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DISINTEGRATION AND SOLUBILISATION OF ACTIVATED SLUDGE FROM RUBBER PROCESSING INDUSTRY BY OZONATION

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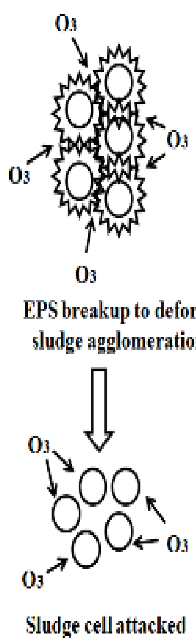
Graphical abstract



Abstract

The activated sludge system is an effective biological treatment applied widely in both industrial and municipal wastewater treatment facilities worldwide. Yet, the big issue arising from this process is the huge amount of sludge production at the end of the process. Thus, this study was aimed at studying the sludge disintegration and solubilisation efficiency of activated sludge from rubber glove processing industry using ozonation. The ozonation was conducted in batch process, and the sample was analyzed after 10, 20, 30, 40, 50 and 60 minutes of contact time. It was found that ozone was able to cause the bacteria in the activated sludge to undergo lysis, which solubilised the organic matter. The settling property was improved with the SVI reduced from 103 to 79 mL/g. Ozonation at 40 minute with ozone consumption of 22.7 $mgO_3/gTSS$ was able to reduce SS, FS, and VSS to 16.6%, 19%, and 14%, respectively. The SCOD solubilisation was achieved at a maximum of 71.8%, while the nitrogenous compound of NH_3-N was at 34% due to the release of organic matter from EPS and sludge cells after 60 minutes of treatment. A graph was plotted to evaluate the correlation between sludge disintegration and solubilisation, which showed a positive correlation (R^2=0.970). Thus, ozonation was found to be an efficient method to disintegrate and solubilise the activated sludge from rubber processing industry.

Keywords: Activated sludge, ozonation, disintegration, solubilisation, EPS

Abstrak

Sistem enapcemar teraktif adalah rawatan biologi yang efektif dan diaplikasi secara meluas dalam rawatan air sisa untuk industri dan perbandaran di seluruh dunia. Namun kemunculan isu yang besar iaitu penghasilan sisa enapcemar yang banyak di akhir proses ini. Oleh itu, kajian ini bertujuan mengkaji keberkesanan perpecahan dan pelarutan enapcemar teraktif oleh pengozonan menggunakan sampel dari kilang pemprosesan sarung tangan getah. Pengozonan telah dilakukan secara berasingan dan sampel pada minit ke 10, 20, 30, 40, 50, dan 60 minit telah dianalisis. Ozon didapati memecahkan enapcemar teraktif dan melarutkan bahan organik. Ciri mendapan telah diperbaiki dengan penurunan SVI daripada 103 kepada 79 mL/g. 40 minit pengozonan dengan penggunaan ozon sebanyak 22.7 $mgO_3/gTSS$ telah mengurangkan SS, FS dan VSS masing-masing sebanyak 16.6%, 19% dan 14%. Pelarutan SCOD telah mencapai maksimum 71.8% dan 34% untuk kompaun nitrogen akibat perlepasan bahan organic daripada EPS dan sel enapcemar selepas 60 minit rawatan. Sebuah graf telah diplot untuk mengkaji kaitan antara perpecahan dan pelarutan enapcemar, yang menunjukkan perkaitan positif (R^2=0.970). Pengozonan didapati telah menjadi cara yang efisien untuk memecah dan melarut enapcemar teraktif daripada kilang pemprosesan sarung tangan getah.

Kata kunci: Enapcemar teraktif, pengozonan, perpecahan, pelarutan, EPS

1.0 INTRODUCTION

The activated sludge process is a well-known biological pollutant removal method in wastewater treatment and thus become a conventional method in this area [1]. This method utilises bacteria as the 'agent of degradation' through suspended growth treatment and is applied worldwide [2]. Despite its efficiency in removing pollutant, this process has a major drawback. It produces excessive sludge, which can cost up to 65% of total cost in handling operation and plant maintenance [3]. Therefore, various techniques have been studied to overcome this matter. Pérez-Elvira and co-workers [4] have categorised sludge minimization strategy into three options: in the water line, in the sludge line or in the final waste line. Water line strategy can include floatation unit [5] and recycle line [6,7]. The sludge line strategy is conducted by reducing the final stream of sludge before further treatment. For example Tokumura *et al*. [8] and Kim *et al*. [9] utilised wasted sludge from secondary settling tank for their respective studies. Meanwhile, treatment in the final waste line is similar to a post-treatment to get a stable final sludge before it is disposed off. However, sludge minimization at the water line is more practical since the amount of sludge wasted can be controlled before it was produced. Therefore, numerous researchers have been looking into this matter using various treatments. Guo *et al*. [2] have categorised four types of possible treatments: uncoupling metabolism, worms' predation, lysis-cryptic growth, and the novel sludge reduction process.

