Image Analysis of Sludge Aggregates in Palm Oil Mill Effluent (POME)

By

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Dissertation submitted in partial fulfilment of
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CERTFICATION OF APPROVAL

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Approved by,

(Dr. Taslima Khanam)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

JANUARY 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

Ian Norman Bin Rizal Song

ABSTRACT

Palm oil mill effluent (POME) is a wastewater produced by palm oil milling activities which generated from crude oil clarification process, sterilization process and cracked mixture separation process. Palm oil mill effluent (POME) is a highly polluting material and activated sludge system is now commonly used for the process of purification of the wastewater. Activated sludge system is a complex ecosystem in which the efficiency is quite dependent on the operating conditions of the process. Due to its complexity, any imbalance between the different types of microorganism may take place and affect the efficiency of the plant with profound economic and environmental consequences. To observe and regulate activated sludge system, bright field microscopy is acquired to monitor the sludge aggregates in palm oil mill effluent (POME). Furthermore, the association of image processing and analysis methodologies with microscopy allows a precise evaluation of the activated sludge status. The most common problems on activated sludge is filamentous bulking due to the extensive growth of the filamentous bacteria which led to poor sludge settling ability and poor thickening characteristics of the sludge thus increase the sludge volume index (SVI). Therefore, the main objectives of the research work is to develop image analysis algorithm by using Matlab in order to identify aggregates characterization in activated sludge system and to develop a correlation between image data obtained and sludge volume index (SVI) for POME. In this research work, two samples will be obtained which are fresh POME collected from the mill and POME undergo Fenton Reagent process. A few process will be prepared for the sample collected namely calculating sludge volume index, image acquisition using microscopy, image processing and analysis, identify image analysis parameter and plot the correlation between SVI with determine parameter. By conducting this research, image analysis algorithm can be developed to monitor sludge aggregates in POME. Thus, this algorithm can be used to identify bulking problems in the POME and establish the true nature of the phenomenon occurring inside the activated sludge system which normally will affect the quality of the effluent.

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CHAPTER 1: INTRODUCTION

1.1 Background of Study

In Malaysia, the palm oil industry is considered as one of the major agro-industries. Malaysia currently account for 39% of world palm oil production and 44% of world exports. The palm oil mill effluent (POME) is an oily wastewater produced by palm oil processing mills which generated from three major sources namely Hydrocyclone waste, sterilizer condensate and separator sludge (Borja and Banks, 1994). Palm oil mill effluent is a highly polluting material and waste water treatment systems for POME are now standards in the main areas of production (Chavalparit, 2006). Process for the purification of wastewater commonly used the activated sludge system (Metcalf and Eddie, 1992; Horan, 1991).

The activated sludge system is a complex ecosystem constituted mainly of bacteria and protozoa (Amaral et al., 2004). The efficiency of activated sludge system is quite dependent on the operating conditions namely sludge flocculation, stability, aggregates size, morphology, density and chemical composition (Mesquita et al., 2013). In activated sludge systems, a suitable balance between the different types of bacteria is essential to ensure an effective pollution removal, good sludge settling abilities and low suspended solid level in the final discharge (Mesquita et al., 2009). To observe and regulate activated sludge system, microscopy observations are becoming ever more important steps.

There are many range of options fitted for most microscopic such as bright-field, phase-contrast or fluorescence microscopy (Mesquita et al., 2013). Furthermore, the association of image processing and analysis methodologies with microscopy allows a precise evaluation of the activated sludge status (Li and Ganczarczyk, 1991; Grijspeerdt and Verstraete, 1997). Image analysis can further characterize and relate sludge volume index (SVI) parameters with structure of activated sludge system. In this study, bright-field microscopy technique is being used to identify aggregated and filamentous biomass morphology and content in the POME. This can be subsequently used to monitor bulking event in the plant. Image analysis by using Matlab 7.3 (The

Mathworks, Natick, USA) can be developed to further recognition aggregates bacteria in grayscale images (Amaral et al., 2004).

According to Eikelboom (2010) aggregates were categorized according to their size: small aggregates (D_{eq}< 25 µm); intermediate aggregates (25 <D_{eq}< 250 µm); large aggregates (D_{eq}> 250 µm) where D_{eq} represents the equivalent diameter. Image analysis dataset will further used to predict the correlation between total aggregates area per volume (TA/Vol) and SVI ($mLg^{-}1$) (Mesquita et al., 2011). By plotting the data, it can identify the correlation between all the variables and thus can be used to elucidate several disturbance that may occur in an activated sludge system. If the correlation is below the threshold limits, the amount of filamentous bacteria will not influence the SVI value. This correlation can also provide further valuable information regarding the biological system in the activated sludge system. This study can also highlighted the benefits of combining SVI determination with sludge aggregates in order to form the phenomena that occurred in the biological system (Mesquita et al., 2008).

1.2 Problem Statement

Activated sludge system is a complex ecosystem compromise of a different types of microorganisms. Due to its complexity, any imbalance between the different types of microorganism may take place and effect the efficiency of the plant with profound economic and environmental consequences. This will eventually cause the reduction of effluent quality. The acceptable range of a good SVI which most plant seem to produce a clear, high-quality effluent is with a SVI in the range of 100 to 200 mL/g. Within this range, the sludge usually forms a uniform blanket before settling as it settles slower and traps more particulate matter. The most common problems on activated sludge is filamentous bulking due to the extensive growth of the filamentous bacteria which led to poor sludge settling ability and poor thickening characteristics of the sludge thus increase the sludge volume index (SVI). In order to maintain the SVI values, the operator would have to regulate the waste sludge rate to effectively creating a less dense particle that settles slightly slower or creating a denser particle that has rapid settling ability which will affect the effluent quality. Furthermore, image analysis algorithm of sludge aggregates for monitoring bulking problem in activated sludge that relates to SVI value are not well developed.

1.2 Objectives

The main objective of this study is in order to:

1. To develop image analysis algorithm in order to extract size of aggregates in activated sludge system.
2. To compare the similarity of results between the existing ImageJ software with self-develop coding in Matlab.
3. To determine the SVI of POME Influent and Effluent to estimate the sludge settling ability

1.3 Scope of Studies

The scope of are as following:

1) Monitoring sludge aggregates using Light Microscopy
2) Development of Image processing algorithm for aggregates characterization using Matlab.
3) Proposed method will be applied for monitoring sludge aggregates of Palm Oil Mill Effluent.

CHAPTER 2: LITERATURE REVIEW

2.1 Palm Oil Mill Effluent (POME)

The palm oil mill effluent (POME) is an oily wastewater produced by palm oil processing mills which generated from three major sources namely Hydrocyclone waste, sterilizer condensate and separator sludge (Borja and Banks, 1994). POME is a highly polluting material and waste water treatment systems for POME are now standards in the main areas of production (Chavalparit, 2006). According to Aljuboori (2013) 5-7.3m^3 of water is required to produce 1 tonne of crude palm oil (CPO) in which more than 50% of the water will end up as POME. Without proper wastewater treatment plant implemented in palm oil mills this huge amount of POME being produced will easily pollute the water sources nearby (Lam et al., 2011).

2.2 Activated Sludge System

Process for the purification of wastewater commonly used the activated sludge system (Metcalf and Eddie, 1992; Horan, 1991). The activated sludge system is a complex ecosystem constituted mainly of bacteria and protozoa (Amaral et al., 2004). The efficiency of activated sludge system is quite dependent on the operating conditions namely sludge flocculation, stability, aggregates size, morphology, density and chemical composition (Mesquita et al., 2013). In activated sludge systems, a suitable balance between the different types of bacteria is essential to ensure an effective pollution removal, good sludge settling abilities and low suspended solid level in the final discharge (Mesquita et al., 2009). One of the main problems in activated sludge system is the sludge settling ability and is generally measured by using the sludge volume index (SVI) (Mesquita et al., 2013). It is also known that formation of pinpoint flocs (PP), filamentous bulking, and viscous or zoogleal bulking (ZB) are common malfunctions that affect the activated sludge system settling ability (Mesquita et al., 2011). Pinpoint flocs phenomenon is related to the formation of small and mechanically fragile spherical flocs, presenting low settling properties. Zoogleal or viscous bulking occurs by an extensive amount of extracellular material (exopolysaccharides) thus increasing the SVI and lowering the sludge settling ability.

According to Mesquita et al., (2011) in activated sludge system the most common sludge bulking problem is the filamentous bulking. Filamentous bulking normally occurred when different types of filamentous microorganisms overgrow consequently leading to poor sludge settling ability and poor thickening characteristics. (Mesquita et al., 2011).

2.3 Activated sludge monitoring through microscopic examination

Microscopy observations played a vital role method to monitor and control activated sludge systems, these method are becoming widespread for the characterization of activated sludge microbial aggregates. Microscopes allow the visualization of activated sludge and is quite effective in identifying the nature of the aggregated biomass and the type and abundance of filamentous microorganisms (Jenkins et al., 2003). At most, microscopes are now fitted with a range of technique such as bright-field, phase contrast or fluorescence microscopy.

2.3.1 Bright-field microscopy

Bright field microscopy can be used to assess the morphology of the activated sludge. It may also provide useful data on the sludge state, especially in conventional activated sludge system where flocs-forming bacteria dominate, increasing the aggregates size and compactness. Bright field microscopy proved to be more accurate in determining aggregate's borders and assessment of short protruding filaments. The down side of bright field microscopy is lack of contrast thus this will hinders the visualization of the transparent nature of majority microbial cells, including filamentous bacteria (Mesquita et al., 2013).

2.3.2 Phase-contrast microscopy

Phase-contrast microscopy is helpful in identifying specific characteristics in filamentous bacteria identification that are hardly visualized in bright-field such as the presence of branching or sheath. It can also use to visualize activated sludge internal structures without staining and contrast filamentous bacteria. Phase contrast microscopy was also identified to favor the valuation of long protruding filaments thus stimulating filamentous bulking conditions identification (Mesquita et al., 2013)

2.3.3 Fluorescence microscopy

Fluorescence probes is an attractive method to solve the problems for analyzing microbial populations. In activated sludge system, the combination of fluorescence microscopy and fluorescent in situ hybridization probes is known as a powerful method for in situ identification of microorganisms. However, in fluorescence microscopy, the quantification may be complex and subjective using labor-intensive counting and non-uniform fluorescence intensity values can cause glitches to automatic quantification procedures (Mesquita et al., 2013)

2.4 Image Processing and Analysis

Image analysis has become a vital tools with a large field of application. This is due to is capability to remove human physical analysis which can avoid tedious and highly time consuming task and the likelihood to extract quantitative data (Amaral et al., 2004). Image analysis by using Matlab 7.3 (The Mathworks, Natick, USA) can be developed to further recognition both aggregates and filamentous bacteria in grayscale images (Amaral et al., 2004). Nowadays, image analysis procedures is considered to be a feasible tools to characterize quantitatively aggregates and filamentous bacteria and subsequently used to prevent bulking events in the plants in the future (Mesquita et al., 2009). Image analysis may offer powerful information by combining the settling properties and the parameters obtained in which can enabling immediate interventions on the biological system. A study developed by Sezgin (1982) established that the sludge volume index (SVI) is directly influenced by flocs size and filamentous bacteria contents. A basic image processing procedure can be done by the example from Mesquita et al., (2009) which start with image acquisition, background correction, image pre-processing and segmentation. The segmentation is divided into aggregates segmentation and filaments segmentations. According to Eikelboom (2010) aggregates were categorized according to their size: small aggregates (D_{eq}< 25 µm); intermediate aggregates (25 <D_{eq}< 250 µm); large aggregates (D_{eq}> 250 µm) where D_{eq} represents the equivalent diameter. Image analysis dataset will further used to predict the correlation between total filaments length per volume (TL/Vol) and SVI (mLg^{-1}), total aggregates area per volume (TA/Vol) and SVI ($mLg^{-}1$) and total filaments length per total aggregates ratio (TA/TL) and SVI ($mLg^{-}1$) (Mesquita et al., 2011). By plotting the data, it can identify the correlation between all the variables and thus can be used to elucidate several disturbance that may occur in an activated sludge system. If the correlation is below the threshold limits, the amount of filamentous bacteria will not influence the SVI value. This correlation can provide valuable information regarding the biological system in the activated sludge system (Mesquita et al., 2008).

CHAPTER 3: METHODOLOGY

3.1 Materials

3.1.1 Biomass Sampling

There will be two sampling to be collect:

1. Influent: Fresh POME will be collected from FELCRA Nasaruddin, a palm oil mill in Bota, Perak.
2. Effluent: Sample will be taken after the sample undergo Fenton Reagent Process. Fenton Reagent Process is normally used to oxidize contaminants or wastewater by using a mixture of hydrogen peroxide and ferrous iron. It is will normally produce a very reactive hydroxyl radical based on the catalyzed decomposition hydrogen peroxide by iron (II).

3.2 Method

3.2.1 Measuring Sludge Volume Index

The total suspend solids (TTS) for both of the sample was measured by weight (grams) and further used to analyze the sludge volume index (SVI) in a 1L cylindrical column for 30 min. The sludge volume index was determined by using equation:

$$SVI = \frac{h_{30}}{h_0 \,.\, TSS}$$

Where h_0 and h_{30} are the height in the time 0 and 30 min, respectively.

3.2.2 Bright field image acquisition

1. A large diameter of recalibrated micropipette with sectioned tip at the end will be used to deposit samples on microscope slides. A large diameter of micropipette will be used to allowing the passage of larger aggregated to flow.
2. Each slide a volume of 50µL of sample will covered with a 20mm x 20mm cover slip. A total of three slides per sample will be taken.
3. The slides were then captured by using light microscopy. The light microscopy used will be MEIJI Microscopy MX 4300L which is available at Environment Lab Block 5, Chemical Engineering Department
4. Image will be captured in the upper, middle and bottom of the slide in order to enhance the representativeness of the microbial community in the samples.
5. A new sample from the same batch will be repeated 3 times to ensure the integrity of the data.

3.2.3 Measuring Total Suspended Solids (TSS)

1. Measure the weight of the filter paper. Each filter paper is measured 3 times to ensure the integrity of the data.
2. Fold the filter paper exactly in half, the folded in quarters.
3. The filter paper cone is then fitted to the glass funnel.
4. Using a wash bottle filled with distilled water, wet the filter paper and carefully press it so that it makes maximum contact with the funnel.
5. The effluent is then slowly and carefully poured into the funnel taking care not to fill the funnel above the edge of filter paper.
6. Once the effluent have been filtered. Remove the filter and placed the filter on metal plate and place it in an oven for at least 1h at 103°C to 105°C.
7. Measure the weight of the filter paper. Each filter paper is measured 3 times to ensure the integrity of the data.
8. Repeat steps for influent.

3.2.4 Image Processing

The image processing and analysis algorithm to identify size of sludge aggregates will be develop in Matlab 7.3 (The Mathworks, Inc., Natick) language. The image processing procedures are:

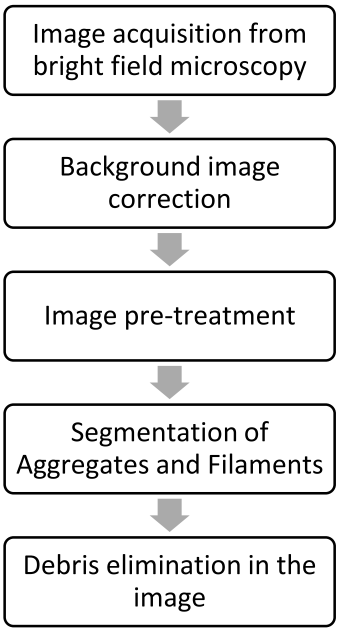

Figure 1 Procedures of the image processing

3.2.5 Image Analysis Parameters

There will be 1 parameter to be determine directly from the image analysis algorithm. Total aggregates area per volume TA/Vol ($mm^2 \mu L^{-1}$). The volume for the parameter will be fixed according to the volume (μL) taken during the image acquisition.

