sample image to understand the whole analysis process.

7.2.1   Flocs morphological characterization

The first part of MATLAB code processes the image in 200x magnification. On the Figure 10, there is a sample microscopic image of the activated sludge coming from data set C. It is composed of flocs with various size and shape. The algorithm has to isolate them from the background and determine which of them are suitable for an analysis. Once they are known, their morphological characteristics are computed and presented. Entire procedure consists of several steps which are introduced end explained below.
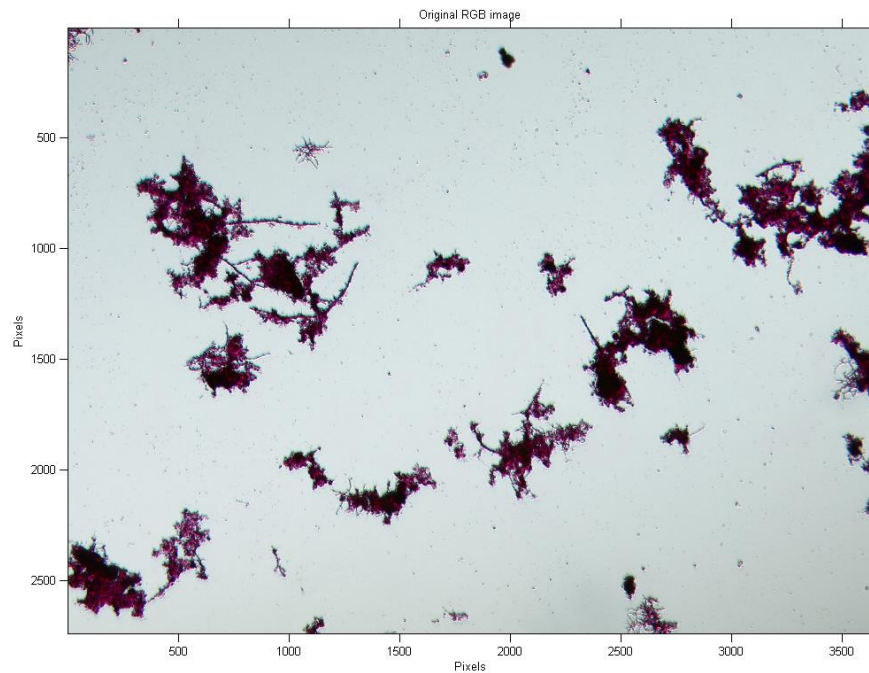

**Figure 10 - Original RGB image of the AS flocs (200x)**

7.2.1.1   Image pre-processing

Before any operation it is necessary first to transform an RGB image into the grayscale one. Each colour channel is multiplied by a coefficient and their sum results to grayscale image. The coefficients are 0,2989 * R + 0,5870 * G + 0,1140 * B.

Due to the non-homogenous microscope backlight and the impurities on the optical system, a mask image, Figure 11, which has been acquired without any sample, is subtracted from the grayscale image to reduce inaccuracy. The pre-processed image is shown on Figure 12.
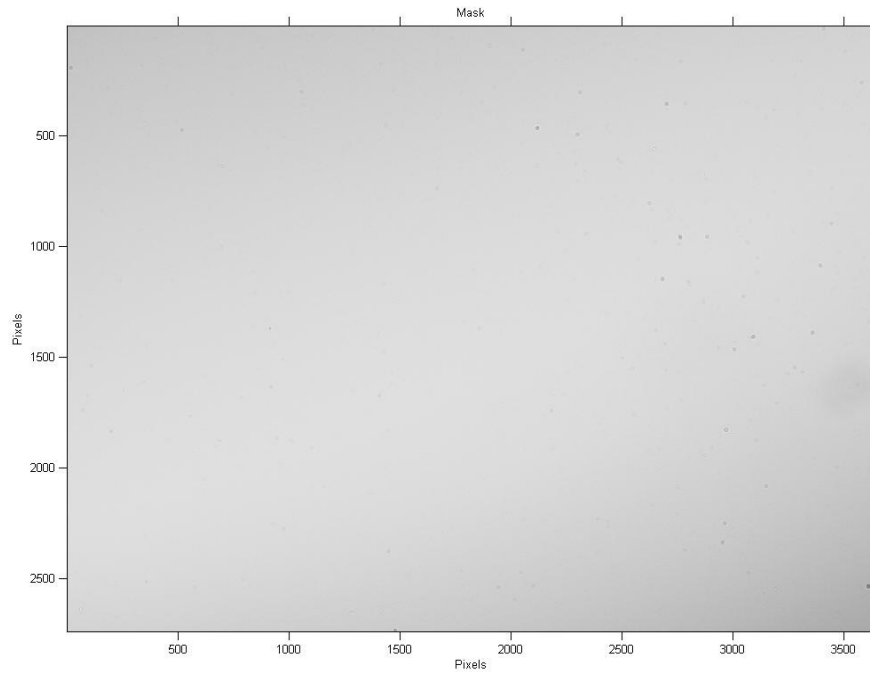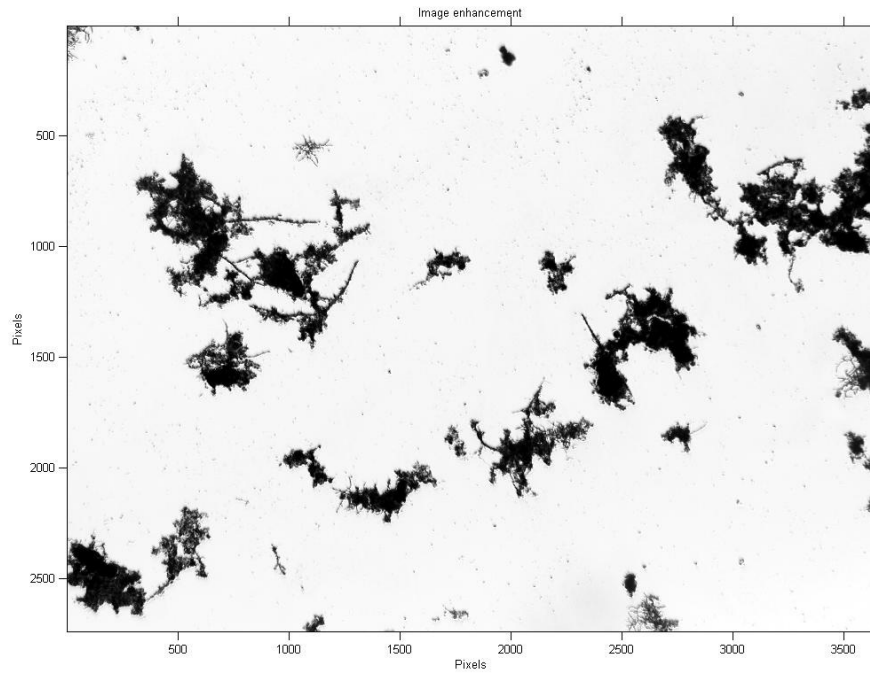
Figure 11 - Mask image to be subtracted
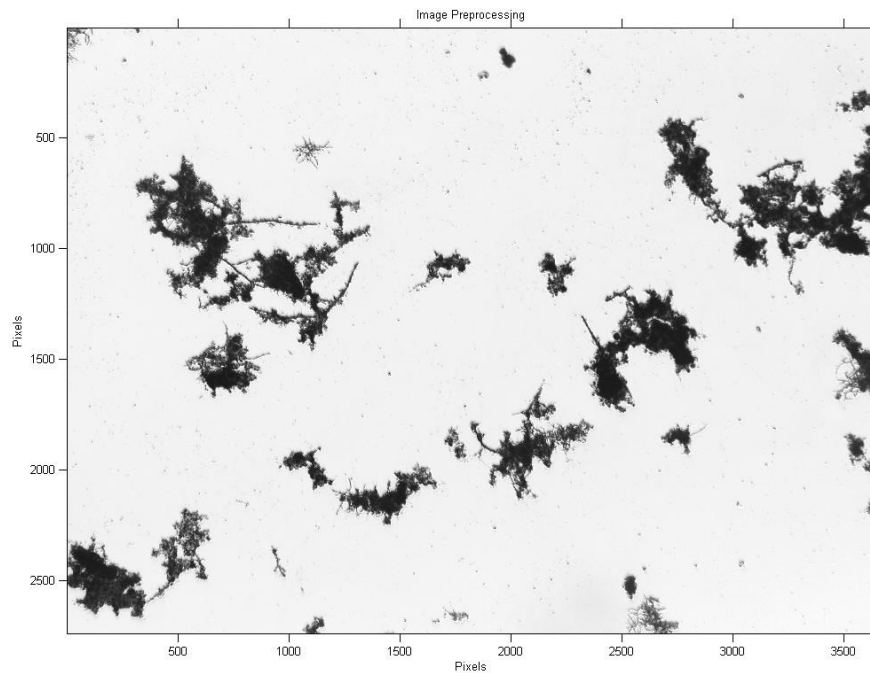
Figure 12 - Pre-processed grayscale image

7.2.1.2 Image enhancement

To enhance the image, contrast stretching algorithm according to the equation (1) and median filtering are applied to the image.