The lysis-cryptic growth is a strategy that involves sludge cell lysis (lysis) that releases soluble organic materials, and the growth of new cell from the soluble substrate (cryptic). A part of the nutrients are utilised by the autochthonous cell for self-metabolism maintenance, and the other part is for the growth of new cells [10]. The damaged cell structure contributes to sludge reduction volume, and releases soluble organic product for further oxidation, and at a certain point, up to mineralization. However, lysis stage is limited-step, which decelerates further degradation step [10]. Various studies have conducted different methods to promote sludge lysis,such as potassium permanganate oxidation [11], ultrasonically [12; 13], thermally [14] and ozonation [15]. Ozonation was found to be the best water line sludge reduction method [4] and has captured the attention of researchers to overcome this issue. Muller [16] also agreed that ozonation is able to achieve a great degree of sludge disintegration. Mines *et al*. [17] and Tsuno and Nagare [18] have shown that ozonation promises good potential in sludge reduction, and there is always room for improvement towards better performance.

Sludge floc consists of the agglomeration of microbial aggregates bounded with extracellular polymeric substances (EPS). The EPS is a high molecular complex polymer that consists mainly of protein and carbohydrate [19]. It keeps the cells from dewatering [20], from harmful toxic substances [21], and it can also accelerate the formation of microbial aggregates [22]. The introduction of ozone can destroy the agglomeration, EPS, and cell wall, and thus solubilises the organic substances into the liquid phase. Zhang *et al*. [23] had successful reduced the sludge mixed liquor suspended solid (MLSS), and mixed liquor volatile suspended solid (MLVSS) to 13.5% and 12%, respectively with improved sewage sludge settleability. Meanwhile, the improvement in sludge solubilisation was up to 30%, which was achieved by Chu *et al*. [6] using bubble contactor ozonation. In addition, the ozonation was proved to be more efficient in total solid (TS) reduction compared to aerobic digestion of sludge [24].

Changes in sludge solubilisation can be used to observe the efficiency of the oxidation process. A reliable parameter to show solubilisation of organic matters from sludge cell is soluble COD (SCOD). Meanwhile, the total suspended solid (TSS) shows the amount of sludge cells that are present in the sludge supernatant. The reduction of TSS and increases of SCOD would indicate that the breakage of sludge cells and the release of organic substrate have occurred.

Although numerous studies have been conducted on sludge reduction, they were mostly focused on activated sludge from municipal treatment plants. Only a small number of studies have looked into industry activated sludge as their subject. Therefore, this study aims to evaluate the disintegration and solubilisation efficiency of sludge ozonation using activated sludge generated from a rubber gloves processing industry as subject sample.

2.0 METHODOLOGY

2.1 Sludge Collection

The sample was collected from return activated sludge (RAS) line of Shorubber (M) Sdn Bhd, a rubber glove processing industry in Arau, Perlis, Malaysia. The wastewater treatment plant has adopted the extended activated sludge process as part of their treatment. A high-density polyethylene (HDPE) bottle was washed with 0.1N nitric acid (HNO3), and rinsed with tap water for several times. The final rinsing was with distilled water before it was used to collect the sample from the return sludge line. The activated sludge sample was stored at 4 °C in a freezer until further usage. An analysis of the raw activated sludge sample showed that it contained 40.7±7.8 mg/L, 42.3±5.75 mg/L, and 1,100 mg/L of SCOD, NH3-N, and TCOD, respectively. The TSS and VSS were 9.212±0.172 and 5.65±0.189 g/L respectively. Initial pH of this sample was 7.31 with SVI of 103.5 mL/g.