3.3 Process Flow of the study

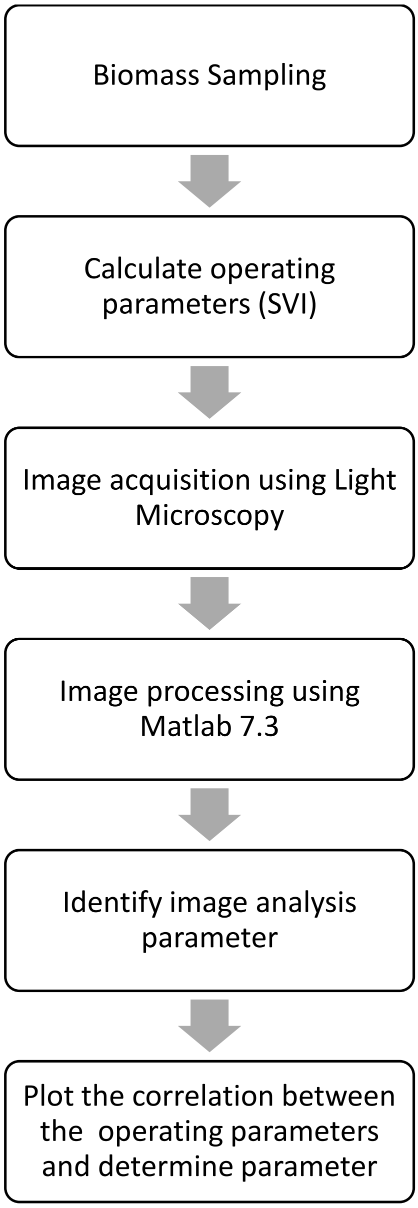

Figure 2 Process Flow of the Study

3.4 Project Key Milestones

Highly achievable, satisfactory and extremely relevant project milestones are important to keep the project in the track. The milestones for this study in the first few weeks are explained as follow:

a) Progress Report Preparation (Week 1-Week 7)
   Preparation of progress report includes, summary of project progress and future work is added to the report. The student will modify the previous interim proposal according to the comments and feedback from the proposal defence with better understanding and knowledge of study.

b) Experimental Activities (Week 1- Week7)
   Student is obligatory to meet the supervisor to get the main ideas on the study. Project started with reading the journals and books that relevant to the study before proceeding to the extended proposal preparation.

c) Progress Report Submission (Week 8)
   Student is required to send in the Progress report to the supervisor during week 8. Amendment will be made to the report if there are any changes.

d) Pre-Sedex (Week 11)
   Students is required to develop a poster for a short presentation to report on the progress of their work to a panel of internal examiners

e) Dissertation Report Submission (Week 13)
   To submit a complete draft of dissertation to Supervisor. Supervisor will examine that report and make comments any changes needed to be made.

f) Technical Paper Submission (Week13)
   To submit a complete technical paper which consist of 5 pages two column with reference to the FYP guidelines provided

3.5 Project Timeline

Table 1 Gantt chart for Final Year Project II

No.	Detail	Week														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	FYPII Activities: Experimental Work															
2	Progress Report Submission															
3	FYPII Activities: Simulation Work															
4	Pre-EDX															
5	Submission of Draft															
6	Project work continue: Analysis and reporting															
7	Submission of Softbound															
8	Submission of Technical Paper															
9	Oral Presentation															
10	Submission of Hardbound															

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Sludge Volume Index

Influent obtained = 1 liter

Effluent obtained = 84 milliliter

After 30 minutes of settling

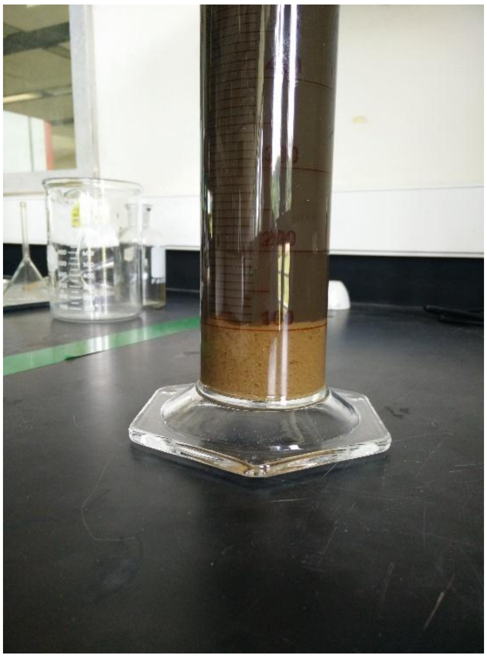

Figure 3 Influent settling after 30 min

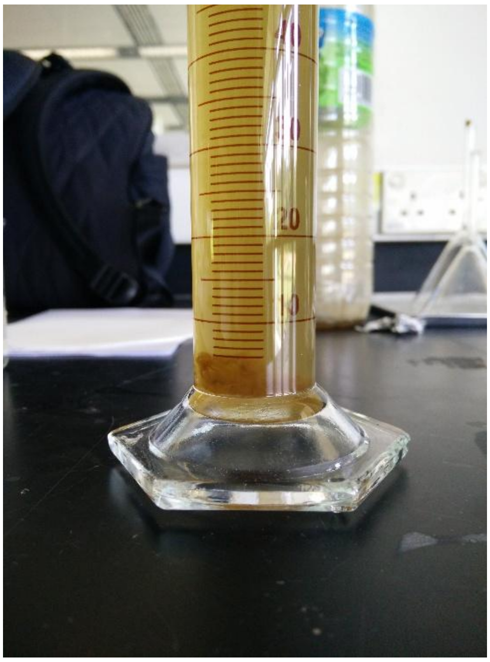

Figure 4 Effluent settling after 30 min

Influent sludge = 110 mL

Effluent sludge = 3 mL

4.1.1 Formula to measure SVI

$$\mathrm{SVI} = \frac{(\text{settled volume of sludge, mL/L})(10^3\ \text{mg/g})}{(\text{suspended solids, mg/L})} = \frac{\text{mL}}{\text{g}}$$

Calculations for SVI is done at the end of experiment after total suspended solids are calculated.

4.2 Light Microscopy image

Image are taken for influent and effluent using Meiji Microscopy MX4300L.

Sample Image of Influent under light microscopy

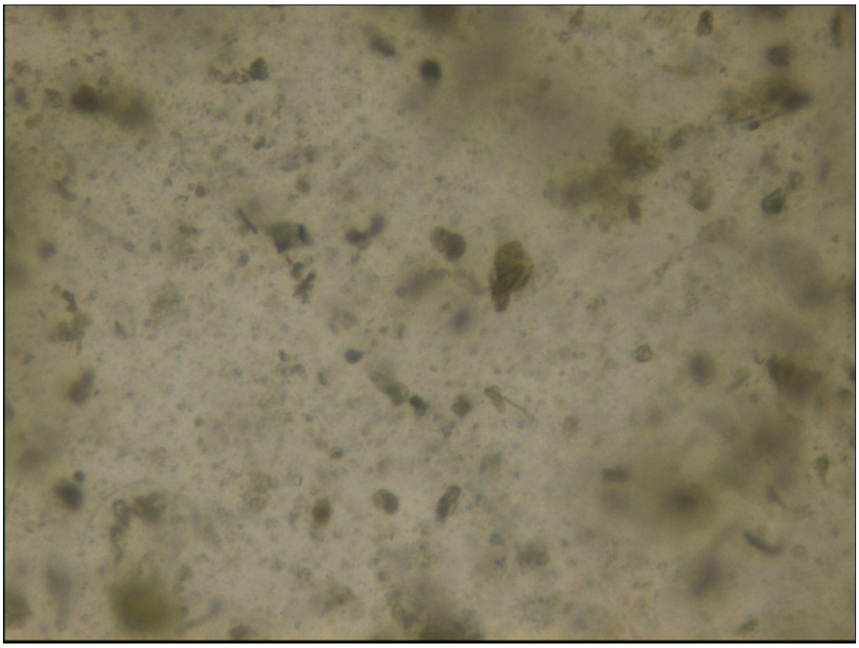

Figure 5 Influent under Light Microscopy x10 Magnification

Sample image of Effluent under light Microscopy

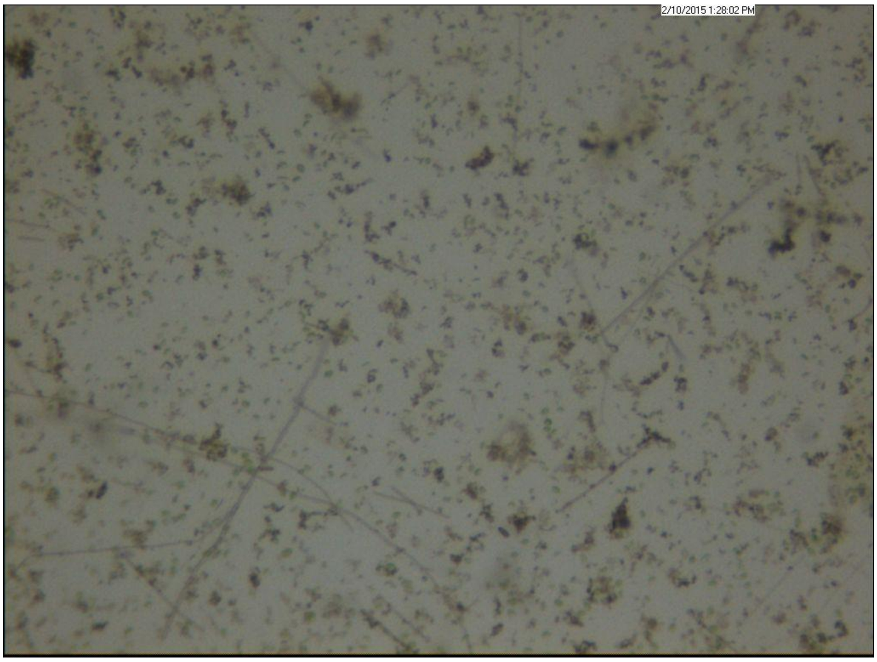

Figure 6 Effluent under Light Microscopy x10 Magnification

4.3 Total Suspended Solids

<u>Influent 100mL</u>

Table 2 Total Suspended Solids for Influent

	Trial 1	Trial 2	Trial 3	Average
Filter Paper	1.5689 g	1.5692 g	1.5695 g	1.5692 g
Filter Paper + Sludge	3.0202 g	3.0247 g	3.0277 g	3.0242 g
Sludge Weight	1.4513 g	1.4555 g	1.4582 g	1.4549 g

<u>Effluent 84mL</u>

Table 3 Total Suspended Solids for Effluent

	Trial 1	Trial 2	Trial 3	Average
Filter Paper	1.5554 g	1.5552 g	1.5540 g	1.5549 g
Filter Paper + Sludge	1.5767 g	1.5863 g	1.5902 g	1.5844 g
Sludge Weight	0.0213 g	0.0311 g	0.0362 g	0.0295 g

Figure 7 Influent filter paper after filtration

Figure 8 Effluent filter paper after filtration

4.3.1 Formula to measure TSS

$$mg\ total\ suspended\ solid/L = \frac{(A - B)\ X\ 1000}{sample\ volume,\ mL}$$

Where:

A = weight of filter + dried residue, mg, and

B = weight of filter, mg

Calculation for TSS

Influent

Weight of filter + dried residue = 3024.2 mg

Weight of filter = 1569.2 mg

Sample volume = 100 mL

$$mg\ total\ suspended\ solid/L = \frac{(3024.2 - 1569.2)\ X\ 1000}{100}$$

$$= 14550 mg\ total\ suspended\ solid/L$$

Effluent

Weight of filter + dried residue = 1584.4 mg

Weight of filter = 1554.9 mg

Sample volume = 84 mL

$$mg\ total\ suspended\ solid/L = \frac{(1584.4 - 1554.9)\ X\ 1000}{84}$$

$$= 351.19\ mg\ total\ suspended\ solid/L$$

Calculation for SVI

Influent

Settled volume of sludge = 110 mL/L

Suspended Solids = 14550 mg/L

$$\mathrm{SVI} = \frac{(\text{settled volume of sludge, mL/L})(10^3\ \text{mg/g})}{(\text{suspended solids, mg/L})} = \frac{\text{mL}}{\text{g}}$$

$$\mathrm{SVI} = \frac{\left(110\,\frac{\text{mL}}{\text{L}}\right)\left(10^3\,\frac{\text{mg}}{\text{g}}\right)}{(14550\,\frac{mg}{L})}$$

$$\mathrm{SVI}\ =\ 7.56\ \text{mL/g}$$

Effluent

Settled volume of sludge = 35.71 mL/L

Suspended Solids = 351.19 mg/L

$$\mathrm{SVI} = \frac{(\text{settled volume of sludge, mL/L})(10^3\ \text{mg/g})}{(\text{suspended solids, mg/L})} = \frac{\text{mL}}{\text{g}}$$

$$\mathrm{SVI} = \frac{\left(35.71\,\frac{\text{mL}}{\text{L}}\right)\left(10^3\,\frac{\text{mg}}{\text{g}}\right)}{(351.19\,\frac{mg}{L})}$$

$$\mathrm{SVI} = 101.69\ \text{mL/g}$$

Sludge Volume Index

Table 4 Sludge Volume Index Result

Sample	Sludge Volume Index
Influent	7.56 mL/g
Effluent	101.69 mL/g

Total Suspended Solids

Table 5 Total Suspended Solids Result

Sample	Total Suspended Solids
Influent	14550 mg/L
Effluent	351.19 mg/L

Discussion

From the calculation of total suspended solid above, the value of effluent which are 351.19 mg of total suspended solids/L is relatively lower than the value of influent which are 14549 mg of total suspended solids/L. These result shows that the effluent which undergo Fenton Process managed to reduce the total suspended solids in the POME. These results also prove that the Fenton Process is effective in pollution removal, display good sludge settling abilities and low suspended solids in the POME.

Once the total suspended solids is calculated, sludge volume index is then calculated. The SVI is calculated using the formula with reference to the standard method for the examination of water and wastewater. From the SVI calculation above, the SVI value for Influent is 7.56 mg/L and Effluent is 101.69 mg/L. A desired value for SVI is below 100, it is considered a good settling sludge. While SVI above 150 are usually associated with filamentous growth (Parker et.al, 2001). From the result above, effluents value shows a desired value while Influent shows an error in the results. The errors in the Influent is due to high amount of suspended solids thus increase the filtration times. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter and produce an error in the calculations.