Figure 13 - Enhanced grayscale image

7.2.1.3 Image segmentation

Enhanced image is put into the segmentation procedure to distinguish between the foreground flocs and light background. To find the threshold separating these two categories, the Otsu's method is used. The grayscale image is converted to binary one composed only of 0s and 1s by the means of equation (2). The result is on Figure 14.
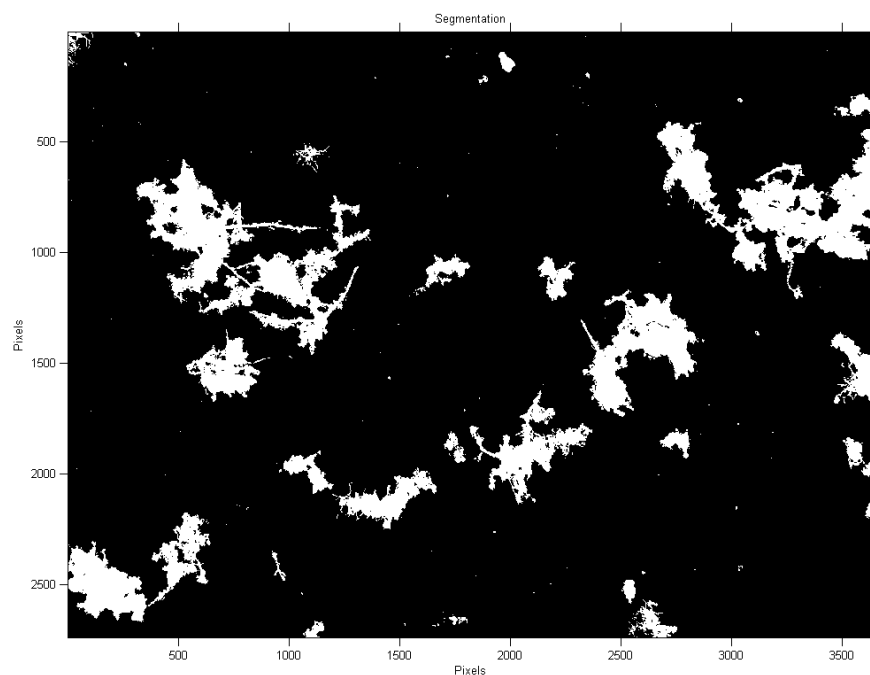
Figure 14 - Binary image segmented with threshold found by Otsu's method

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7.2.1.4 Debris removal and small holes filling

The binary image has to be cleaned from debris and small holes that arose after the process of segmentation. Each connected component in the image is labelled by classical method introduced in chapter 2.6.2. In this way, it is possible to identify all of the objects in the image and remove ones that are smaller than given limit. This operation applied to the inversion of the binary image can be also used to remove/fill small holes in the objects. Limits for debris removal and holes filling have been set to 500 pixels. They have been determined experimentally. Result of this operation is shown on Figure 15.
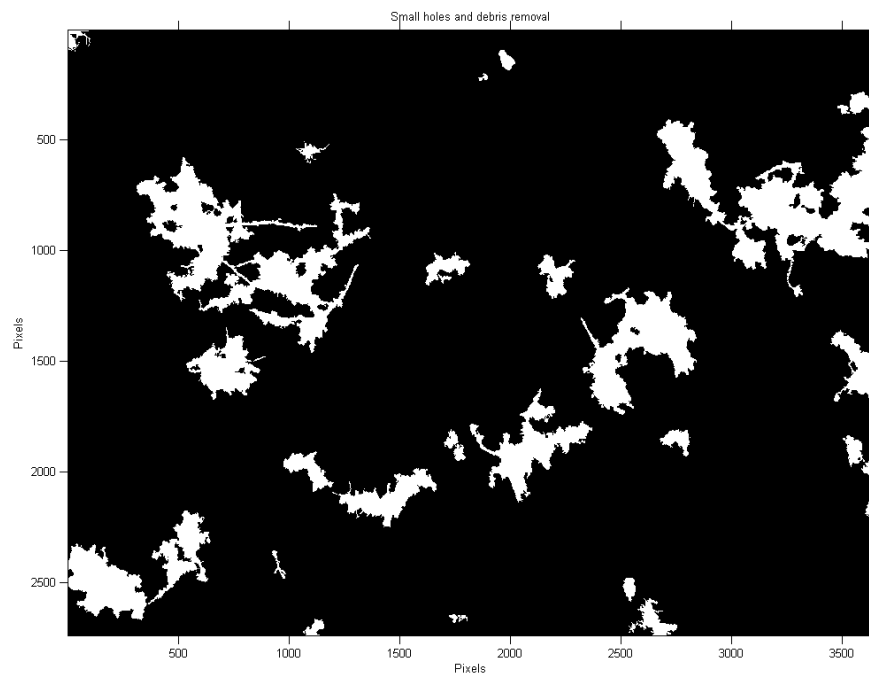

Figure 15 - Binary image cleaned from small debris and holes

#### 7.2.1.5 Unwanted objects removal

Once the image is cleaned, it is labelled again to identify remaining objects. The ECD according to equation (7), which can best represent the dimension, is calculated for each object on the image. With knowledge of a pixel size, the real size of the objects (in μm) can be determined. All of them which are larger than 10 μm are retained to be able to detect the dispersed growth according to the chapter 1.5.2.2, rest is removed. The objects those share more than 30 pixels with the image frame are also removed as they are not entire and it can introduce errors in characterization. There is the resulting binary image on the Figure 16 representing a set of 18 flocs with their known ECD. In addition, PA and RG (according to equation (12)) are determined for each object to be

able to distinguish between flocs and standalone filaments. The RG is the best suitable parameter how it was mentioned in the chapter 3.1.9.
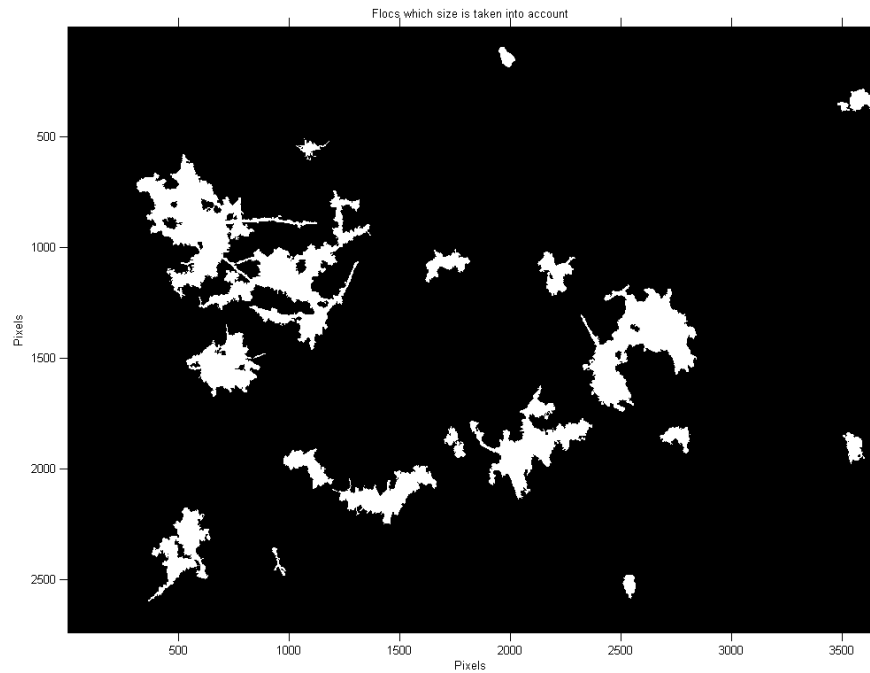
Figure 16 - Small flocs and flocs on the image frame removal

The same image but with highlighted integer labels assigned to each connected component can be seen on Figure 17.
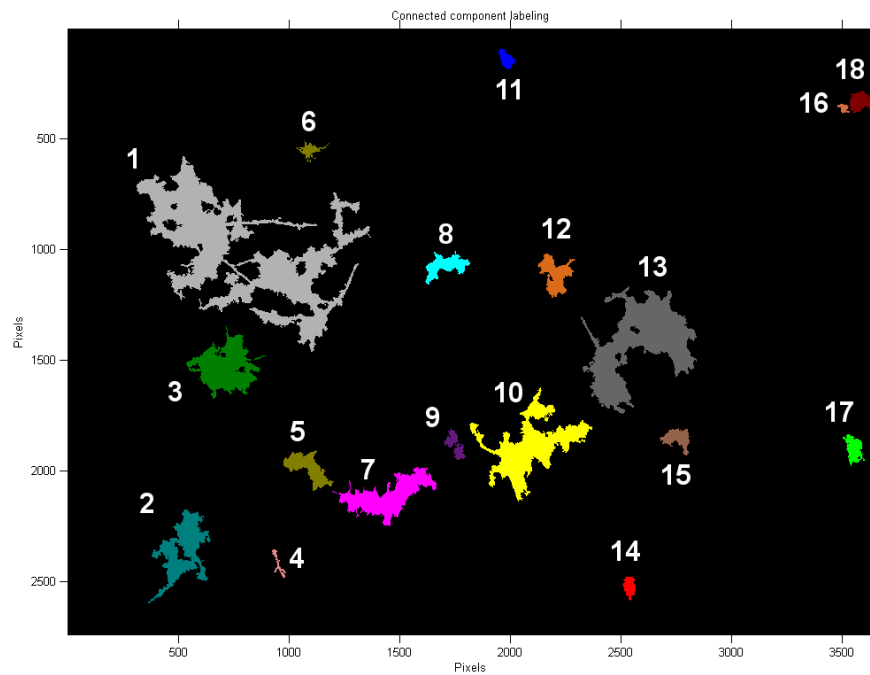
Figure 17 - Labelled connected components

7.2.1.6   Selection of the flocs to be morphologically analyzed

Now it is possible to select the flocs to be morphologically analyzed. In general, it is inherent to image analysis that smaller objects produce larger measurement errors. In the paper [20], it is noted that the objects with projected area smaller than 0,1 % of the total number of pixels in the image should not be taken into account. In accordance with this fact, the flocs with ECD smaller than 50 μm are removed and only the larger ones are considered for the morphology analysis. Figure 18 shows the flocs satisfying this assumption.
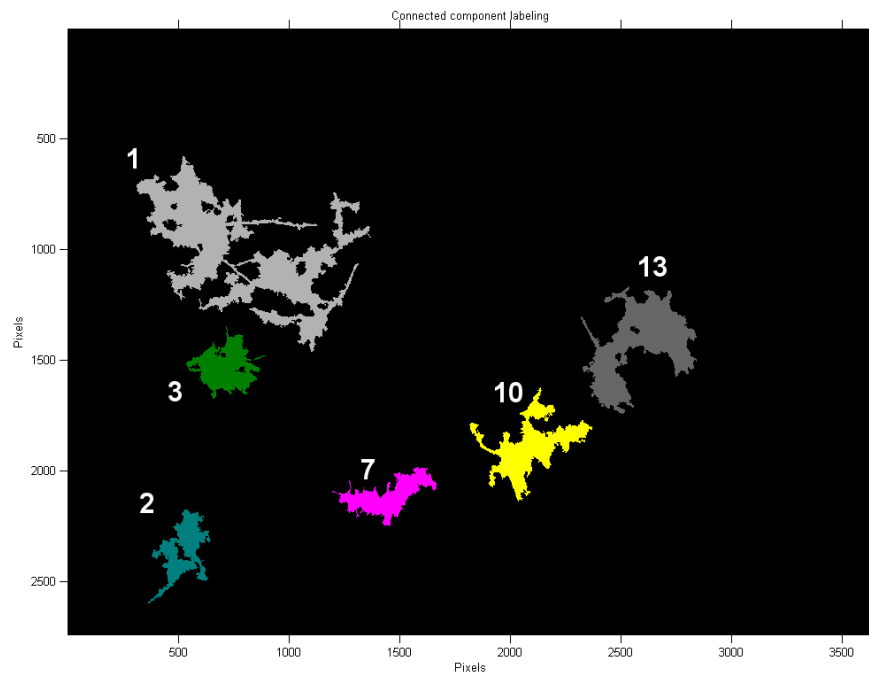

Figure 18 - Morphologically analyzed flocs

7.2.1.7   Flocs characterization

As it can be seen on Figure 18, there are 6 identified flocs that satisfy the given criteria. For all the flocs, the morphological parameters based on Euclidian and fractal geometry introduced in the chapter 3.1 are determined. They are AR, 2D-porosity, RN, FF and FD. PA, ECD and RG have been already determined in previous step.