2.2 Batch Ozonation

A batch ozonation experiment was carried out in a capped cylindrical glass reactor that measures 80 mm in diameter and 500 mm in height, with a working activated sludge volume of 1,200 mL. The activated sludge was allowed to mix to ensure homogeneity using a magnetic stirrer during the ozonation process. Purified oxygen (99%) at a flow rate of 2 L/min was flowed into the ozone reactor (A2Z Model Z-3G) with the output concentration set at 60%. Ozone flowed into the sludge reactor via a porous fritted glass diffuser, and off-ozone was trapped in a conical flask with 2% of potassium iodide. Ozonation was carried out for 10, 20, 30, 40, 50, and 60 minutes. After ozonation ended, the oxygen gas was kept flowing for 3 minutes to allow removal of any residue ozone along the tube, and inside the reactor. The ozonation process was carried out triplicated and the value present was the average.

2.3 Sample Analysis

Sludge settlability was measured by the sludge volume index (SVI). The disintegration of sludge floc was reflected by the total suspended solid (TSS), and volatile suspended solid (VSS). The released organic matter in the supernatant was measured by SCOD and NH3-N that indicated the breakage of cell walls. Ozone concentration was measured using the Iodometry Method.

TSS and VSS were measured according to the Standard Method [25]. Ozonated activated sludge sample was centrifuged in the IEC MicroCL 21R Centrifuge at 12 X 10,000 g relative centrifuge force (RCF) for 10 minutes at room temperature. The resulting clear supernatant was used to measure SCOD. The unfiltered sample was used to measure TCOD and NH3-N. The sample to measure COD was prepared and digested as per Closed Reflux, Colorimetric Method according to Standard Method. NH3-N was measured according to the Nessler Method. The concentration of COD and NH3-N were measured using HACH 2800 spectrophotometer. The pH of activated sludge was measured using a HI 2211 pH meter (HANNA Instrument). Ozone consumption (mgO_3/gSS) was calculated according to Nagare *et al.* [26] as in Equation 1. Sludge disintegration and solubilization efficiency (mg/L) were calculated according to Yan *et al*. [27] in Equation 2 and 3 respectively. SS_i and SS_t represent the initial and final SS concentration. $SCOD_t$ represents soluble COD at t time, $SCOD_i$ represents the initial SCOD, while $TCOD_i$ represents the initial total COD of the supernatant.

$$\text{Ozone consumption} = \frac{(O3in\text{-}O3out)\,(mg)}{SSi\left(\frac{g}{L}\right) X\, sludge\ volume\ (L)} \quad (1)$$

$$\text{Sludge disintegration} = \frac{SSi\text{-}SSt}{SSi} \quad (2)$$

$$\text{SCOD solubilisation} = \frac{SCODt\text{-}SCODi}{TCODi} \quad (3)$$

3.0 RESULTS AND DISCUSSION

3.1 Sludge Volume Index

Sludge flocs consist of microorganism cells bonded together with extracellular polymeric substances (EPS). Ozone attacking the sludge will cause the disintegration of the EPS and microbial cells, consequently. As a result, smaller particles are obtained. Then, the organic matters in the EPS and in the biomass are released into activated sludge liquid phase as solubilised material. Sludge volume index (SVI) is a parameter related to the disintegration and solubilisation of sludge where it can be see visually. The disintegrated suspended solid will settle at the bottom of the measuring cylinder. Meanwhile, the supernatant above it represents the amount of solubilised material. Figure 1 below shows the settled sludge to represent settleability. SVI is defined as the volume of settled sludge (mL) occupied by 1 g of dry solid after 30 minutes of settling time. Therefore, the decreasing value of SVI indicates the decreasing volume of sludge to be occupied by 1 g of dry solid due to disintegration. The decreasing volume of sludge will simultaneously increase the solubilisation of organic matters, and this will be discussed in the following Section 3.5.

Figure 1 The settleability of ozonated sludge floc from 10 to 60 minute (left to right)

Meanwhile, Figure 2 below shows the SVI values obtained in this study. Raw sludge has the SVI value of 103 mL/g, whereby this value then decreased with ozonation time. The lowest SVI value obtained from this experiment was 79 mL/g at 60 minutes of ozonation. As expected, the SVI value decreased with ozonation time. The SVI value also represents the sludge quality and settleability. A lower value indicates a compact suspended solid in the supernatant, and therefore, has good settling properties [28].