4.4 Aggregates Size Analysis Using ImageJ

All the influent and effluent image captured by the microscope were then analyze by using ImageJ software. The analysis consist of 7 parameters. Below are the list of parameters involved in the analysis.

i. Particle Count
ii. Total Area of aggregates in the image
iii. Average Size of aggregates in the image
iv. Percentage area of aggregates in the image
v. Perimeter of the total aggregates in the image
vi. Fit ellipse of aggregates
vii. Feret's Diameter of the aggregates

Influent

A total of 77 images of influent taken for each sample. The images was captured by using Meiji MX4300L Microscope. The images were taken with 2 type magnification which are x4 magnification and x10 magnification. The image were then analyze using ImageJ software and further analyze using Microsoft Excel. (APPENDIX 1)

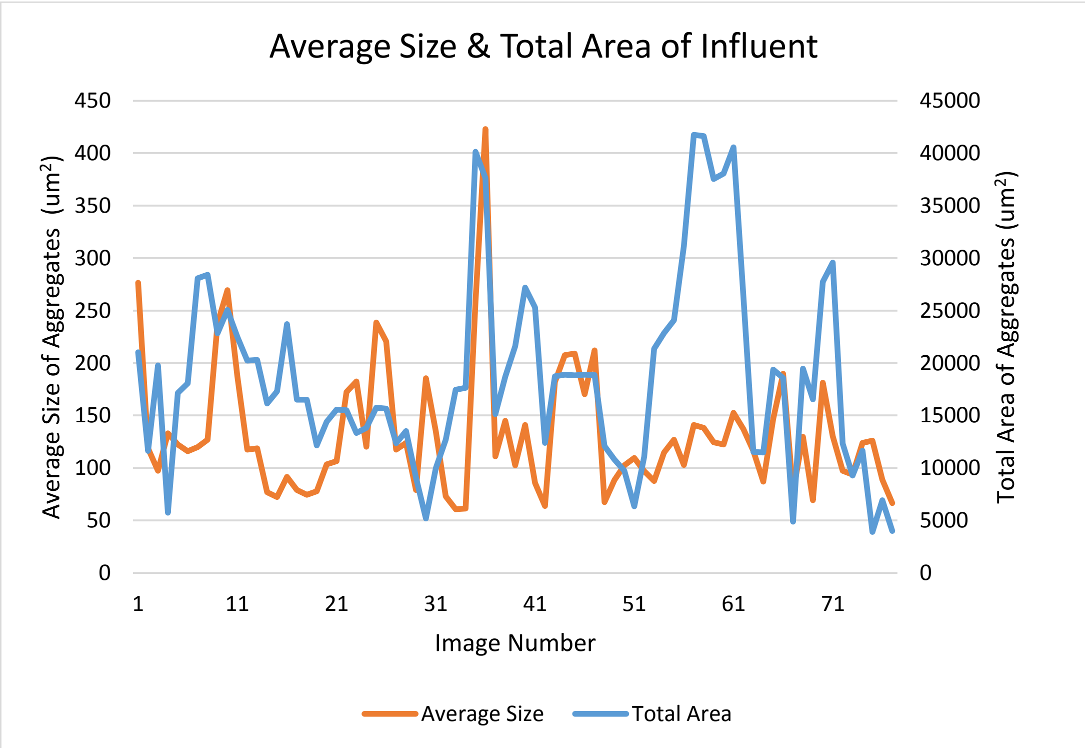

Figure 9 Average Size and Total Area of Influent

Effluent

A total of 72 images of effluent taken for each sample. The images was captured by using Meiji MX4300L Microscope. The images were taken with 2 type magnification which are x4 magnification and x10 magnification. The image were then analyze using ImageJ software and further analyze using Microsoft Excel. (APPENDIX 2)

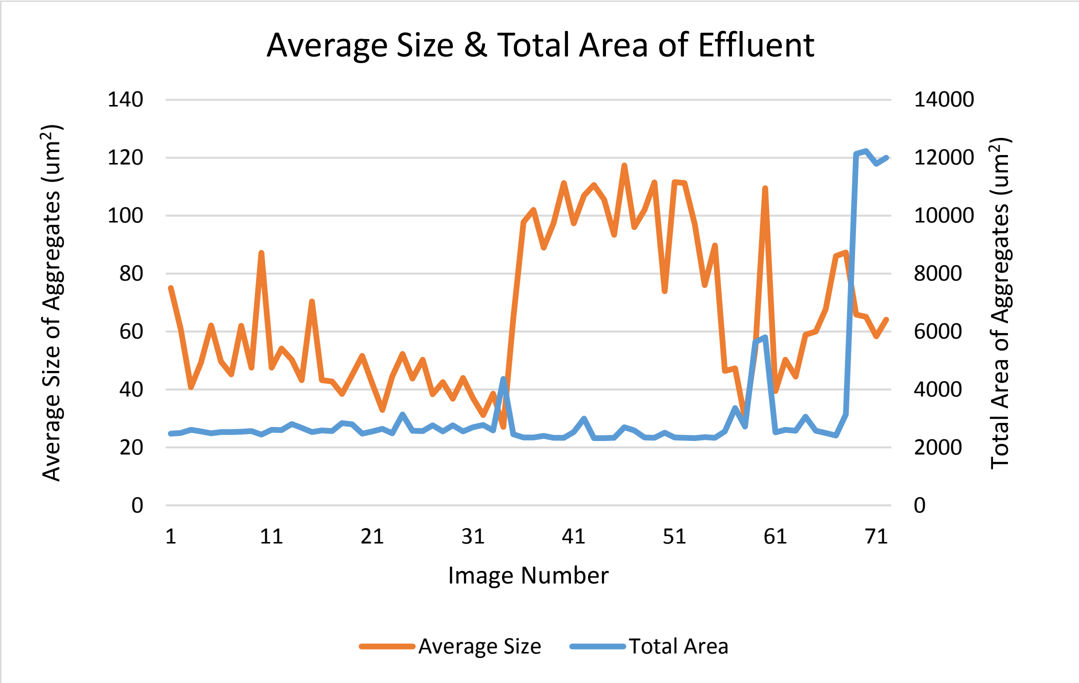

Figure 10 Average Size and Total Area of Effluent

Discussion

From Figure 9 Average size and total area of Influent, it shows that the size of particles and total area of particles per images is not consistent. This is due to many irregular shape and amount of aggregates per image. In contrary with figure 10, the effluent sample which undergo Fenton Process, the average total area of aggregates can be seen is almost the same for all the images which prove that Fenton Process managed to reduce the total area of aggregates in the sample.

Influent

From the total of 77 images captured in influent, ImageJ identified a total of 7504 individual particles. Figure below is the histogram of the total number of particles per image vs the frequency of image.

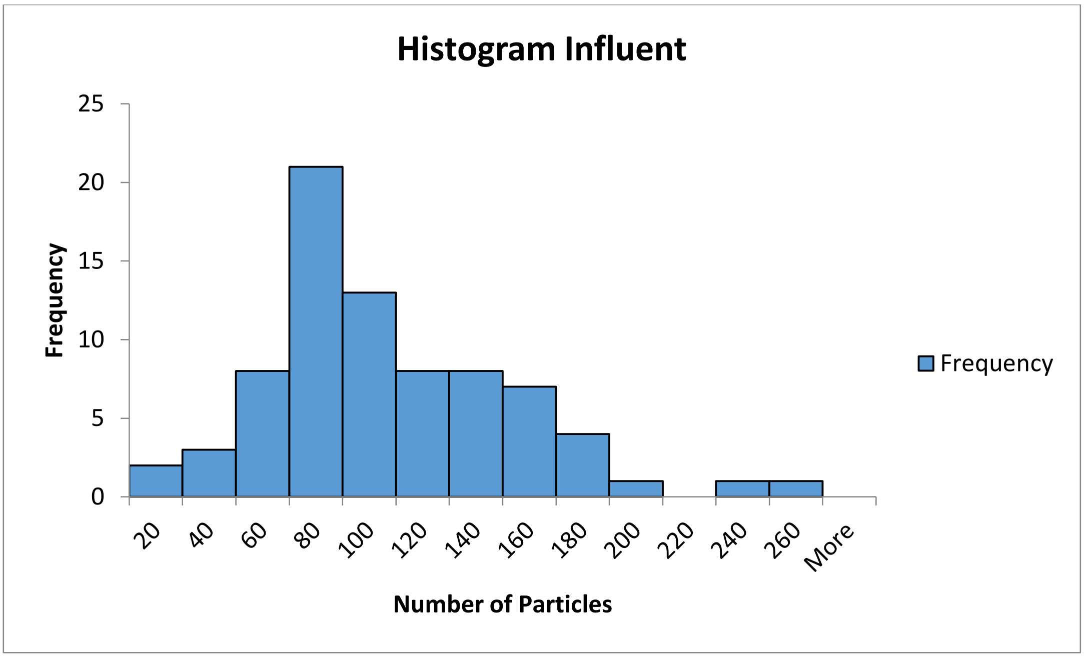

Figure 11 Histogram Analysis for Influent

From Figure 11 above, the highest number of particles recorded per image is 80 particles with a frequency of 20 images. Followed by 120 particles per image with 13 images. This proves that Influent have less number of particles compare to Effluent. Even though Influent have less particles per image, but the aggregates size of Influent is relatively bigger compare to Effluent.

Effluent

From the total of 72 images captured in influent, ImageJ identified a total of 22480 individual particles. Figure below is the histogram of the total number of particles per image vs the frequency of image.

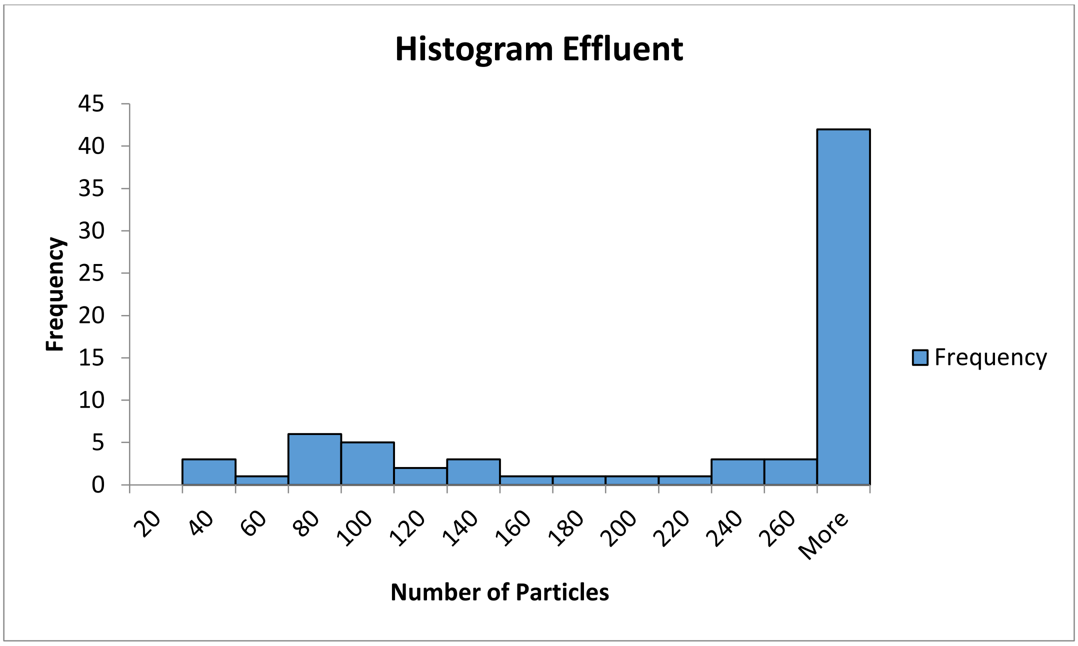

Figure 12 Histogram Analysis for Effluent

Based on Figure 12 above, the highest frequency of images contains number of particles are 260 and above. This shows that effluent contains more number of particles per image compare to influent. With refer to Figure 14 below, the high number of particles per image is due to small size particles which dominated the image. This is because the effluent undergo Fenton Process have purify the POME content by filter out the large size of aggregates.