The function for flocs characterization goes through the labelled image in iteration and calculates the parameters for each floc. Determined parameters of the morphologically analyzed flocs along with the PA, ECD and RG of the excluded flocs are presented in Table 2.

First it is possible to see that only flocs larger than 50 μm are morphologically analyzed – highlighted ones. Results show that there are no regular and smooth flocs in the image. The flocs have high irregular and ragged contour differentiating from the circle shape. The size of the flocs varies from 10,0 to 136,2 μm. In addition, it can be seen that the object 4 has high value of RG. It mostly means it is a standalone filament.

Label number	1	2	3	4	5	6	7	8	9
Projected area [px]	256672	41664	49193	1932	16335	4030	43183	12854	5779
Circle diameter [um]	136,191	55,000	59,762	11,905	34,524	17,143	55,952	30,476	20,476
Aspect ration [-]	0,500	0,551	0,872	-	-	-	0,392	-	-
2D-porosity [-]	0,049	0,055	0,040	-	-	-	0	-	-
Roundness [-]	0,243	0,370	0,750	-	-	-	0,270	-	-
Form factor [-]	0,017	0,070	0,111	-	-	-	0,095	-	-
Gyration radius [-]	1,135	0,939	0,766	1,670	0,960	0,924	1,034	0,958	0,987
Fractal dimension [-]	1,155	1,162	1,176	-	-	-	1,159	-	-
Label number	10	11	12	13	14	15	16	17	18
Projected area [px]	74402	3937	15467	120819	4264	8205	1384	7349	7651
Circle diameter [um]	73,333	16,905	33,571	93,571	17,619	24,524	10,000	23,095	23,571
Aspect ration [-]	0,647	-	-	0,643	-	-	-	-	-
2D-porosity [-]	0	-	-	0,014	-	-	-	-	-
Roundness [-]	0,373	-	-	0,395	-	-	-	-	-
Form factor [-]	0,054	-	-	0,081	-	-	-	-	-
Gyration radius [-]	0,975	0,796	0,853	0,946	0,794	0,845	0,799	0,816	0,756
Fractal dimension [-]	1,159	-	-	1,131	-	-	-	-	-

Table 2 - Flocs characteristics

7.2.2 Flocs composition characteristics

The second part of the MATLAB code deals with floc composition. It tries to extract information from the more detailed view of the floc. It investigates images in 500x optical magnification and searches for occurrence of the filamentous organisms. They can lie alone in the image or can be found in the flocs creating inter-floc bridges or open-floc structures. The code determines a ratio of the projected area of the filaments, floc-forming bacteria colonies and ESP to the total projected area of the floc. Sequence of the operations is presented on the sample image coming from data set D, see Figure 19. There are filamentous organisms outgoing from the flocs, dark floc-forming bacteria colonies (compact cores) inside the flocs and EPS with red colour all around. These connected parts have to be decomposed to single components.
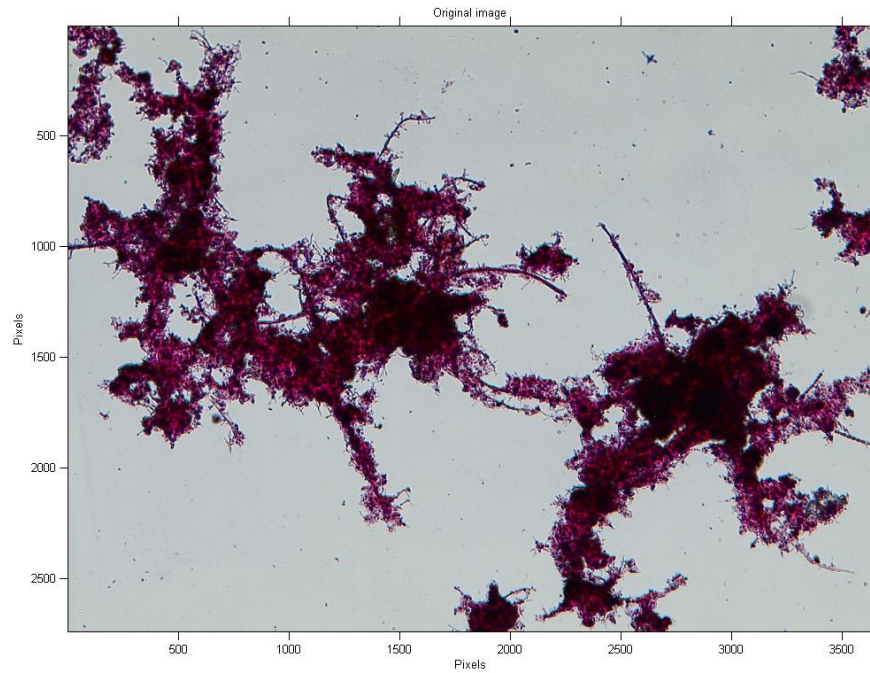
Figure 19 - Original RGB image of the AS flocs (500x)

7.2.2.1   Image pre-processing and enhancement

Like in chapters 7.2.1.1 to 7.2.1.4, the image is pre-processed, enhanced, transformed to the grayscale image, segmented and cleaned from the debris. The result is possible to see on Figure 20.
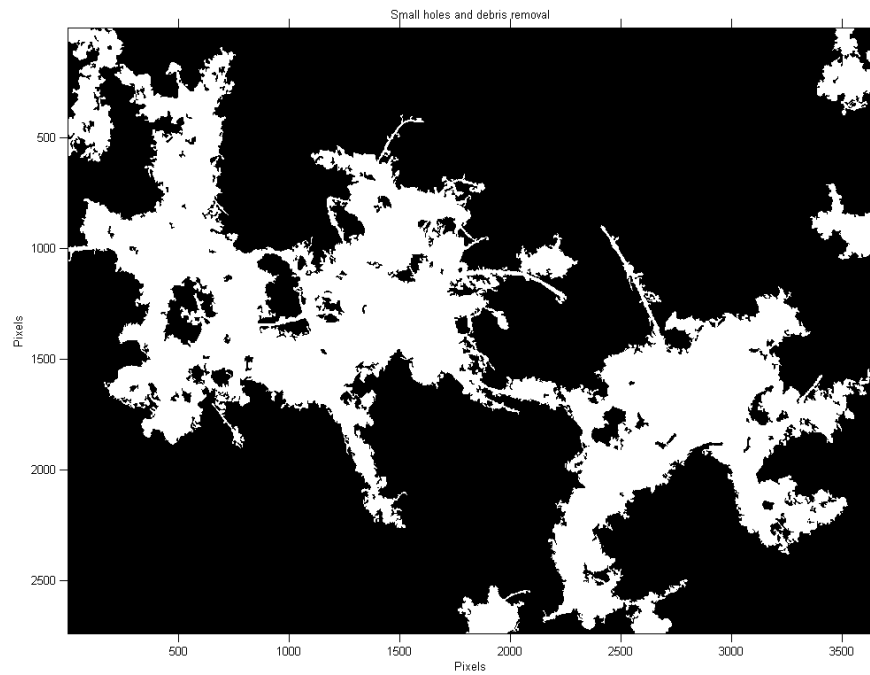
Figure 20 - Binary image with segmented flocs

7.2.2.2   Filaments separation process

Morphological operations are used to separate filaments from the rest of the aggregations. Sequence of the operations is described on Figure 21.
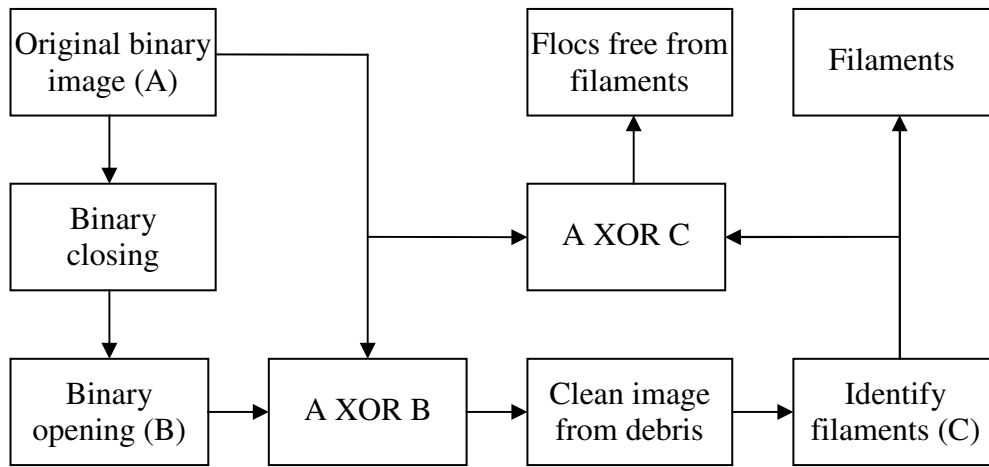

Figure 21 - Filaments separation process

Binary image is first closed by disk shape structuring element with radius of 3 pixels to fill gaps. Then it is opened by the same shape but with radius of 20 pixels to roughly separate the objects on the boundaries from the aggregation. These operations are performed according to the equations (5) and (6). Difference between the original binary image and the opened one is shown on Figure 22.
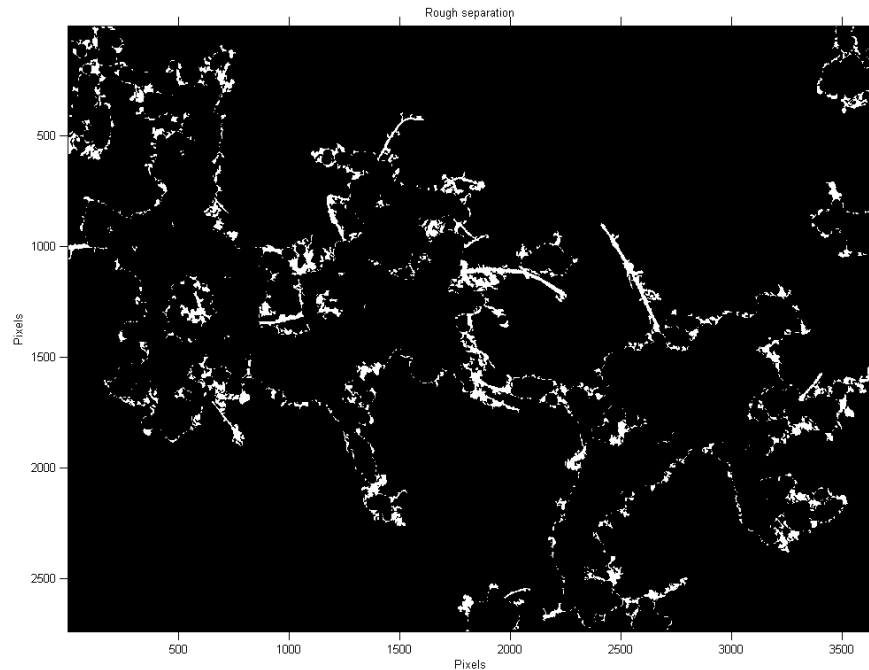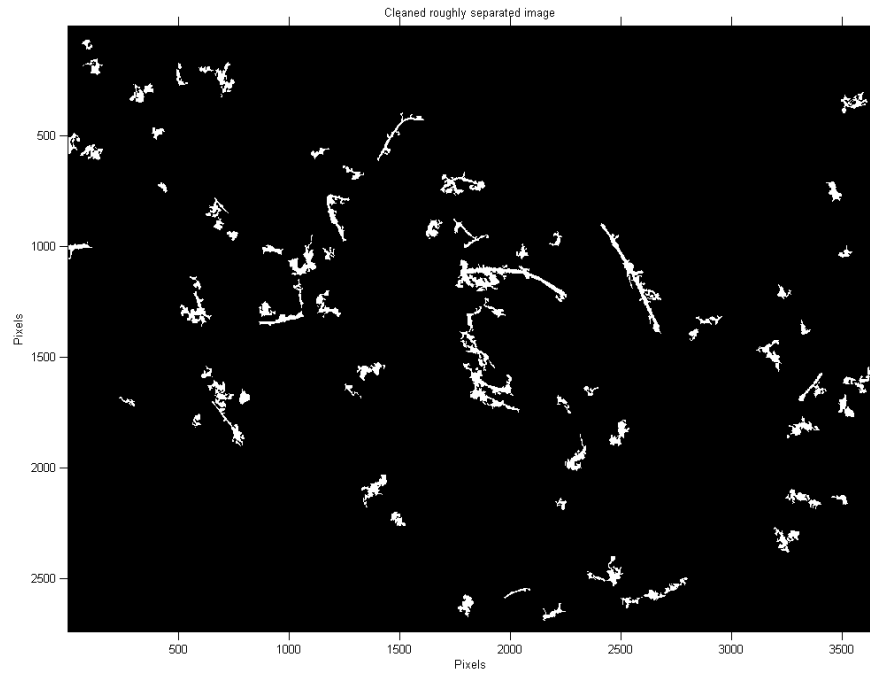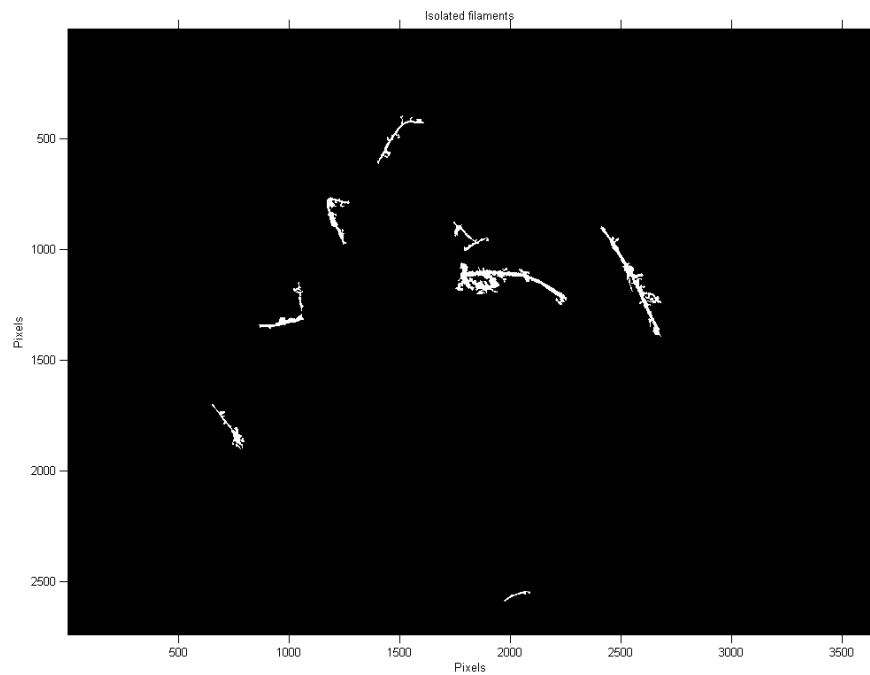

Figure 22 - Rough separation of the boundary objects

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Figure 23 - Cleaned roughly separated image

The image is cleaned from small debris so that only larger objects including filaments left. These objects are shown on Figure 23.
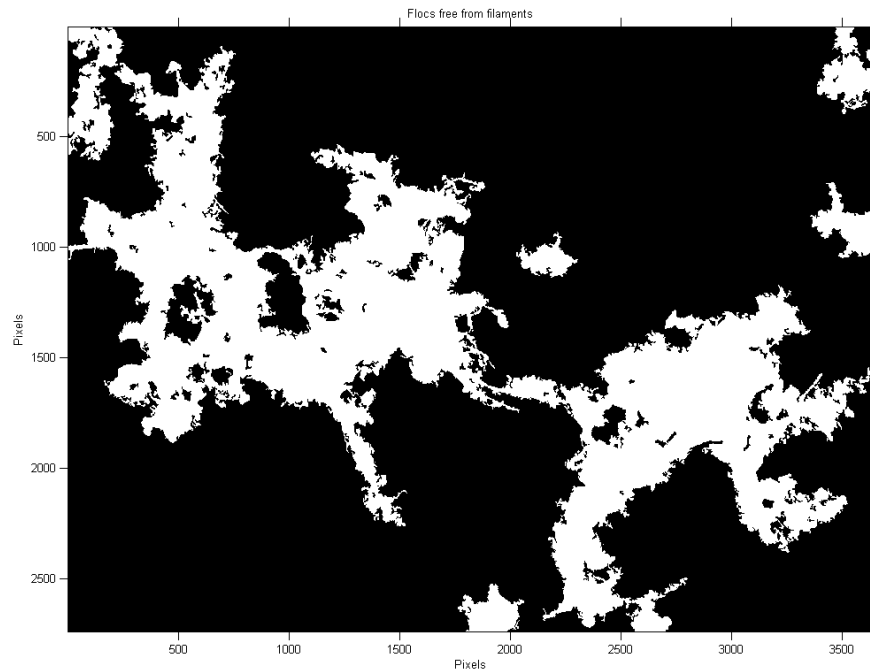

Figure 24 - Separated filaments

For each object on the image, RG according to equation (12) is calculated. This parameter is concluded in [21] as the most suitable parameter to identify filaments. All

objects with RG greater than 1,3 and with PA at least 400 pixels are marked as filaments, see Figure 24. These values have been determined experimentally.

Difference between the original binary image and the one with separated filaments gives the composition of single flocs free from any filamentous objects, see Figure 25.

Figure 25 - Single flocs free from filaments

7.2.2.3 Decomposition to floc-forming bacteria colonies and EPS
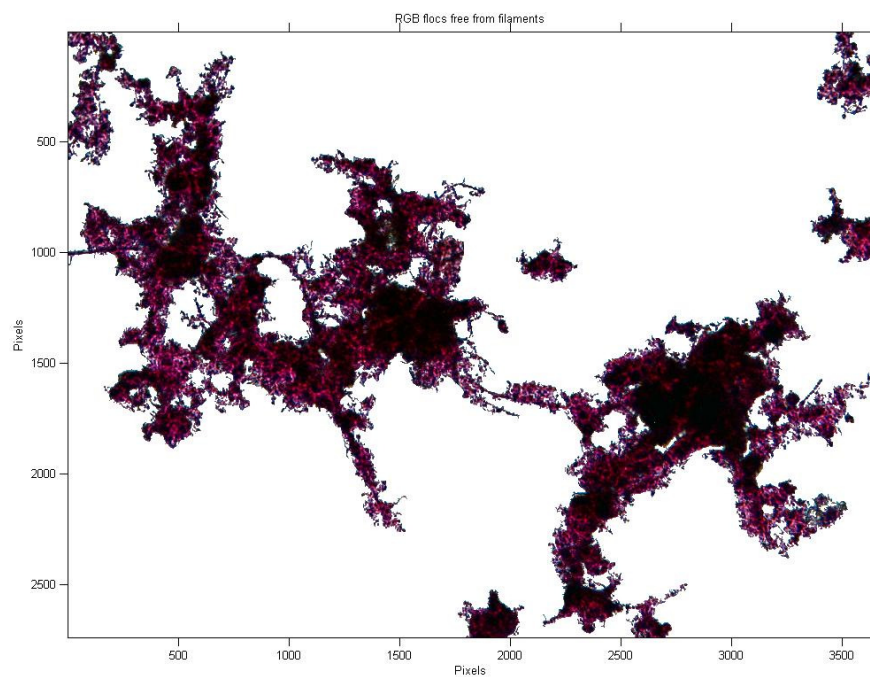
Figure 26 - RGB flocs free from filaments

Once the filaments are separated, the rest of the flocs can be decomposed to floc-forming bacteria colonies (compact cores) and EPS.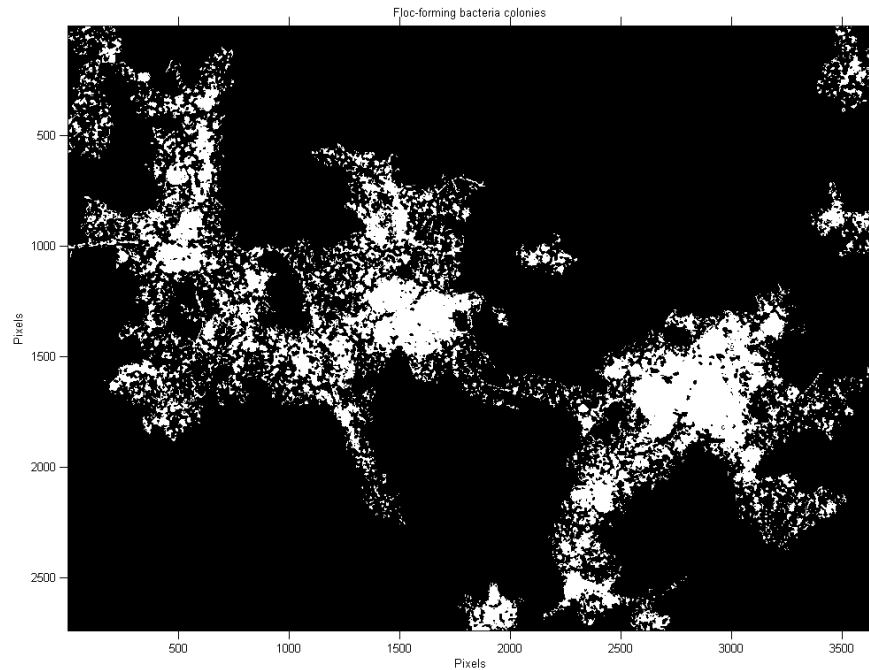 For this purpose the original RGB image is masked by binary one with separated flocs to obtain Figure 26. The flocs are investigated within this constraint to find given components.