4.5 Aggregates Size distribution Analysis Using ImageJ

Influent

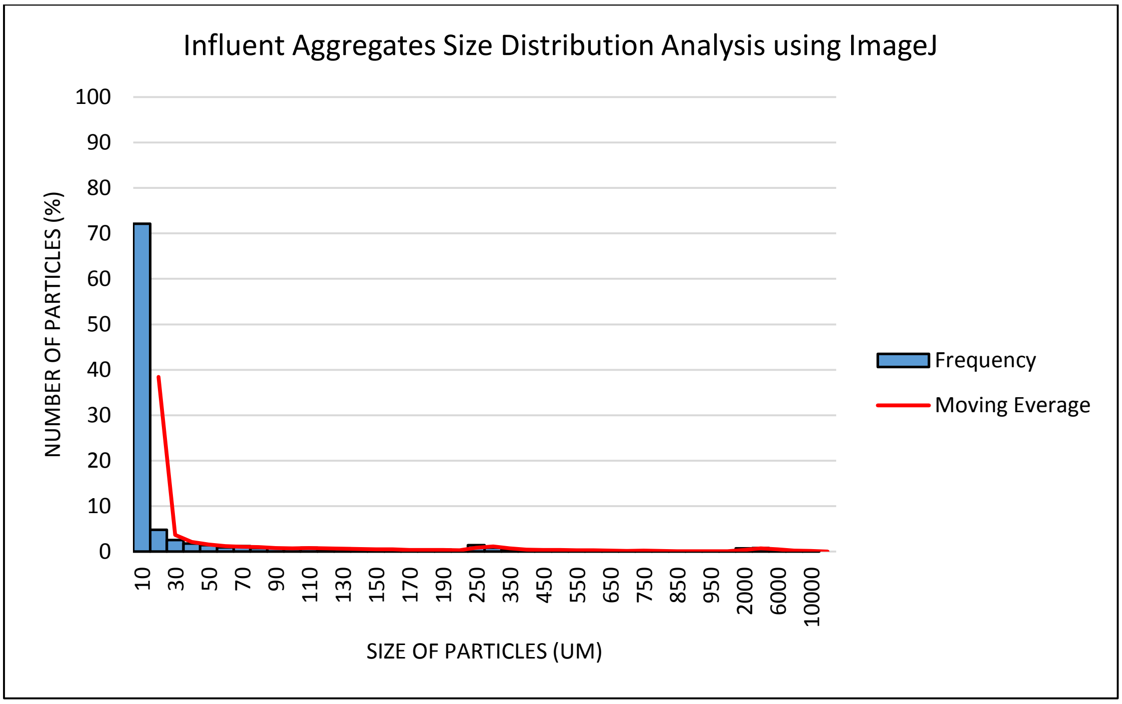

Figure 13 Influent Individual Particle Analysis

Effluent

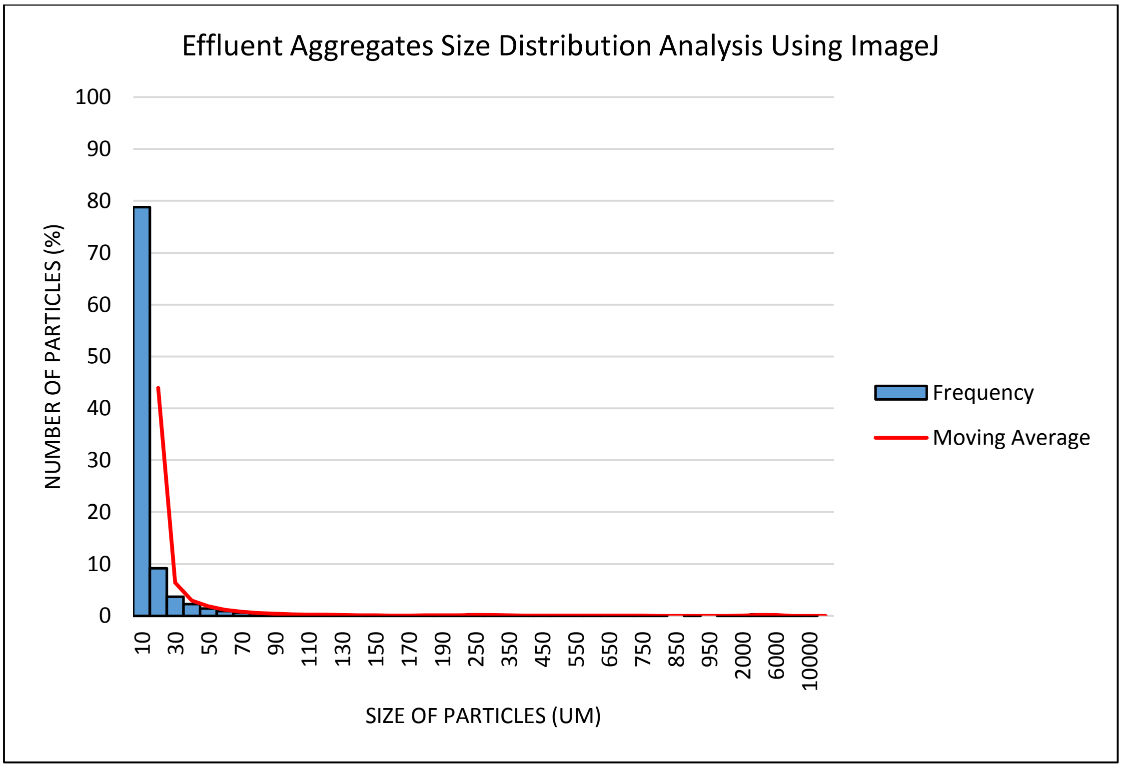

Figure 14 Effluent Individual Particle Analysis

Discussion

Figure 13 and Figure 14 is the aggregates size distribution analysis using ImageJ for Influent and effluent respectively. From Figure 13, it shows that around 70% of aggregates have the particle size 10 um^2 and below. Meanwhile, it also display large size of aggregates which is around 250-360 um^2 and 6000 um^2. This proves that the Influent contains aggregates size which differ in size and volume. In contrary with Figure 14, the effluent contains around 79% of number of particles which have size 10 um^2 below. In also display no large aggregates in the sample. This proves that the Influent which undergo Fenton Process managed to filter out the large aggregates which are contains in the sample. From the Figure 14 also, it can be concluded that the Effluent contains more number of particles in small sizes compare to Influent which display in Figure 13.

4.6 Aggregates Size Distribution Analysis using Matlab

Influent

A Matlab analysis is conducted using the same image sample from ImageJ. From the Figure below, it shows that aggregates particles with the equivalent diameter of 10 um and below have the most frequency which is 80% out of the 3913 particles identified. The difference in amount of particles identified in Matlab and ImageJ is due to the different threshold value set between the two software. Based on the figure below also it can be concluded that Influent contains aggregates which are large in size which are equivalent to 100 um and above. This proves that the influent contains waste that are large in size that may affect the SVI values.

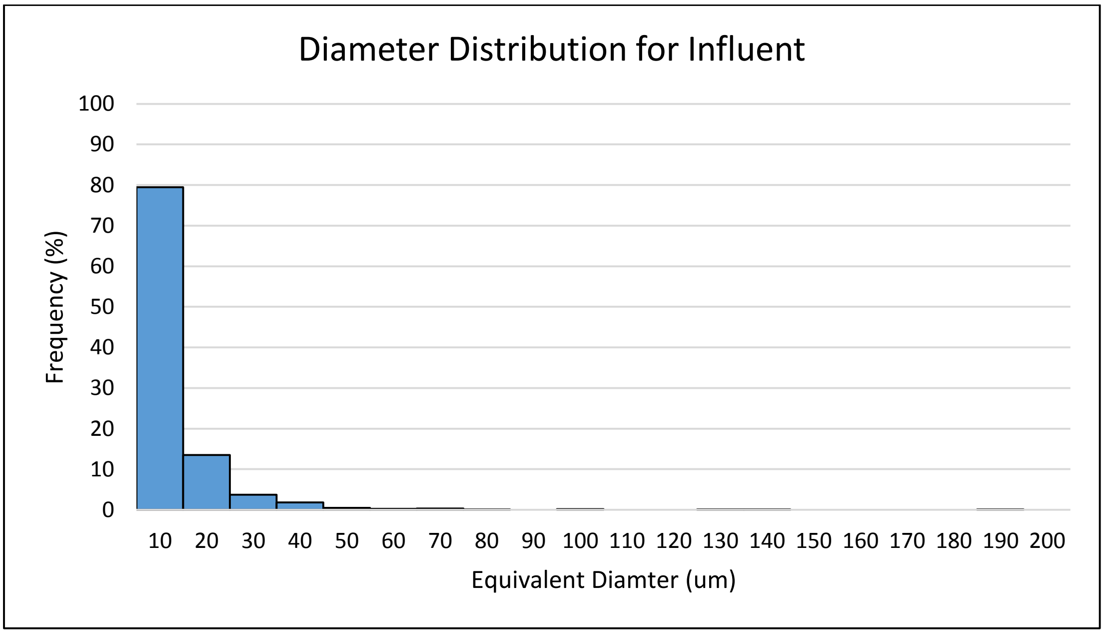

Figure 15 Diameter Distribution for Influent using Matlab

Effluent

A Matlab analysis is conducted using the same image sample from ImageJ. From the Figure below, it shows that aggregates particles with the equivalent diameter of 10 um and below have the most frequency which is 98% out of the 21085 particles identified. The difference in amount of particles identified in Matlab and ImageJ is due to the different threshold value set between the two software. Based on the figure below also it can be concluded that effluent contains no aggregates which are larger than equivalent diameter of 80 um. This proves that Fenton Process managed to reduce the aggregates size in the effluent.