Figure 27 - Floc-forming bacteria colonies

To identify colonies of the floc-forming bacteria, red channel from RGB image is taken as the EPS has the highest intensity while regions representing bacteria are dark. This grayscale image is multiplied by 3, determined experimentally, and segmented by threshold found by Otsu's method. Obtained binary image shows the desired colonies see Figure 27.

Difference between binary images representing colonies and flocs free from filaments gives binary image of EPS shown on Figure 28.
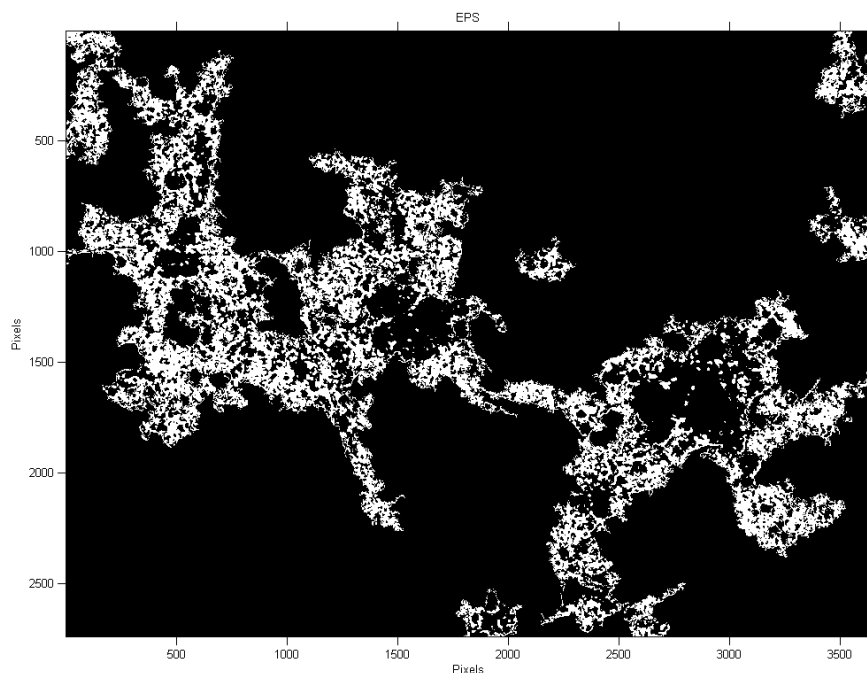
Figure 28 - EPS

7.2.2.4 Floc composition characteristics

Once the particular components are classified, it is possible to calculate their PA and express their ratios to the total PA of the flocs. This information characterizes the entire composition and can describe relationship in the aggregations. The projected areas along with the relative areas of the composition are presented in Table 3.

	Filaments	Floc-forming bacteria	EPS	Total
Projected area [px]	59280	1154872	1365310	2579462
Relative area [%]	2,30%	44,77%	52,93%	100,00%

Table 3 - Flocs composition characteristics

7.3 Morphological and composition data processing

The image analysis code introduced in chapter 7.2 has been applied to all data sets (A – H) of acquired images to get overall characteristics describing particular samples. Data set E has been chosen to present computed results as there is the largest number of analyzed flocs, see below.

Prior to data interpretation, it has been necessary first to determine in which way the morphological parameters and flocs composition could be expressed. The code has been applied to images in 200x magnification to determine size and morphological parameters and afterwards to images in 500x magnification to determine composition

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parameters. On the base of these results, histograms have been calculated to show how the probability of each parameter is distributed. Each histogram has been visually evaluated and the corresponding probability distribution has been estimated. Properties of the distributions have been used to describe the particular parameter. As the number of analysed objects has been relatively high, the results can be considered as statistically significant.

7.3.1 Floc size distribution

There are 1155 flocs in the set identified by the algorithm. Sizes (ECD) of these flocs range from 57 to 797 pixels that correspond to range from 14 μm to 190 μm. Graph 1 shows the flocs size distribution expressed as the frequency of occurrence. The size histogram reflects the log-normal distribution that verifies fact noted in [8]. The red curve represents an expected probability density function with distribution parameters μ and σ. They are the mean and standard deviation (STD), respectively, of the associated normal distribution.
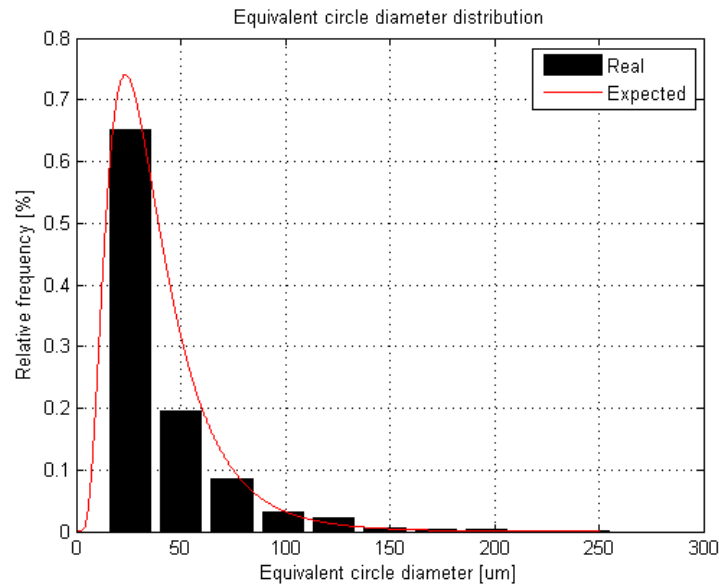
Graph 1 - ECD distribution

The mean value and STD of the log-normal distribution can be used to describe the flocs sizes in the data set. In this case, the mean value equals approximately to 39 μm and STD to 25 μm. It is evident that approximately 90 % of all flocs are up to 100 μm. From this fact it is possible to deduce that the small flocs mostly dominate.

7.3.2 Morphological parameter distribution

Due to the error minimization, flocs smaller than 50 μm have been excluded. As well as the objects with RG larger than 1,3 as they can represent standalone filaments. Total number of 255 flocs has satisfied these assumptions and been considered for the morphological analysis.

7.3.2.1 Form factor distribution

Graph 2 shows a FF histogram. Like in previous case it reflects the log-normal distribution. As the mean value equals to 0,084, it can be deduced that the flocs have rugged contour and are highly irregular because of their large perimeter.
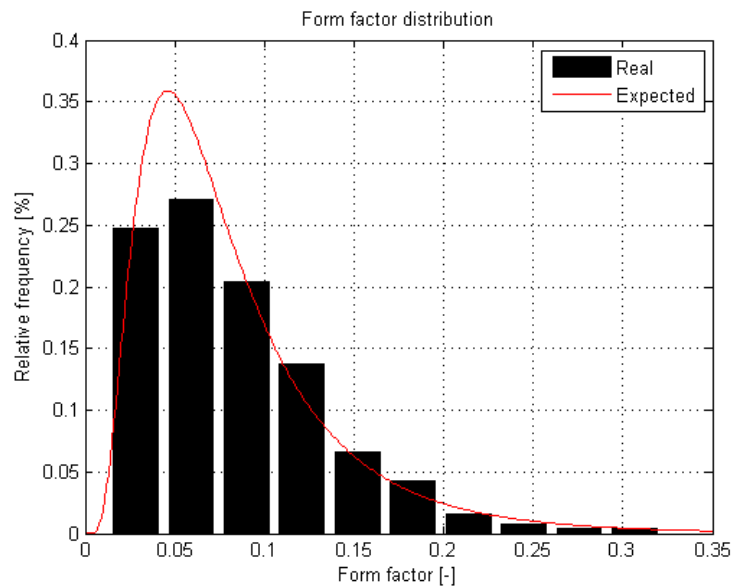
Graph 2 - FF distribution

7.3.2.2 Fractal dimension distribution

Graph 3 shows a FD histogram that reflects the normal Gaussian distribution. The mean value equals to 1,145 and STD to 0,024.
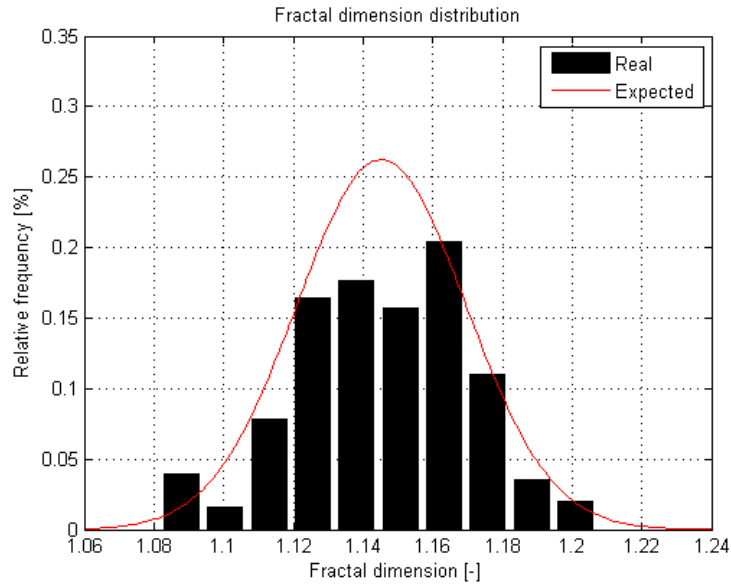
Graph 3 - FD distribution