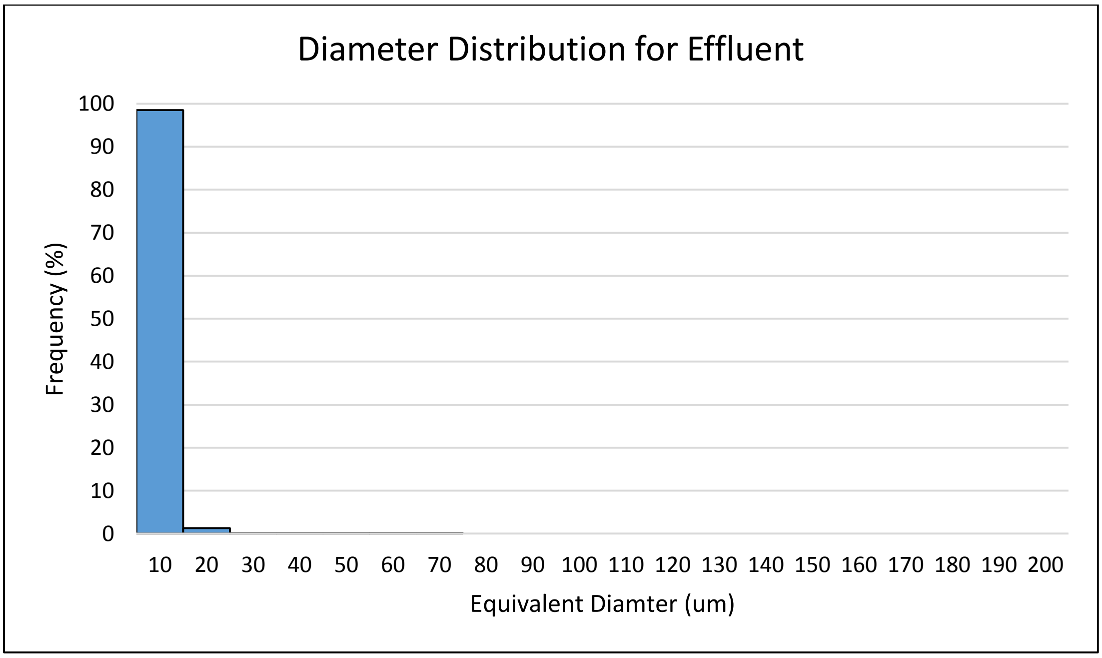

Figure 16 Diameter Distribution for Effluent using Matlab

4.7 Comparison Images of ImageJ and Matlab

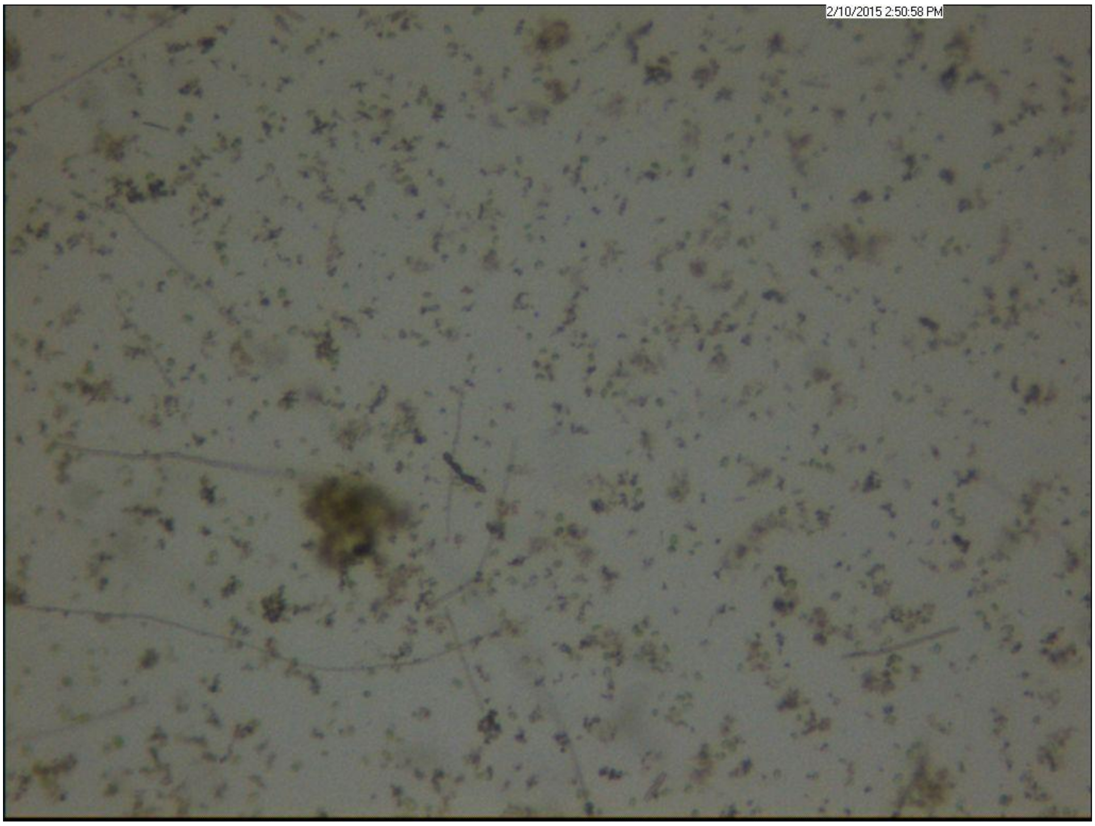

Figure 17 Original Image from Light Microscopy

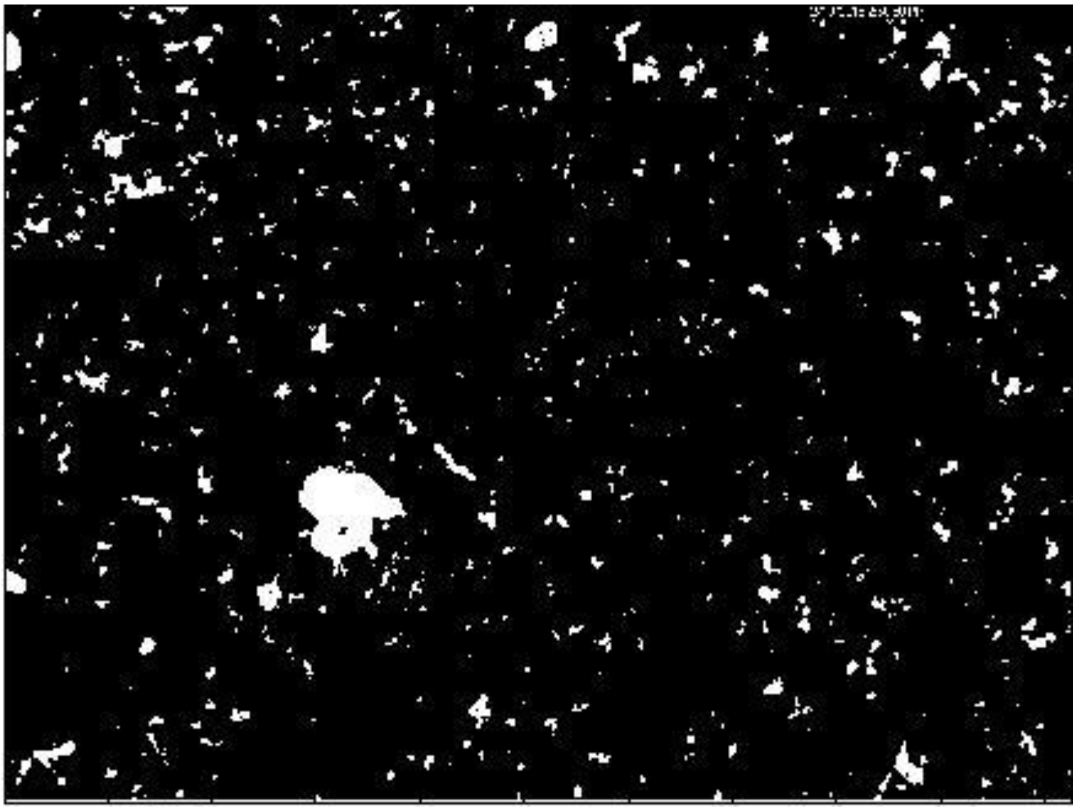

Figure 18 Threshold using Matlab

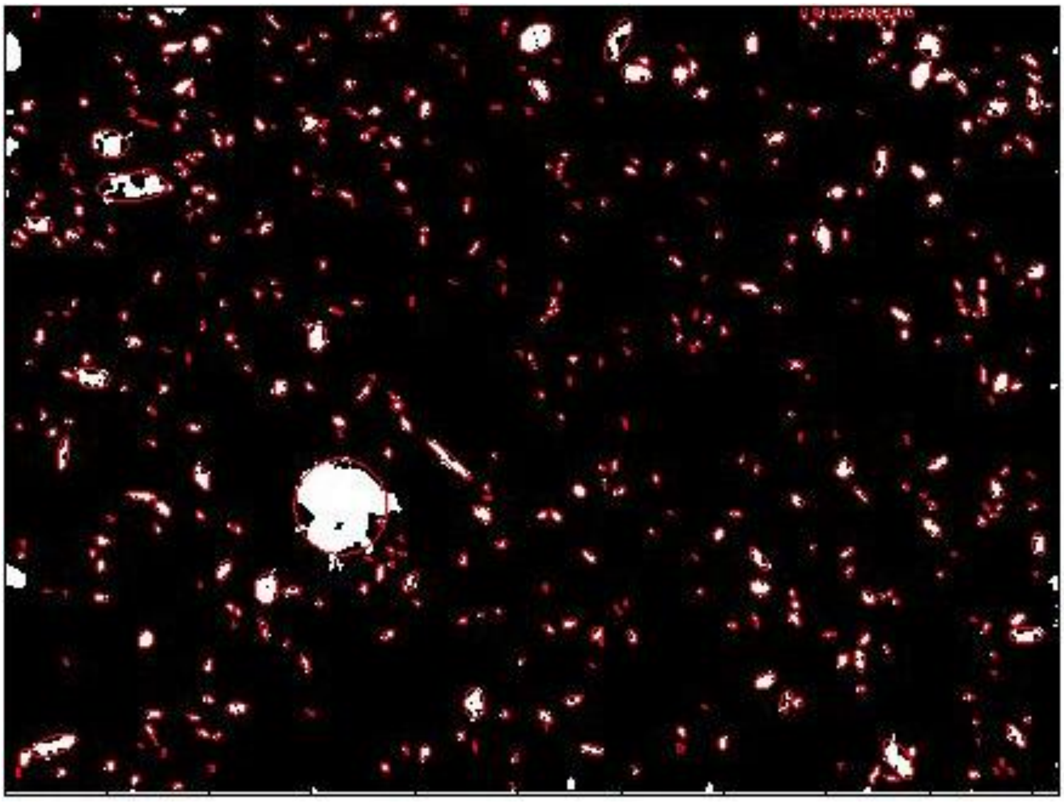

Figure 19 Identifying Particles using Matlab

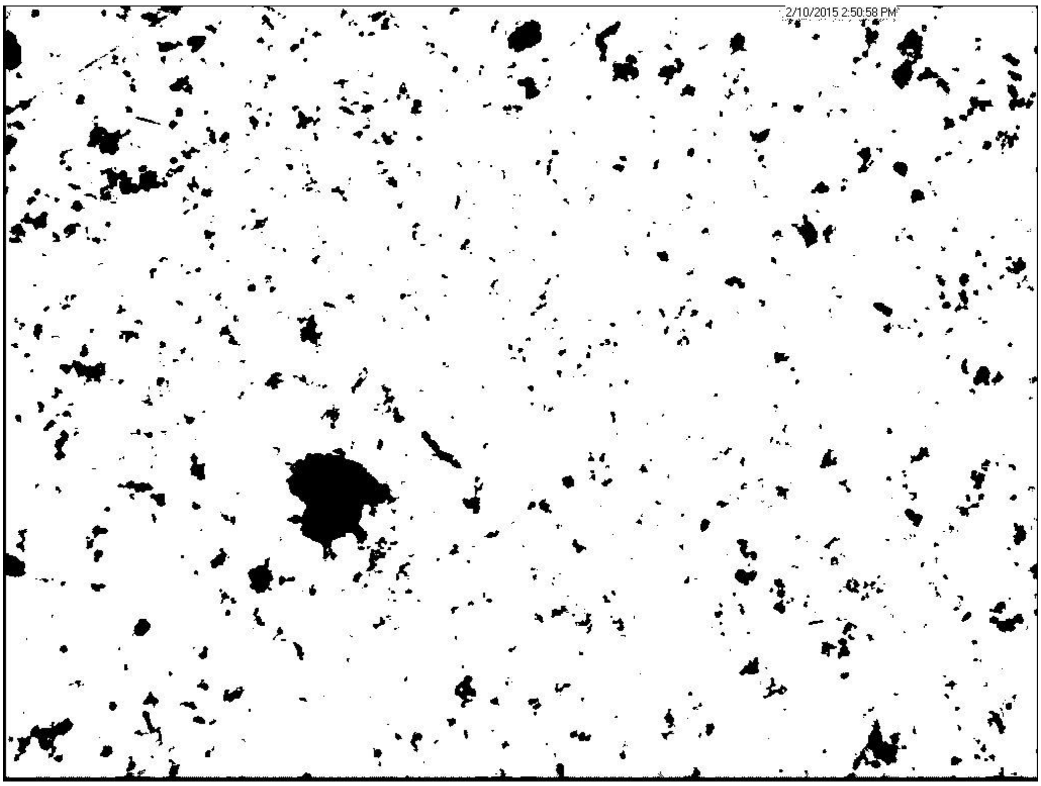

Figure 20 Threshold using ImageJ

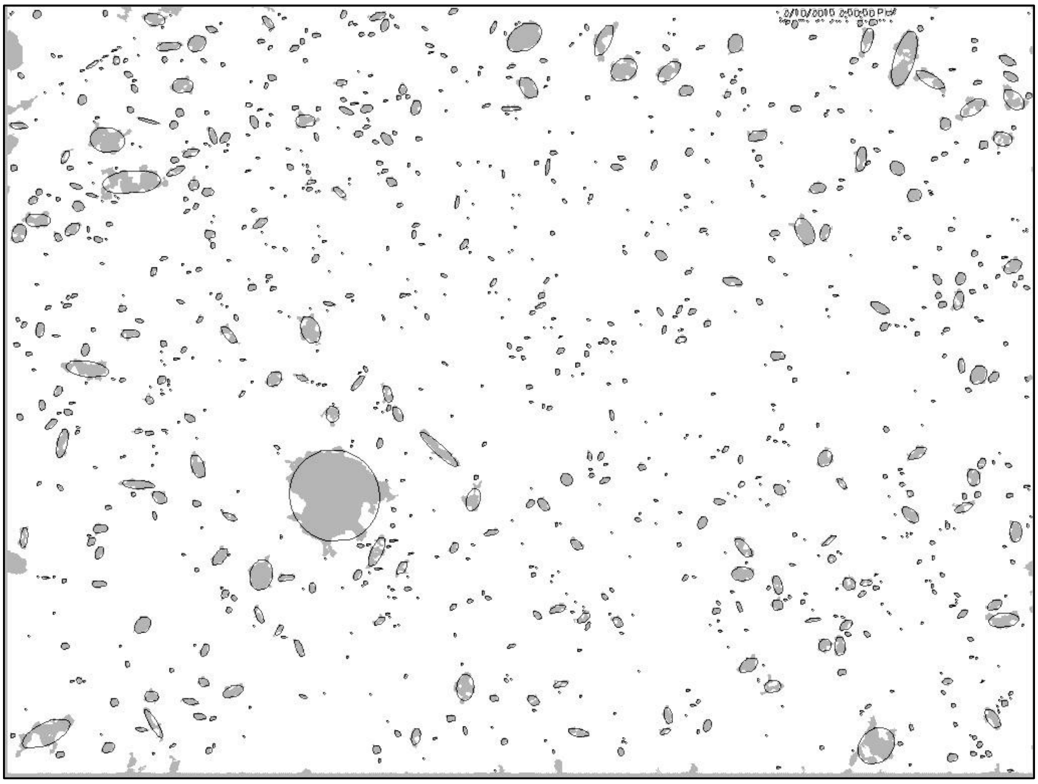

Figure 21 Identifying Particles using ImageJ

Discussion

From the comparison of Tresholding in Figure 17 and Figure 19, is shows that the two software Matlab and ImageJ are using a vice versa color identification. In Matlab, particles are label as white color while in ImageJ particles are label as black color. Even though there may different in the color scheme of the two software, the results display remain the same between the two software. Refer to Figure 18 and Figure 20, the particles are label by using ellipse. This process is by labelling particles into ellipse to make it easier to identify the diameter or length of individual particles as each particles have different sizes and shapes. For the Tresholding values, both software Matlab and ImageJ is set with the value 85. This is to ensure the consistency of data between those two. Finally, it can be concluded that, the both software gave the same result, thus the Image Analysis Algorithm using Matlab is correct.

4.8 Scale identification using ImageJ Software

Scale identification is important to identify the area of an aggregates in the image. Different magnification of microscopy will produce different length of pixels in the image. Below are the steps required to identify the length of pixel per micrometer.

Step 1: Open image file location

File > Open (Select the image)

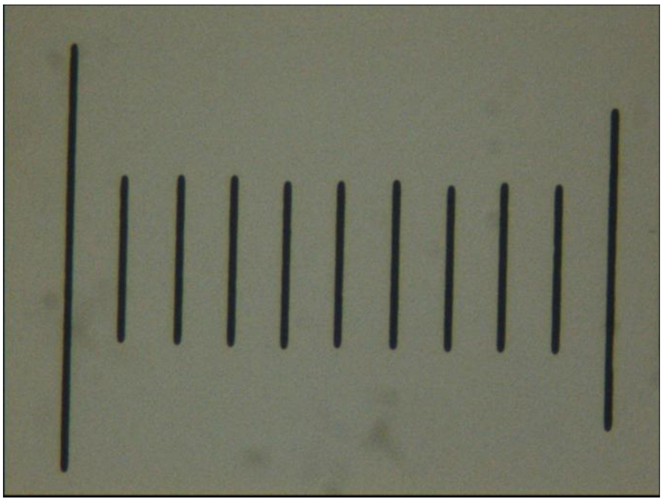

Figure 22 Example of Selected Image (magnification x10)

Step 2: Convert image type to grayscale

If the current image type is under the format of RGB Colors, the image need to convert to 8 – bit grayscale. This will converts the image to 256 shades (8-bit) of gray.

Image > Type > 8-bit

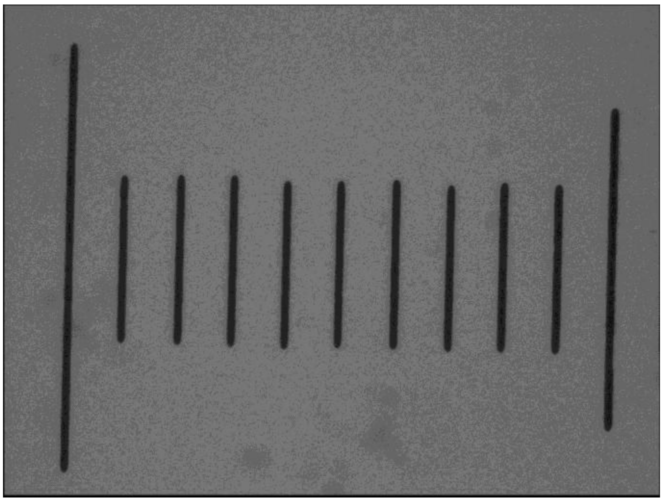

Figure 23 After converting to 8-bit grayscale

Step 3: Tresholding (Binary Contrast Enhancement)

There are two methods to create a threshold binary image

1. Automatic: Process > Binary > Make Binary
2. Manually: Image > Adjust > Threshold

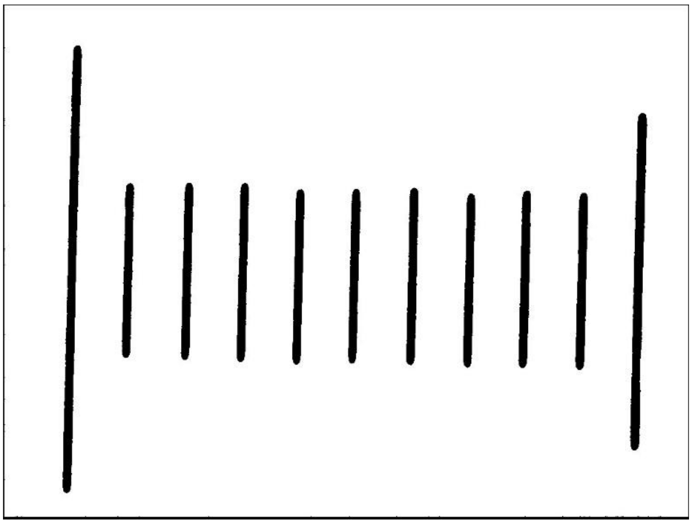

Figure 24 After threshold process

Step 4: Draw a line between two points

Click the line selection tools at tool bar. Then manually draw a straight line between the two points in the image

Step 5: Set Scale for the image

This functions is to define the spatial scale of the active image so measurement results can be presented in calibrated units, such as mm or µm. The distance in pixel will be automatically filled in based on the length of the line selection in Step 4

Analyze > Set Scale

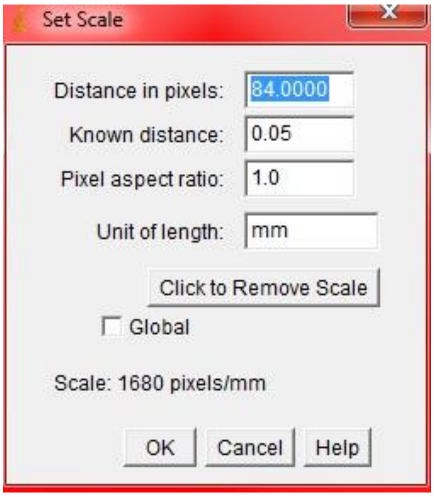

Figure 25 Set Scale table in ImageJ

Step 6: Insert Scale Bar into the image

This function is to integrate a scale bar into the image. The scale bar is measured according to the scale defined in Step 5

Analyze > Tools > Scale Bar

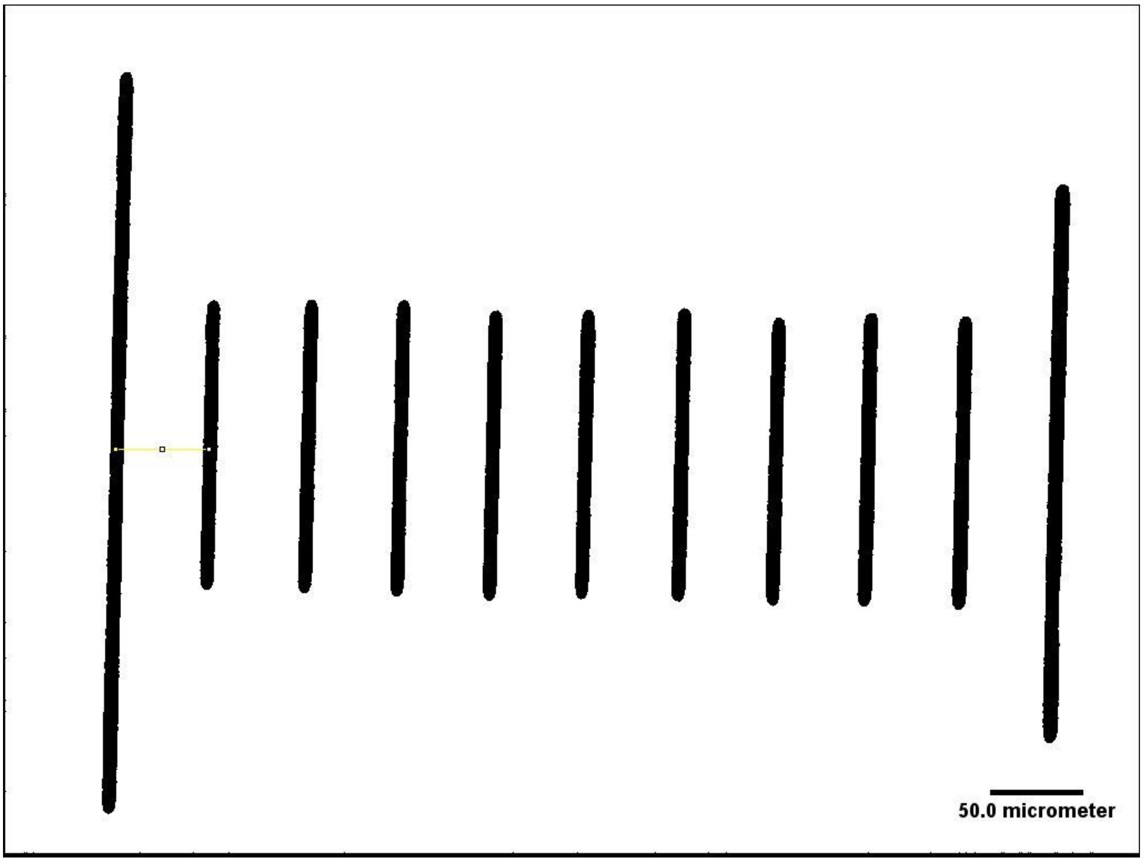

Figure 26 Integrated Scale Bar in the image

Below are the table for scale identification for different magnification images. Based on data provided from ImageJ, it showed that image with magnification of 4x will produce a length of 1.467 µm for every 1 pixels of the image. Image with magnification of 10x will produce a length of 0.586 µm for every 1 pixels of the image. To further ascertain the correlation between the two magnification data, manual calculation is being made.

Table 6 Scale Identification Data

Magnification	Known Distance (mm)	Distance in Pixel	Distance in µm	Scale pixels/mm	Scale pixels/ µm	Distance µm / pixels
4x	0.01	6.8354	9.96	683.54	0.6835	1.463
	0.05	34.0147	49.99	680.29	0.6803	1.47
	0.1	68.0294	99.96	680.29	0.6803	1.47
Average				**681.3733333**	**0.681366667**	**1.467666667**
10x	0.01	17.3754	9.78	1737.54	1.7375	0.576
	0.05	84.0106	49.99	1680.212	1.6802	0.595
	0.1	170.6667	100.2	1706.667	1.7067	0.586
Average				**1708.139667**	**1.708133333**	**0.585666667**

Sample Calculation

Based on the data with known distance 0.1mm:

Magnification 4x = 68.0294 pixels

Magnification 10x = 170.6667 pixels

For Magnification 4x calculation:

$$Magnification\ 4x = 68.0294\ pixels$$

$$Maginification\ 1x\ = 68.0294\ pixels\ \div 4$$

$$Magnification\ 1x = 17.0035\ \text{pixels}$$

For Magnification 10x calculation:

$$Magnification\ 10x = 170.667\ pixels$$

$$Maginification\ 1x\ = 170.667 pixels\ \div 10$$

$$Magnification\ 1x = 17.0667\ \text{pixels}$$

For the calculation above, the two data correlate with each other as they both give the same answer for Magnification 1x = 17 pixels. This also can proved that the calculation from ImageJ software is correct for Scale identification. With the base line of Magnification 1x is identified, the base line can be integrated to be used to any magnification in the microscope.

4.9 Aggregates area identification and measurement using ImageJ

Step 1: Open image file location

File > Open (Select the image)

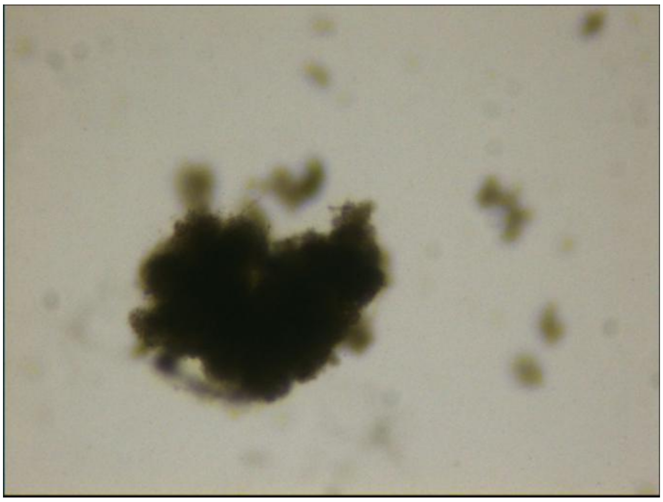

Figure 27 Sample image

Step 2: Convert image type to grayscale

If the current image type is under the format of RGB Colors, the image need to convert to 8 – bit grayscale. This will converts the image to 256 shades (8-bit) of gray. In this scale 0 = pure black and 255 = pure white

Image > Type > 8-bit

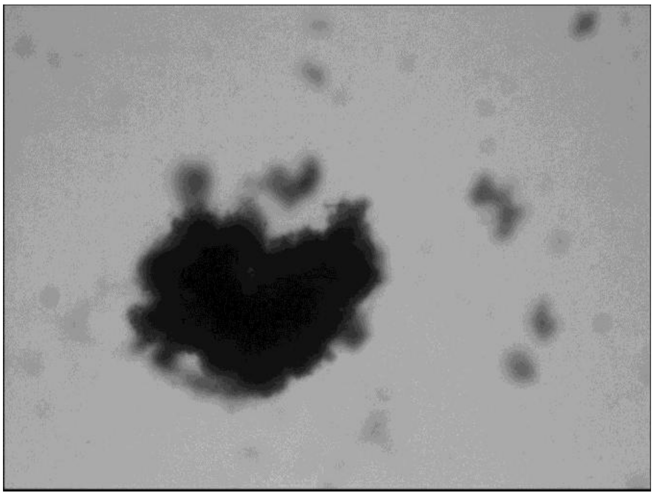

Figure 28 Grayscale Image

Step 3: Tresholding (Binary Contrast Enhancement)

Tresholding is also known as “Segmentation”. Tresholding works by separating the pixels which fall within a desired range of intensity values set by the users or systems. The separating of the pixels will produce a black and white image.

There are two methods to create a threshold binary image

1. Automatic: Process > Binary > Make Binary
2. Manually: Image > Adjust > Threshold

Both of this method will produce almost similar result

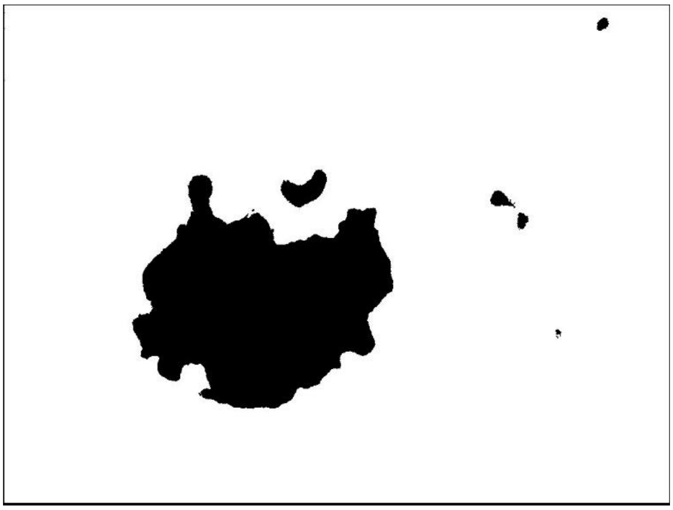

Figure 29 Automatic Tresholding image

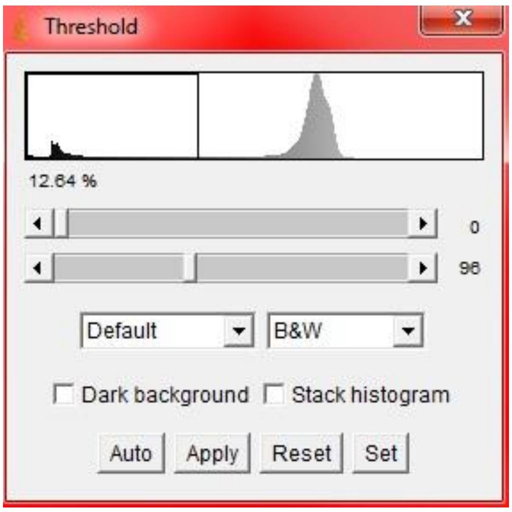

Figure 30 Manual Tresholding Windows

There are more process available in ImageJ commands to create or process binary (black and white) images. Some of the sample and process are listed below.

Outline

This command generates a one pixel wide outline of foreground in a binary image. The line is drawn inside the object

Process > Binary > Outline

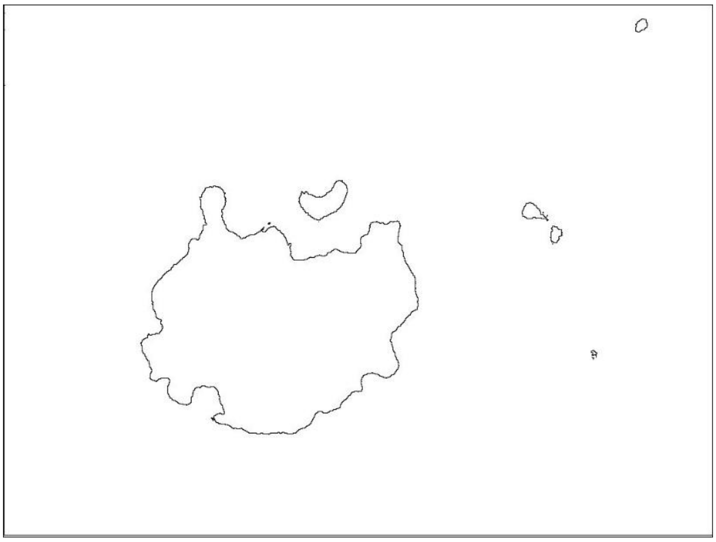

Figure 31 Sample outline image

Skeletonize

This command repeatably remove the pixels from the edges of the objects in a binary image until they are reduced to single pixel wide shape

Process > Binary > Skeletonize

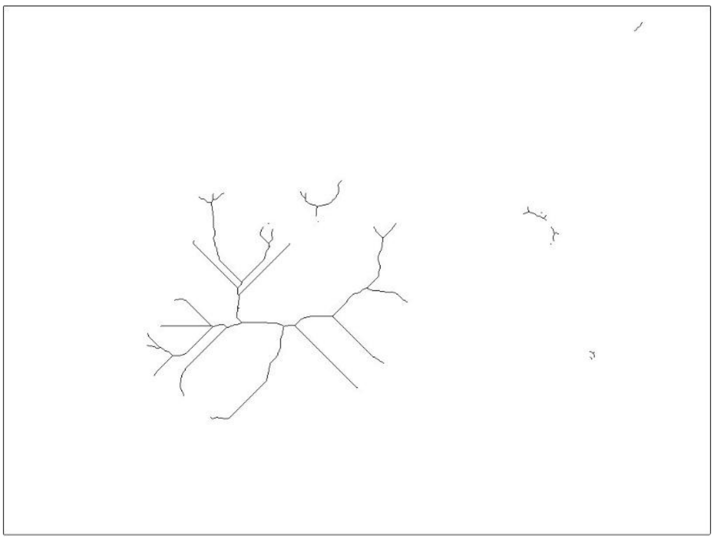

Figure 32 Sample Skeletonize image

Distance Map

This command generates a Euclidian distance map (EDM) from a binary image. Each foreground pixel in the binary image is replaced with a gray value equal to that pixel's distance from the nearest background pixel (for background pixels the EDM is 0). The Ultimate Points, Watershed and Voronoi commands are all based on the EDM algorithm

Process > Binary > Distance Map

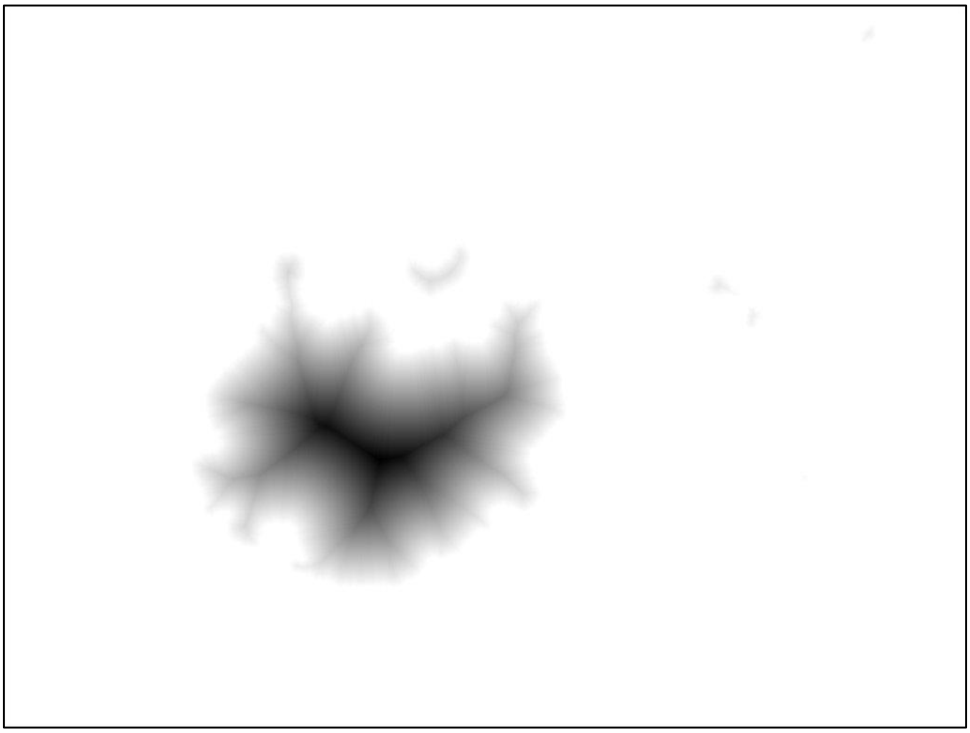

Figure 33 Sample Distance Map image

Functions of ultimate points, watershed and voronoi:

i. Ultimate Points

   Generates the ultimate eroded points (UEPs) of the Euclidian distance map (EDM) from a binary image. Ultimate Eroded Points are maxima of the EDM. In the output, the points are assigned the EDM value, which is equal to the radius of the largest circle that fits into the binary particle, with the UEP as the center.

ii. Watershed

Watershed segmentation is a way of automatically separating or cutting apart particles that touch. It first calculates the Euclidian distance map (EDM) and finds the ultimate eroded points (UEPs). It then dilates each of the UEPs (the peaks or local maxima of the EDM) as far as possible — either until the edge of the particle is reached, or the edge touches a region of another (growing) UEP. Watershed segmentation works best for smooth convex objects that don't overlap too much.

iii. Voronoi

Splits the image by lines of points having equal distance to the borders of the two nearest particles. Thus, the Voronoi cell of each particle includes all points that are nearer to this particle than any other particle. When particles are single points, this process is a Voronoi tessellation (also known as Dirichlet tessellation)

<u>Step 4: Set Scale into the image</u>

Refer to 4.8 Scale identification using ImageJ, insert the relevant value into the table provided with refer to the magnification of the image.