7.3.2.3   Roundness distribution

Graph 4 shows an RN histogram. Like FF histogram, it is expressed by the log-normal distribution. The mean value equals to 0,353 and shows how the flocs deviate from the circle. In this, case they are highly elongated as the circle has value 1.
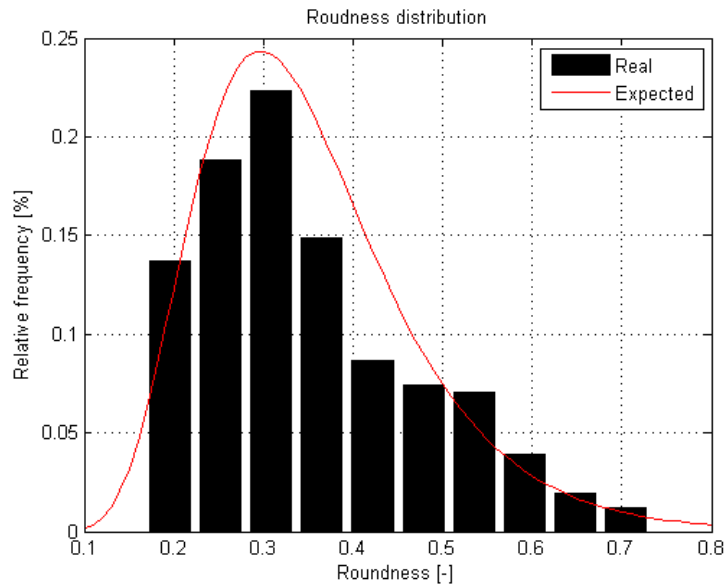
Graph 4 - RN distribution

7.3.2.4   Aspect ratio distribution

Graph 5 represents an AR histogram with corresponding normal Gaussian distribution. The mean value 0,560 shows how the flocs are elongated.
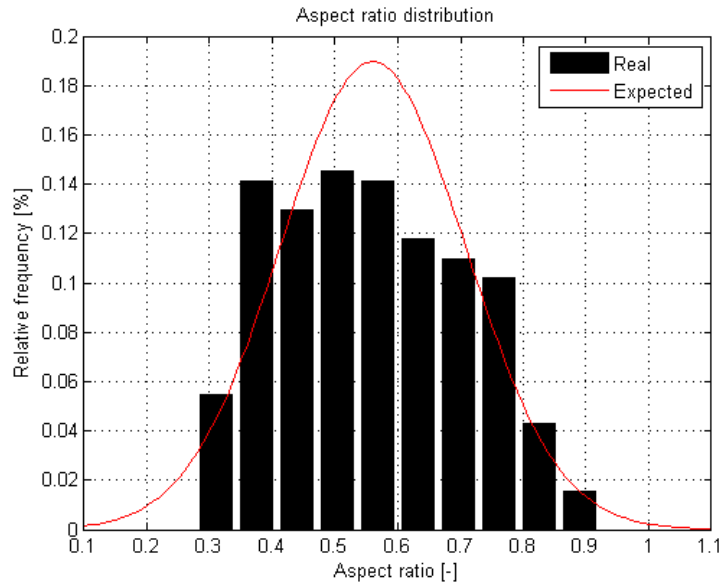
Graph 5 - AR distribution

7.3.2.5   Reduced radius of gyration
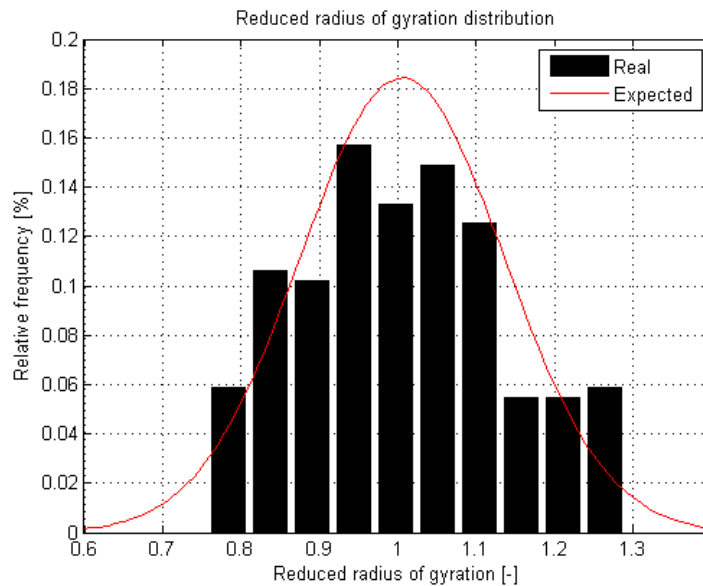
Graph 6 - RG distribution

There is an RG histogram along with the corresponding normal distribution on Graph 6. The mean value is equal to 1,006 with STD of 0,130. It shows how the mass of flocs is dispersed from the centre of gravity. As the circle has RG of 0,707, it follows that flocs in this data set differ from circle shape as they are more dispersed.

7.3.2.6   2D-porosity distribution

There is a 2D-porosity histogram on the Graph 7. It can be seen that the most of the flocs are nonporous or slightly porous. The arithmetic average of data is 0,028.
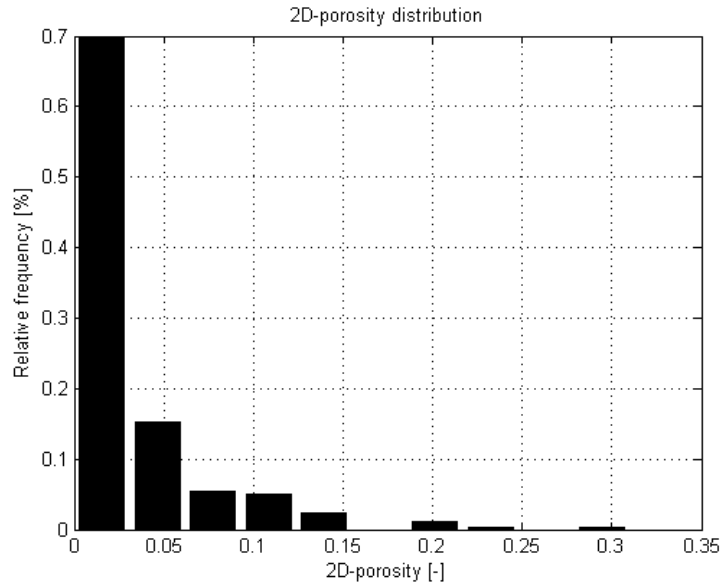
Graph 7 - 2D-porosity distribution

7.3.2.7 Overall morphological characteristics

Overall morphological characteristics for all data sets are shown in the Table 4. Highlighted column presents data set E which results are introduced above. Average time needed for morphologically characterization of one floc is equal to 10 seconds. With knowledge of number of flocs which have been characterized, it is possible to express the total time required for calculation of whole data set.

| Data set	A	B	C	D	E	F	G	H
Nb of flocs	539	876	907	897	1155	538	703	381
ECD mean [um]	49,919	43,207	40,733	46,710	38,982	42,531	44,230	59,087
ECD STD [um]	35,384	25,330	24,551	31,272	24,634	31,624	31,282	46,770
Nb of flocs	219	257	224	256	255	238	196	166
FF mean [-]	0,078	0,103	0,087	0,073	0,084	0,085	0,078	0,083
FF STD [-]	0,050	0,067	0,059	0,057	0,059	0,065	0,053	0,064
FD mean [-]	1,157	1,141	1,148	1,153	1,145	1,142	1,142	1,142
FD STD [-]	0,020	0,024	0,022	0,024	0,024	0,020	0,024	0,024
RN mean [-]	0,352	0,356	0,366	0,354	0,353	0,369	0,367	0,396
RN STD [-]	0,121	0,134	0,135	0,132	0,123	0,138	0,131	0,147
AR mean [-]	0,573	0,553	0,574	0,564	0,560	0,583	0,577	0,600
AR STD [-]	0,157	0,155	0,157	0,151	0,147	0,153	0,160	0,149
RG mean [-]	1,010	1,003	0,997	1,011	1,006	0,999	0,995	0,969
RG STD [-]	0,123	0,136	0,132	0,136	0,130	0,137	0,125	0,131
2D-poro mean [-]	0,034	0,023	0,030	0,035	0,028	0,040	0,035	0,044

Table 4 - Overall morphological characteristics

7.3.3　Flocs composition distribution

Distributions of relative area of the filaments, EPS and floc-forming bacteria with respect to the total area of the flocs are presented on the following graphs. Total area of the flocs represents 100 %.
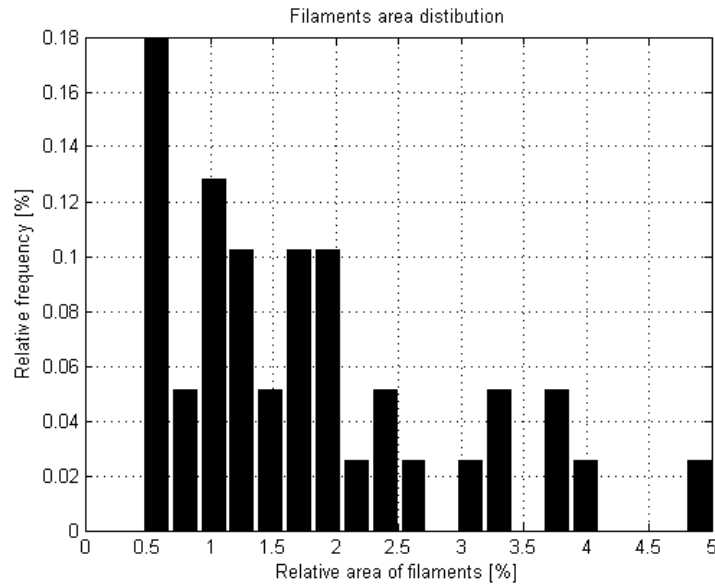
Graph 8 - Filaments relative area distribution

As the distribution on Graph 8 shows, occurrence of the filaments is very low in the data set E. Relative area ranges between 0,45 to 5,00 % of total area of the flocs. The average is equal to 1,77 %.
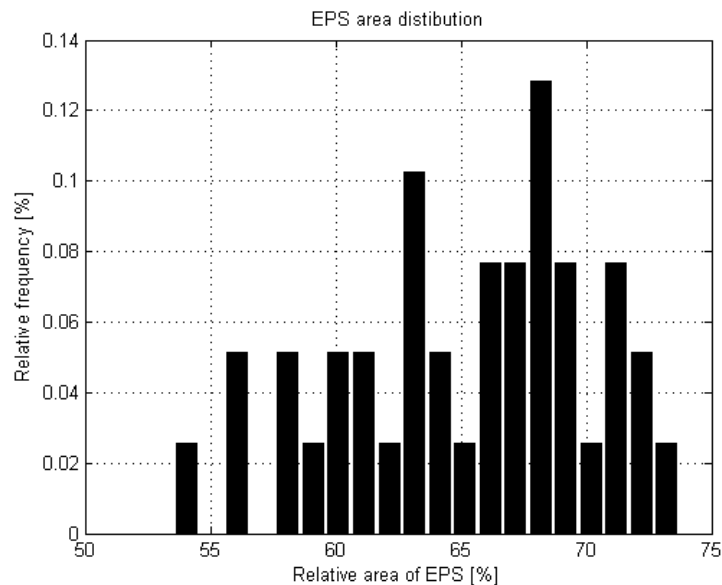
Graph 9 - EPS relative area distribution

There is the EPS relative area distribution presented on the Graph 9. The average of the data is equal to 65,18 % that shows the majority of EPS in the samples.

Graph 10 shows the distribution of the floc-forming bacteria area in the samples. The average equals to 33,06 %.

Graph 10 - Floc-forming bacteria area distribution

7.3.4   Overall composition characteristics

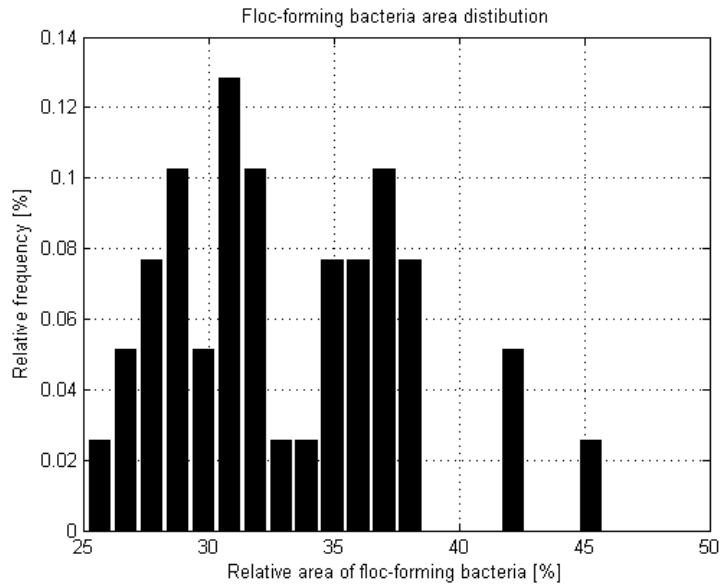Overall composition characteristics for all data sets are shown in the Table 5. Highlighted column represents data set E which results are introduced above. Average time spent by computation of one image equals to 28 seconds.

| Data set	A	B	C	D	E	F	G	H
Filaments [%]	1,33	1,06	1,54	1,44	1,77	1,64	1,17	1,35
EPS [%]	63,59	66,40	60,87	59,14	65,18	58,53	62,81	58,16
Floc-forming bacteria [%]	35,08	32,54	37,60	39,42	33,06	39,83	36,03	40,49

Table 5 - Overall composition characteristics

8   DISCUSSION OF THE RESULTS

It this chapter, results coming from laboratory investigation and from image analysis are compared. As the SVI is the most important laboratory parameter and the others are accompanying just in case of future detailed evaluation by an expert or WWTP operator, only SVI is compared with all the parameters obtained from the image analysis on following graphs.

First it is necessary to note that good operating situation in the WWTP in Liberec have not allowed obtaining activated sludge in extreme conditions (with wider range of SVI) so only narrow set of SVI (from 78 to 92 ml/g) can be compared with image analysis parameters. It is not sufficient for more complex comparison but it is possible to find at least the basic relationships. As the values of SVI are so close to each other and every measurement naturally brings errors, it is possible to assume that the values of the same morphological parameter will not differ to much as well.

8.1   Morphological parameter relation

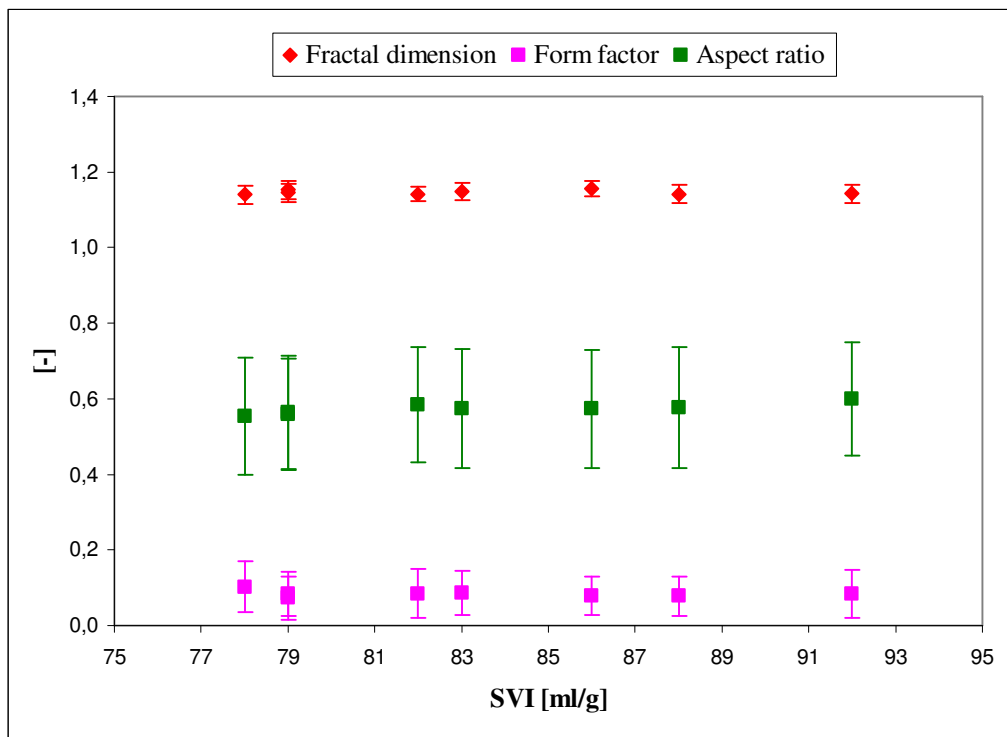Graph 11 shows a relation between SVI and size of the flocs expressed by ECD along with their STD.
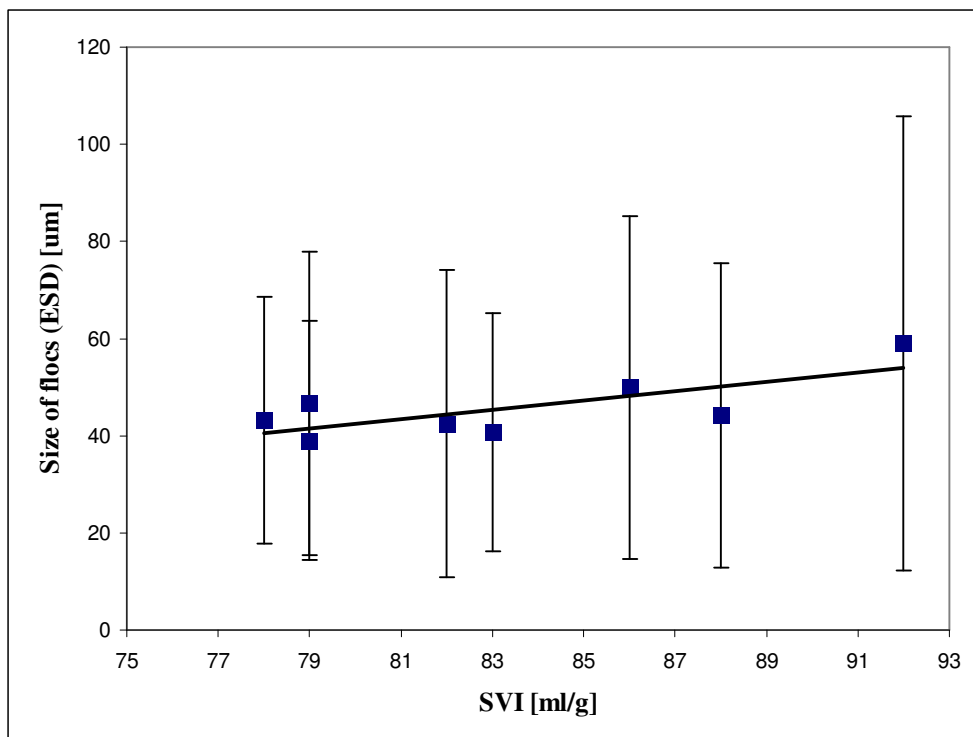
Graph 11 - SVI vs. size of the flocs

SVI ranges from 78 to 92 ml/g and mean value of sizes from 39 to 59 μm. Generally both of the parameter are low that indicates predominance of smaller flocs as it is mentioned in chapter 4.2. Inserted linear regression can show the growing trend of SVI in dependence on the floc size but too narrow range of values cannot verify this assumption precisely.

The ECD parameter together with its STD can warn of unwanted conditions cause by dispersed grown or pinpoint flocs mentioned in chapter 1.5.2 where the predominant size of the flocs is important.

A relation between SVI and FF, FD and AS is presented on Graph 12. It clearly shows that the mean values of the same parameter, in narrow SVI range, do not vary from each other to much – it satisfies the assumption above. Relative low STD of FD and FF can indicate that there can be very close relationship between these parameters and SVI, but other investigation is necessary.