Analyze > Set Scale

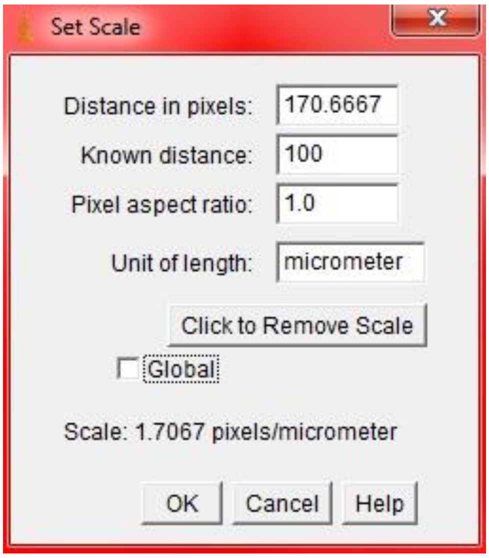

Figure 34 Set scale into the image windows

Step 5: Area measurement

1. This command will measure all the relevant data selected from the windows below.

   Analyze > Set Measurement

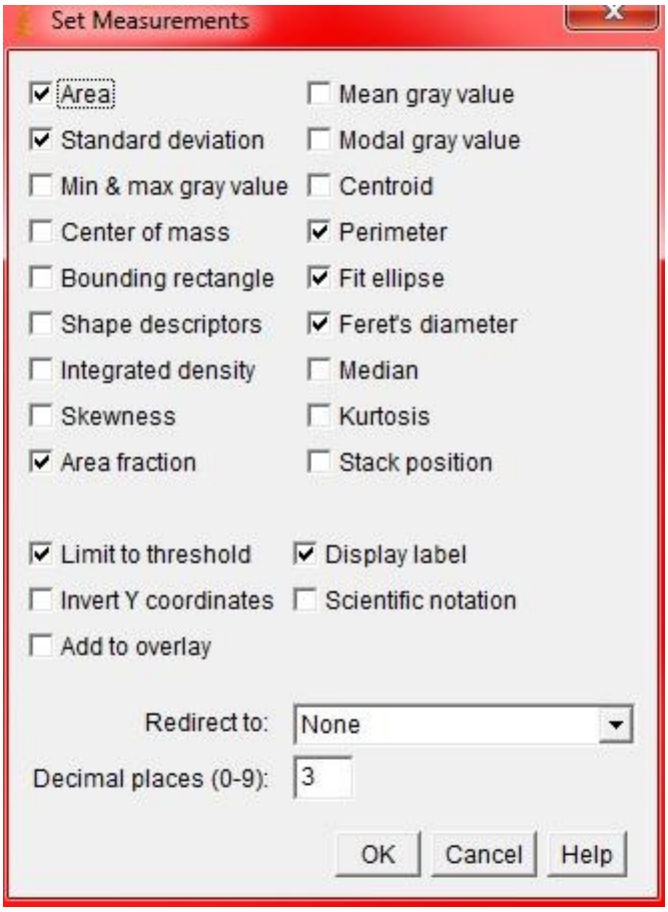

Figure 35 Set measurement windows

2. This command will produce the result based on the selected data in the set measurement windows.

   Analyze > Measure

Table 7 Measurement results

Area	Perim.	Major	Minor	Angle	Feret	%Area	FeretAngle	MinFeret
34139.32	3584	233.515	186.145	6.401	1280	12.644	143.13	768

From the results above, the total area of aggregates in the image is 34319 μm^2. While the ferret diameter is 1280 µm.

Step 6: Analyze Particle

This command will counts and measures objects in the image. It works by scanning the image or selection until it finds the edge of an object

Analyze > Analyze Particles

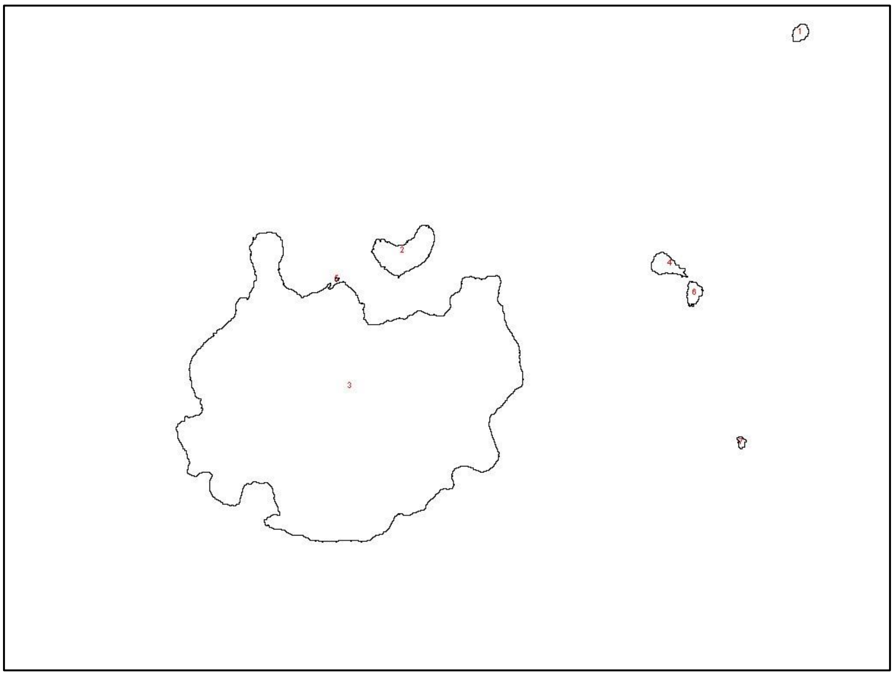

Figure 36 Image containing numbered outline of the measured particles

Based on figure 17, the command will automatically outline the particles and numbered the particle individually

Table 8 Particle Analysis Result

	Area	Perim.	Major	Minor	Angle	Feret	%Area	FeretAngle	MinFeret
1	2545.051	2098.627	63.608	50.944	170.108	750	100	143.13	450
2	96.474	37.809	13.132	9.354	50.373	14.554	100	49.899	10.358
3	803.032	141.198	42.982	23.788	22.08	44.47	100	18.435	29.726
4	30316.07	976.187	224.625	171.84	7.348	250.179	100	32.276	200.839
5	221.443	73.901	22.615	12.467	157.444	26.832	100	148.392	15.125
6	123.596	52.038	15.846	9.931	82.039	17.243	100	99.782	10.978
7	28.839	24.089	7.409	4.956	97.931	8.531	100	105.945	6.166

Based on table 4, the command will produce a summary of result which contains all the data selected from the set measurement windows in step 5. The total area for all particles is 34134.51 µm^2. There is a difference of area 4.81 µm^2 between table 4 and table 3. The difference is due to limits set in the software which is to only identify particles with area 5 µm^2 and above inside the image. Based on the limits set, it can be concluded that both measurement produce the same results.

CHAPTER 5: CONCLUSION AND RECOMMENDATION

Conclusion

By conducting this study, image analysis algorithm can be developed to monitor sludge aggregates in Palm Oil Mill Effluent (POME). Thus, this algorithm can be used to identify bulking problems in the POME and establish the true nature of the phenomenon occurring inside the activated sludge system which normally will affect the quality of the effluent. From the experimental data of Total Suspended Solids, it show that influent have a higher amount of suspended solids in the POME. The SVI calculated for influents display an error as due to the prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter and produce an error in the calculations. The effluent result for SVI shows an ideal value for a typical good SVI. From the image comparison data between Matlab and ImageJ, it can be proven that the coding for Matlab is correct and can be used to identify the aggregates size in the POME. This also proves that the POME undergo Fenton Process have less large particles in the solution and contain better SVI values compare to Influent which have many large particles in the solution.

Recommendation

To provide a comprehensive procedure to measuring Total Suspended Solids and Sludge Volume Index. To prepare different type of POME which have undergo different purification process. To obtain different POME sample from different Palm Oil Mill. To identify the effect of filaments in the POME. To use different method of Image Acquisition and Image Analysis.

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APPENDIX

APPENDIX 1: INFLUENT IMAGE ANALYSIS DATA X10 MAGNIFICATION

SAMPLE A TRIAL 1 X10 MAGNIFICATION

	Column1	Column2	Column3	Column4	Column5	Column6	Column7	Column8	Column9	Column10	Column11	Column12	Column13	Column14
No	Slice	Count	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Feret	FeretX	FeretY	FeretAngle	MinFeret
1	2-10_15-45-31	76	21012.235	276.477	8.433	51.914	7.753	4.906	56.916	17.188	358.293	211.148	114.885	10.735
2	2-10_15-45-38	98	11617.986	118.551	4.663	44.826	8.821	4.966	64.986	15.897	297.026	217.559	110.395	9.406
3	2-10_15-45-45	203	19776.65	97.422	7.937	34.519	7.891	4.364	58.051	11.898	321.429	225.598	110.648	6.863
4	2-10_15-45-52	43	5725.195	133.144	2.298	63.925	6.501	3.815	46.654	21.907	425.591	170.234	120.058	12.971
5	2-10_15-45-59	140	17144.853	122.463	6.881	42.235	8.929	5.201	53.059	14.433	347.211	168.799	106.979	8.636
6	2-10_15-46-6	156	18064.572	115.799	7.25	39.384	8.541	5.049	49.435	13.491	332.606	158.374	107.851	8.082
7	2-10_15-46-13	234	28076.297	119.984	11.269	31.089	6.897	4.076	58.761	10.326	304.18	216.318	110.304	6.117
8	2-10_15-46-20	224	28415.608	126.855	11.405	31.772	7.121	4.229	68.725	10.586	300.04	218.628	112.03	6.288
9	2-10_15-46-27	96	22835.832	237.873	9.165	38.866	6.87	4.563	48.413	13.451	285.132	116.472	115.902	8.341
10	2-10_15-46-34	93	25059.569	269.458	10.058	42.966	8.174	4.917	57.763	14.896	278.648	180.976	115.755	8.91
11	2-10_15-46-41	120	22498.422	187.487	9.03	34.218	6.66	4.221	50.075	12.052	336.696	162.222	110.513	7.349
12	2-10_15-46-48	172	20227.48	117.602	8.118	35.14	7.529	4.553	55.109	12.001	321.687	185.965	109.313	7.251
13	2-10_15-46-55	171	20293.378	118.675	8.145	35.688	7.621	4.634	62.643	12.153	319.931	190.874	113.48	7.333
14	2-10_15-47-2	210	16148.148	76.896	6.481	28.895	6.281	3.555	66.225	10.002	312.564	175.89	114.604	5.732
15	2-10_15-47-9	239	17307.064	72.414	6.946	27.299	6.033	3.242	57.028	9.418	289.489	183.859	109.54	5.245
16	2-10_15-47-16	259	23712.464	91.554	9.517	23.067	4.971	2.959	52.742	7.77	344.686	152.502	108.812	4.581
17	2-10_15-47-23	209	16515.022	79.019	6.628	30.718	6.614	3.837	57.19	10.429	288.968	163.406	109.818	6.181
18	2-10_15-47-30	222	16500.131	74.325	6.622	29.428	6.348	3.751	61.783	10.058	290.976	179.473	113.994	5.967
19	2-10_15-47-37	156	12127.744	77.742	4.868	31.331	5.823	3.534	57.313	10.57	283.46	163.029	114.547	6.4
20	2-10_15-47-44	139	14394.25	103.556	5.777	35.258	6.568	3.72	56.115	11.977	329.754	163.62	111.924	6.909
21	2-10_15-47-51	146	15556.651	106.552	6.244	36.39	7.223	3.92	58.179	12.528	324.734	129.864	113.751	7.081
22	2-10_15-47-58	90	15505.96	172.288	6.223	47.121	7.949	4.479	66.293	15.542	266.135	264.365	107.326	9.141
23	2-10_15-48-5	73	13320.242	182.469	5.346	50.967	8.136	4.52	50.195	17.216	259.951	229.965	108.2	10.115
24	2-10_15-48-12	115	13815.11	120.131	5.545	32.599	5.987	3.617	59.817	11.628	277.864	125.72	115.578	7.006
25	2-10_15-48-19	66	15744.207	238.549	6.319	66.237	10.365	5.533	55.302	21.007	378.663	138.746	108.567	12.17
26	2-10_15-48-26	71	15655.498	220.5	6.283	60.31	9.97	5.214	65.336	19.789	391.239	141.192	113.376	11.426
27	2-10_15-48-33	105	12346.664	117.587	4.955	37.854	7.14	3.919	53.88	13.472	276.168	150.119	116.363	7.822
28	2-10_15-48-40	109	13507.481	123.922	5.421	40.676	8.156	4.211	71.749	14.341	263.348	153.579	114.519	8.117
29	2-10_15-48-47	115	9088.521	79.031	3.648	39.334	7.136	4.315	64.579	13.782	304.539	196.68	104.158	8.214
30	2-10_15-48-54	28	5191.675	185.417	2.084	84.349	6.788	4.47	55.224	29.899	200.842	92.511	119.606	18.176
31	2-10_15-49-1	74	9979.093	134.853	4.005	55.307	9.567	4.954	54.167	19.183	318.368	165.596	107.193	10.835
32	2-10_15-49-8	174	12684.391	72.899	5.091	32.46	7.091	3.783	64.604	11.468	353.938	146.374	109.982	6.536
33	2-10_15-49-15	288	17464.838	60.642	7.01	23.941	5.621	3.148	57.103	8.46	342.456	185.161	112.325	4.913
34	2-10_15-49-22	288	17638.454	61.245	7.079	24.255	5.618	3.119	58.354	8.445	332.952	180.953	108.851	4.884
35	2-10_15-49-29	155	40122.936	258.858	16.104	36.549	7.013	4.169	62.233	11.728	264.136	172.189	117.669	7.063