Graph 12 - SVI vs. form factor, fractal dimension and aspect ratio

Graph 13 presents a relation between SVI and RN, RG and 2D-porosity. Like in the previous case, the mean values of the same parameter do not vary too much in the narrow range of SVI. So it would be possible to expect that other activated sludge

sample within the same morphological parameter range can fit into the same SVI range as well, but other investigation is necessary too.
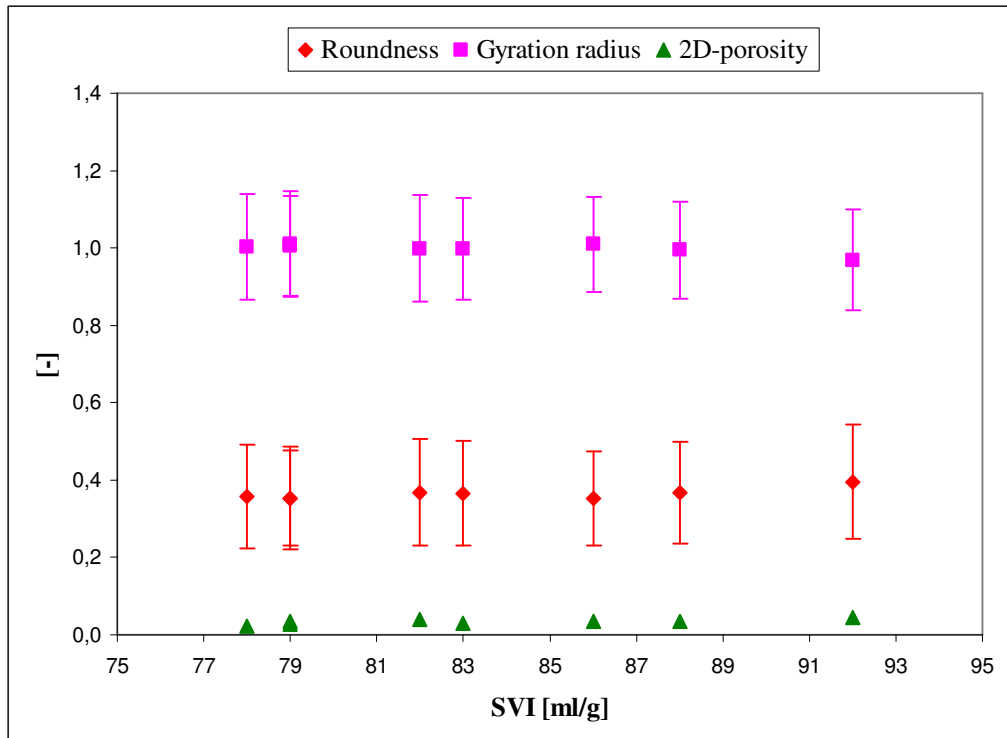
Graph 13 - SVI vs. roundness, gyration radius and 2D-porosity

8.2 Flocs composition relation

Relation between SVI and flocs composition is shown on Graph 14 and Graph 15. SVI is compared with relative areas of filaments, EPS and floc-forming bacteria. Rather low occurrence of filamentous organisms reflects low values of SVI. While filaments are missing, small flocs are formed that satisfy the results in previous chapter as well. How it was mentioned, it is not possible to precisely compare results due to narrow set of SVI values.

Exceeding values of filaments or EPS areas can reflect problems with filamentous or viscous bulking respectively mentioned in chapter 1.5.2. So these parameters are very important to detect the unwanted conditions.
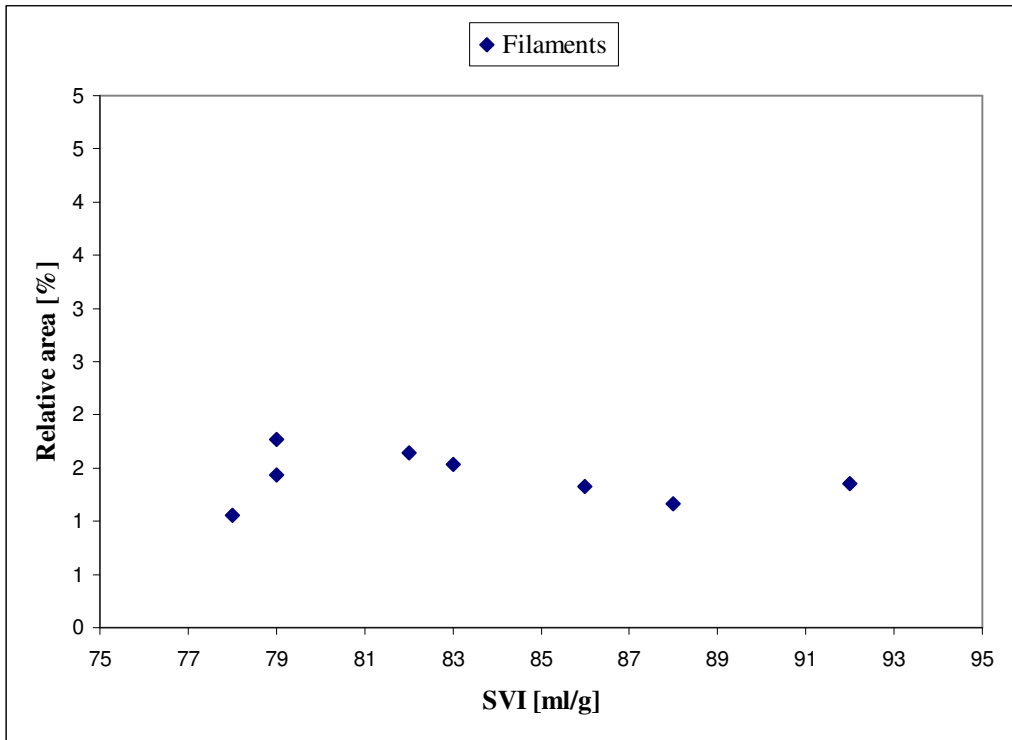
Graph 14 - SVI vs. filaments relative area
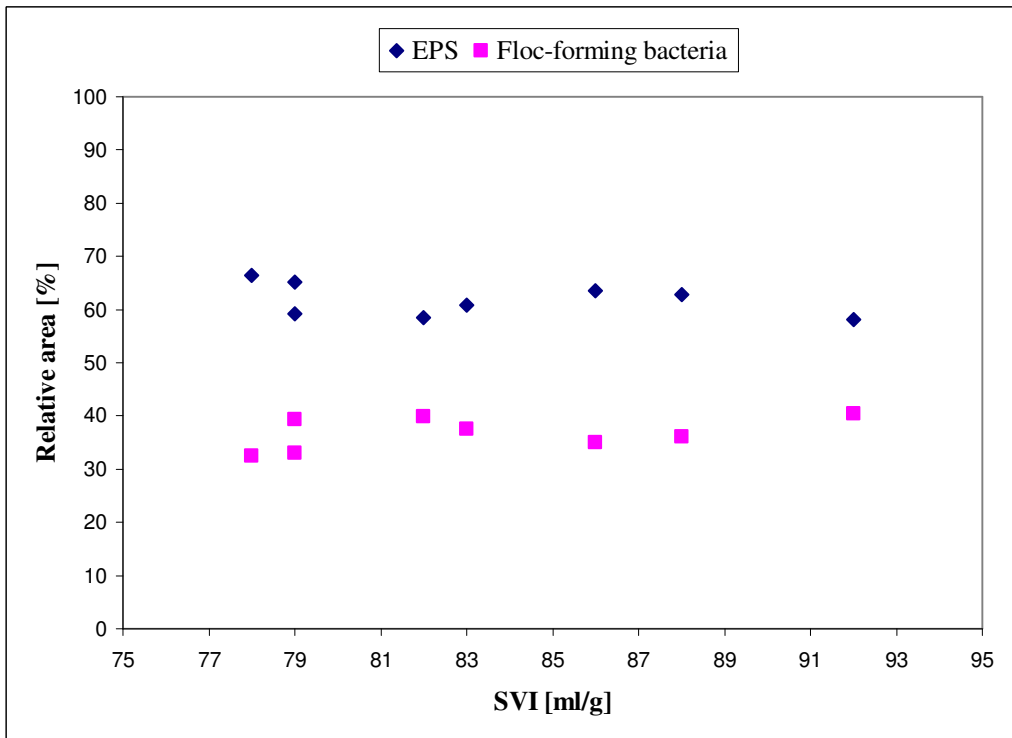
Graph 15 - SVI vs. EPS and floc-forming relative area

CONCLUSION

Within the scope of the diploma thesis, 8 samples of activated sludge have been collected during 3 month time period under various conditions (temperature, load). These samples have been manually processed in laboratory to obtain their characteristics including the most important one – sludge volume index. This parameter can express quality of sedimentation in the final clarifier and efficiency of whole WWTP in the same time. A set of digital microscopic images coming from these samples has been manually acquired in 2 optical magnifications as well. The total data volume of 1,5 GB has been prepared for the image analysis.

An automatic image analysis procedure has been developed for characterization of the activated sludge samples. The MATLAB environment and especially its Image Processing Toolbox have been used to create a program code for image processing and evaluation of the activated sludge samples in digital domain. Selected morphological parameters, like form factor, fractal dimension, aspect ratio, gyration radius etc., are determined for the flocs in 200x optical magnification. Flocs microbial composition – it means ratio of filamentous organisms, EPS and floc-forming bacteria to the total area of the flocs are determined in 500x optical magnification. Program is designed to perform all necessary steps automatically (process all the images presented in the given directory) and after computations give user the found results.

Proposed program has been applied to the set of acquired images. Morphological and composition parameters corresponding to the particular samples have been determined. As the shape of the sludge flocs is related to the settling properties, the determined parameters have been compared with sludge volume index to find any basic relations.

Unfortunately, good operating situation in the WWTP in Liberec have allowed obtaining activated sludge only in good condition with narrow range of SVI (78 – 92 ml/g). In this case, it has not been possible to make complex comparison but on the other hand at least basic relationship have been found and, mainly, the program functionality has been verified. Low values of SVI indicate occurrence of the small flocs and low ratio of the filamentous organisms. This assumption has been satisfied. Morphological parameters such as form factor and fractal dimension show low STD that can indicate very close relationship between them and SVI. All morphological

parameters also show that their mean values do not vary too much in the narrow set of SVI values – they are approximately identical within the SVI range of 14 ml/g. It is a positive effect which can be utilized together with other future investigations.

Activated sludge separation problems, how it was mentioned in chapter 8, can be either detected by ECD parameter together with its STD which expresses the predominant size of the flocs or by parameters describing the flocs composition such as filaments or EPS relative areas. All unwanted problems are introduced in chapter 1.5.2.

Basic database of parameters corresponding approximately to SVI range 80 – 90 ml/g has been gained. As soon as more samples will be available, new parameters can be obtained and the more precisely relationships can be found. On this base, it will be also possible to select the parameters which can characterize the sludge in the best way.

The proposed procedure can be used in future by the plant operators to characterize activated sludge and predicate the unwanted conditions such as bulking. Manual laboratory methods are very time consuming and on the other hand computers can provide fast computations of large amount of data. How it was mentioned in chapter 7.3.2.7, 10 seconds are needed in average for morphologically characterization of one floc. There are 255 flocs in data set E which have been characterized. Total spent time equals approximately to 43 minutes on Intel Core 2 Duo E6300 1,86 GHz. This time is relatively high and together with time for sample preparation, it can be easily compared to standard manual methods. The problem is in the high resolution of the images which equals to 9,98 megapixels. In practical application, lower resolution can be used without loss of precision. Images with resolution of 2400 x 1800 pixels (4,32 megapixels) have been tested and the average time decreased to 4 second that is 17 minutes in total.

In the case of composition characterization, spent time equals in average to 28 seconds per an image. While 50 images are characterized, approximately 24 minutes are needed. If the resolution is decreased to 4,32 megapixels, the average time per image equals to 10 second, that is approximately 8 minutes in total. These results are more positive and they are preferable to standard manual methods apart from objective and unified evaluation.

Proposed procedure can be also rewritten in C or C++ language which is quite faster than interpreted MATLAB script.

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