36	2-10_15-49-36	89	37643.846	422.965	15.109	51.396	8.531	5.564	53.954	16.346	256.559	171.225	110.933	10.234
37	2-10_15-49-43	136	15122.612	111.196	6.07	28.395	5.904	3.145	45.143	10.405	229.769	160.247	111.933	6.03
38	2-10_15-49-50	129	18708.027	145.023	7.509	41.565	7.595	4.32	51.696	13.426	331.287	176.321	113.827	7.849
39	2-10_15-49-57	211	21615.771	102.444	8.676	30.276	6.513	3.599	61.792	10.181	369.053	127.506	109.672	5.884
40	2-10_15-50-4	193	27205.685	140.962	10.919	28.969	5.973	3.767	50.047	9.669	368.548	160.177	116.276	5.861
41	2-10_15-50-11	295	25291.162	85.733	10.151	23.366	5.129	2.984	52.349	7.756	359.056	169.75	117.158	4.582
42	2-10_15-50-18	195	12396.405	63.571	4.975	25.675	4.862	3.061	54.042	8.745	366.872	166.752	114.585	5.393
43	2-10_15-50-25	103	18735.906	181.902	7.52	34.527	6.197	3.893	57.714	12.16	303.592	186.734	110.979	7.352
44	2-10_15-50-32	91	18897.166	207.661	7.585	40.005	7.026	4.263	63.58	13.844	321.594	174.228	112.759	8.202
45	2-10_15-50-40	90	18821.131	209.124	7.554	39.825	6.894	4.282	49.505	13.755	298.087	187.44	114.337	8.241
46	2-10_15-50-47	111	18884.811	170.133	7.58	32.329	5.887	3.662	52.962	11.47	296.209	193.884	112.848	6.878
47	2-10_15-50-53	89	18869.92	212.022	7.574	40.242	6.993	4.354	54.373	13.927	303.435	164.641	115.28	8.352
48	2-10_15-51-1	179	12078.954	67.48	4.848	26.597	4.994	2.948	54.184	9.015	287.313	128.242	113.008	5.394
49	2-10_15-51-8	122	10785.391	88.405	4.329	32.046	5.849	3.292	59.503	11.331	320.224	146.22	114.257	6.69
50	2-10_15-51-15	95	9724.689	102.365	3.903	39.42	6.423	4.048	63.35	13.904	355.108	105.475	111.59	8.473
51	2-10_15-51-21	58	6343.304	109.367	2.546	58.905	8.375	5.412	72.929	20.595	357.225	153.837	98.123	12.537
52	2-10_15-51-29	114	11059.121	97.01	4.439	38.915	7.265	4.217	64.071	13.545	293.504	193.384	116.547	8.011
53	2-10_15-51-36	244	21349.011	87.496	8.569	31.308	7.05	3.875	58.186	10.59	341.804	192.283	106.121	6.118
54	2-10_15-51-43	199	22850.089	114.825	9.171	31.932	6.407	3.846	57.292	10.22	282.668	181.138	114.019	6.255
55	2-10_15-51-50	190	24088.525	126.782	9.668	31.548	6.416	3.836	57.145	10.244	338.569	169.864	119.618	6.16
56	2-10_15-51-57	303	31175.399	102.889	12.512	22.032	5.01	3.037	49.22	7.373	322.261	137.025	118.811	4.381
57	2-10_15-52-4	296	41754.542	141.063	16.758	24.877	5.914	3.487	57.488	8.424	318.798	150.774	113.879	4.922
58	2-10_15-52-11	301	41624.33	138.287	16.706	23.771	5.677	3.352	54.769	8.078	322.501	150.059	115.925	4.754
59	2-10_15-52-18	302	37548.167	124.332	15.07	24.363	5.518	3.258	50.539	7.912	345.649	159.693	114.217	4.762
60	2-10_15-52-25	311	38056.024	122.367	15.274	24.087	5.319	3.213	49.084	7.684	354.189	162.29	112.594	4.724
61	2-10_15-52-32	266	40576.935	152.545	16.286	23.536	5.696	3.339	50.862	8.089	293.485	160.014	113.372	4.844
62	2-10_15-52-39	192	26304.975	137.005	10.558	32.083	6.824	3.917	52.276	10.626	343.016	157.344	108.175	6.327
63	2-10_15-52-46	99	11521.99	116.384	4.624	40.622	6.576	3.915	58.152	13.594	326.683	243.175	113.241	8.239
64	2-10_15-52-53	132	11457.993	86.803	4.599	32.859	6.134	3.063	61.912	11.512	275.757	183.17	121.626	6.426
65	2-10_15-53-0	132	19374.292	146.775	7.776	33.106	6.633	3.329	56.483	11.744	315.149	132.214	119.363	6.417
66	2-10_15-53-7	98	18596.825	189.764	7.464	37.553	6.207	4.033	55.929	12.967	282.363	153.306	113.832	7.727
67	2-10_15-53-14	63	4887.531	77.58	1.962	48.087	5.976	3.755	62.835	17.08	287.052	124.465	115.705	10.235
68	2-10_15-53-21	150	19462.051	129.747	7.811	27.853	5.691	3.168	48.845	9.771	379.461	162.597	114.813	5.806
69	2-10_15-53-28	239	16540.05	69.205	6.638	25.646	5.611	3.144	57.561	8.801	326.942	140.16	112.252	5.205
70	2-10_15-53-35	153	27746.491	181.35	11.136	40.137	8.977	5.301	65.245	13.63	324.755	177.751	108.55	8.263
71	2-10_15-53-42	227	29576.741	130.294	11.871	29.935	6.524	3.893	53.232	9.845	302.216	140.124	111.016	5.932
72	2-10_15-53-49	127	12339.061	97.158	4.952	34.271	6.032	3.491	47.818	11.588	414.038	102.375	120.544	6.817
73	2-10_15-53-56	99	9260.869	93.544	3.717	43.356	7.309	4.155	57.935	14.816	347.219	117.838	111.166	8.683
74	2-10_15-54-3	94	11656.321	124.003	4.678	44.578	8.395	4.821	72.931	15.953	316.114	187.602	122.719	9.331
75	2-10_15-54-10	31	3910.786	126.154	1.57	84.416	8.542	4.961	60.625	30.459	343.311	96.667	104.799	17.687
76	2-10_15-54-17	78	6924.346	88.774	2.779	47.554	7.896	4.56	64.017	17.053	282.132	148.726	108.68	10.006
77	2-10_15-54-24	60	3985.554	66.426	1.6	50.589	6.405	3.978	64.454	18.101	354.314	101.1	100.145	10.97

APPENDIX 2: EFFLUENT IMAGE ANALYSIS DATA X10 MAGNIFICATION

	SAMPLE B TRIAL 3 x10 MAGNIFICATION													
	Column1	Column2	Column3	Column4	Column5	Column6	Column7	Column8	Column9	Column10	Column11	Column12	Column13	Column14
No	Slice	Count	Total Area	Average Size	%Area	Perim.	Major	Minor	Angle	Feret	FeretX	FeretY	FeretAngle	MinFeret
1	2-10_14-48-16	33	2476.239	75.038	0.994	128.734	4.826	3.011	68.047	25.626	388.036	85.436	106.074	15.057
2	2-10_14-48-23	41	2496.833	60.898	1.002	104.911	4.281	2.659	74.916	21.093	348.468	91.253	108.147	12.425
3	2-10_14-48-30	64	2609.302	40.77	1.047	68.857	3.286	2.117	53.346	14.178	410.003	114.948	110.116	8.356
4	2-10_14-48-38	52	2555.444	49.143	1.026	84.439	4.045	2.43	56.908	17.354	381.124	97.213	109.974	10.146
5	2-10_14-48-45	40	2484.16	62.104	0.997	107.564	4.318	2.695	77.07	21.583	392.345	107.803	106.978	12.726
6	2-10_14-48-52	51	2532.316	49.653	1.016	85.765	3.866	2.335	63.478	17.469	371.645	114.824	110.28	10.217
7	2-10_14-48-58	56	2531.366	45.203	1.016	78.278	3.732	2.301	52.733	16.118	347.117	124.262	103.827	9.457
8	2-10_14-49-6	41	2543.405	62.034	1.021	106.043	4.35	2.732	59.321	21.328	359.478	77.662	106.24	12.57
9	2-10_14-49-13	54	2568.433	47.564	1.031	81.902	3.951	2.346	68.837	16.864	389.826	94.53	109.267	9.827
10	2-10_14-49-20	28	2441.073	87.181	0.98	151.278	5.312	3.257	66.788	29.817	399.513	59.603	102.342	17.497
11	2-10_14-49-27	55	2614.055	47.528	1.049	80.749	4.03	2.405	64.186	16.669	279.447	120.637	117.564	9.765
12	2-10_14-49-34	48	2600.748	54.182	1.044	91.963	4.448	2.693	72.118	18.91	407.291	71.953	117.318	11.12
13	2-10_14-49-41	56	2813.649	50.244	1.129	80.902	4.515	2.729	58.36	16.99	301.746	88.39	109.269	9.979
14	2-10_14-49-48	62	2677.735	43.189	1.075	72.794	3.861	2.407	64.798	15.13	365.599	125.201	101.391	8.968
15	2-10_14-49-55	36	2535.801	70.439	1.018	119.45	4.638	2.855	58.934	23.813	304.088	93.529	113.308	13.942
16	2-10_14-50-2	60	2590.293	43.172	1.04	73.985	3.685	2.294	57.776	15.314	393.527	92.601	108.97	8.987
17	2-10_14-50-9	60	2564.315	42.739	1.029	73.598	3.554	2.258	48.478	15.165	246.872	132.011	114.619	8.951
18	2-10_14-50-16	74	2845.648	38.455	1.142	62.142	3.469	2.131	56.084	12.991	313.348	155.138	114.604	7.606
19	2-10_14-50-23	62	2797.808	45.126	1.123	72.629	3.584	2.326	55.781	14.839	321.65	102.723	112.439	8.778
20	2-10_14-50-30	48	2475.606	51.575	0.994	89.736	3.567	2.307	52.936	17.992	285.701	111.623	119.858	10.589
21	2-10_14-50-37	61	2554.493	41.877	1.025	72.604	3.51	2.217	54.682	14.972	297.802	146.391	113.736	8.826
22	2-10_14-50-44	80	2640.034	33	1.06	56.951	3.407	2.044	64.129	12.167	356.3	133.758	115.716	7.141
23	2-10_14-50-51	56	2494.298	44.541	1.001	77.65	3.464	2.12	55.616	15.805	296.248	105.547	113.018	9.239
24	2-10_14-50-58	60	3137.436	52.291	1.259	76.983	4.131	2.423	46.419	15.843	263.346	139.459	108.056	9.258
25	2-10_14-51-5	59	2582.373	43.769	1.036	75.026	3.702	2.222	59.684	15.483	374.82	93.188	114.477	9.047
26	2-10_14-51-12	51	2562.414	50.243	1.028	86.061	3.83	2.407	61.456	17.444	262.096	88.789	110.879	10.3
27	2-10_14-51-19	72	2764.226	38.392	1.109	64.025	3.897	2.347	66.721	13.665	318.855	113.495	112.568	8.033
28	2-10_14-51-26	60	2554.81	42.58	1.025	73.335	3.482	2.052	52.979	15.053	260.71	117.779	112.92	8.753
29	2-10_14-51-33	75	2761.691	36.823	1.108	61.655	3.833	2.263	65.928	13.212	350.702	117.819	112.472	7.701
30	2-10_14-51-40	58	2551.959	43.999	1.024	75.52	3.476	2.188	59.43	15.449	284.538	138.135	114.537	9.082
31	2-10_14-51-47	73	2702.447	37.02	1.085	62.482	3.61	2.217	59.353	13.241	338.282	144.726	102.809	7.843
32	2-10_14-51-54	89	2775.948	31.19	1.114	52.483	3.425	2.158	60.099	11.37	367.614	124.905	107.904	6.787
33	2-10_14-52-1	67	2588.076	38.628	1.039	66.833	3.55	2.139	58.036	13.988	318.237	136.49	112.001	8.201
34	2-10_14-52-8	161	4370.487	27.146	1.754	36.938	4.202	2.056	59.335	9.051	403.301	204.491	100.891	4.885
35	2-10_14-52-15	38	2453.745	64.572	0.985	115.971	4.729	2.684	61.584	22.896	361.181	92.813	106.651	13.252

36	2-10_14-52-22	24	2347.295	97.804	0.942	179.835	5.684	3.45	74.294	34.337	396.562	42.426	111.312	20.116
37	2-10_14-52-29	23	2346.028	102.001	0.942	185.722	5.597	3.455	62.71	35.392	417.792	19.921	113.399	20.734
38	2-10_14-52-36	27	2401.787	88.955	0.964	161.005	5.622	3.412	72.363	31.131	437.742	29.165	110.024	18.231
39	2-10_14-52-43	24	2340.008	97.5	0.939	178.596	5.488	3.341	65.028	34.038	402.261	18.692	107.601	19.922
40	2-10_14-52-50	21	2336.523	111.263	0.938	203.677	6.028	3.742	69.193	38.665	443.269	2.385	110.464	22.655
41	2-10_14-52-57	26	2530.732	97.336	1.016	168.886	6.076	3.845	75.389	32.567	403.271	28.793	106.587	19.223
42	2-10_14-53-4	28	2994.235	106.937	1.202	163.097	7.074	4.291	71.27	31.968	414.691	54.256	107.334	18.872
43	2-10_14-53-11	21	2323.534	110.644	0.933	203.657	6.074	3.633	69.656	38.623	443.35	2.385	108.786	22.547
44	2-10_14-53-18	22	2320.999	105.5	0.932	194.975	5.795	3.551	66.232	36.914	432.715	12.076	110.527	21.599
45	2-10_14-53-25	25	2333.989	93.36	0.937	172.252	5.276	3.268	65.056	32.714	417.961	19.858	116.055	19.165
46	2-10_14-53-32	23	2697.694	117.291	1.083	194.42	7.072	4.019	70.789	37.226	409.668	30.028	108.25	21.828
47	2-10_14-53-39	27	2593.145	96.042	1.041	164.03	6.1	3.433	63.019	31.788	427.444	70.421	100.672	18.569
48	2-10_14-53-46	23	2344.761	101.946	0.941	185.529	5.56	3.468	63.131	35.344	413.608	18.721	111.591	20.734
49	2-10_14-53-53	21	2340.008	111.429	0.939	203.885	6.029	3.724	69.483	38.655	443.269	2.385	110.201	22.655
50	2-10_14-54-0	34	2512.357	73.893	1.008	129.737	5.22	3.259	71.097	25.597	333.017	60.061	110.075	15.056
51	2-10_14-54-7	21	2342.226	111.535	0.94	202.639	6.019	3.727	68.44	38.644	443.35	2.627	107.343	22.655
52	2-10_14-54-14	21	2337.157	111.293	0.938	203.189	6.06	3.688	69.059	38.611	443.43	2.385	108.21	22.601
53	2-10_14-54-21	24	2328.286	97.012	0.934	178.598	5.448	3.316	70.796	33.973	396.022	22.468	111.996	19.87
54	2-10_14-54-28	31	2355.532	75.985	0.945	139.93	4.637	2.885	57.85	26.852	428.74	58.011	111.106	15.763
55	2-10_14-54-35	26	2333.989	89.769	0.937	165.756	5.134	3.211	61.888	31.52	366.706	24.03	110.731	18.513
56	2-10_14-54-42	55	2550.691	46.376	1.024	80.381	3.943	2.32	62.046	16.618	334.311	92.3	113.055	9.703
57	2-10_14-54-49	71	3359.524	47.317	1.348	67.053	4.413	2.481	76.728	14.45	334.413	120.897	113.902	8.351
58	2-10_14-54-56	91	2719.871	29.889	1.092	51.241	3.353	2.007	56.639	11.138	342.265	118.115	103.889	6.506
59	2-10_14-55-2	105	5637.437	53.69	2.263	49.68	4.533	2.719	64.062	11.45	359.767	112.53	104.736	6.76
60	2-10_14-55-9	53	5800.281	109.439	2.328	91.709	5.953	3.308	55.29	19.297	342.201	95.581	106.261	11.07
61	2-10_14-55-16	64	2527.564	39.493	1.014	69.476	3.408	2.008	61.652	14.268	382.519	120.383	112.357	8.297
62	2-10_14-55-23	52	2614.371	50.276	1.049	86.505	4.119	2.525	63.502	17.628	273.249	164.227	111.008	10.381
63	2-10_14-55-30	58	2580.155	44.485	1.036	76.591	3.729	2.315	61.469	15.715	363.125	84.391	112.846	9.261
64	2-10_14-55-37	52	3063.618	58.916	1.23	87.397	4.596	2.737	68.667	18.03	308.125	90.481	113.911	10.546
65	2-10_14-55-44	43	2581.106	60.026	1.036	102.78	4.63	2.547	73.098	20.75	366.935	75.162	108.608	11.873
66	2-10_14-55-51	37	2505.703	67.722	1.006	116.772	4.422	2.812	51.39	23.04	347.151	85.419	115.374	13.568
67	2-10_14-55-58	28	2411.292	86.118	0.968	152.105	5.214	3.222	70.604	29.717	350.303	66.579	106.02	17.433
68	2-10_14-56-5	36	3142.505	87.292	1.261	124.377	5.459	3.687	65.166	24.829	404.794	65.511	109.009	14.942
69	2-10_15-1-52	184	12126.477	65.905	4.867	47.687	8.306	4.622	76.858	12.924	285.002	167.449	101.161	7.472
70	2-10_15-1-59	188	12230.393	65.055	4.909	46.525	8.149	4.585	81.545	12.664	285.741	166.707	103.541	7.338
71	2-10_15-2-6	202	11787.483	58.354	4.731	43.691	8.003	4.521	80.806	12.24	348.965	167.77	101.637	7.11
72	2-10_15-2-13	187	11993.097	64.134	4.814	46.464	8.008	4.498	79.159	12.466	282.423	170.226	99.388	7.208