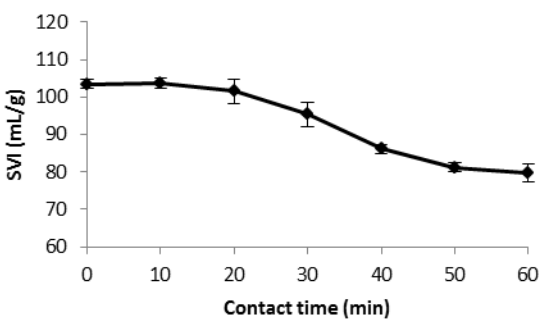


Figure 2 SVI value of ozonated sludge

The SVI values from this process in the range of 50 – 150 mL/g, which fitted with the suggested SVI value for typical sludge in an aeration basin. Within this range, the sludge has a good quality, which represented by its settleability. It is observed that a reduction in settled sludge volume for an ozonated sample was better than in a raw sample of municipal wastewater [24]. The reduction of SVI value from 160 mL/g to 60 mL/g also obtained in a continuous ozonation, which led to the settleability improvement of sludge [26].The reduction of SVI value indicate the changes in the sludge floc into rounder and more compact floc, which would improve its settling properties, and prevents the sludge from bulking[29]. Sludge with SVI value of less than 100 mL/g is good when it is dense and settles rapidly. The dense characteristic is contributed by the balance formation of filamentous organisms and flocs, which would end up in a large and strong floc structure, and clear supernatant [30].

3.2 Sludge Disintegration

The disintegration of suspended solid is a result of sludge ozonation into smaller particles. Figure 3 below shows the concentration of SS, VSS and FS with contact time after ozonation. The SS reduction was observed initially until 40 minutes of the process. At this point, the disintegration was 16.6%, and further ozonation time had only increased the efficiency for 1.2%. This could be due to the optimum ozone dose occured at 40 minutes (38.5 $mgO_3/gTSS$) and further ozonation was unable to disrupt the sludge anymore. This condition can be explained due to presence of hard-to-degrade sludge fraction [31], and increasing the contact time would not improve the efficiency. Zhang *et al*. [23] had also found that ozone dose of higher than 37.8 $mgO_3/gTSS$ was no longer able to cause cell disruption. The sludge disintegration can be related to the SVI value shown in Figure 2, which shows unchanged reduction after 40 minutes of ozonation process. At 40 minutes of ozonation, the ozone consumption for this experiment was 22.7 $mgO_3/gTSS$. This trend was supported by Saktaywin *et al*. [31], whereby found the disintegration of sludge decrease at higher ozone consumption because most of the oxidisable fraction has been reacted. The remaining hard-to-oxidize materials do not react with supplied ozone and therefore decrease the ozone consumption. Meanwhile, Demir and Filibeli [32] claimed that the decrease in the disintegration process may be due to the complexity in the sludge ozonation process. Other than the complexity of sludge matrix itself, the presence of high concentration of carbonate, which is typical in wastewater, reduced the disintegration efficiency [33]. This complexity may refer to the presence of radical scavenger such as lactic acid and sulphate ion from the microbial cells after ozonation at higher ozone dose [27].

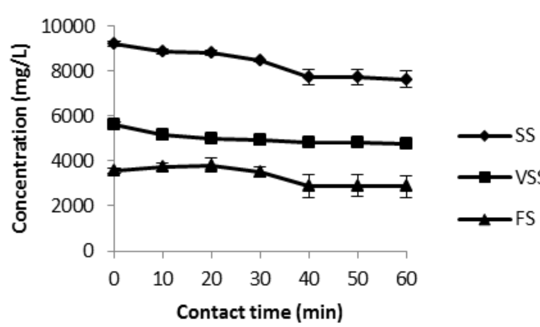


Figure 3 SS, VSS and FS disintegration at different contact time

The volatile suspended solid (VSS) represent the organic part, while the fixed solid (FS) is the inorganic portion in the sludge cells and EPS. Upon ozonation, these values tend to decrease due to their release in liquid phase, as shown in Figure 3. The decreased VSS and FS, from 5,650 mg/L to 4,850 mg/L and 3,562.7 mg/L to 2,889 mg/L, respectively at 40 minutes of ozonation, represent 14% and 19% of reduction, respectively. Further ozonation had shown only a small reduction of ±1%. During the ozonation process, the attached organic and inorganic components to the solid and EPS were separated by the powerful ozone, and hydroxyl radical reaction, thus dissolving these materials into liquid phase [23]. Little increment of VSS/TSS ratio was observed in this process (from 0.63 to 0.65), which is a normal phenomenon in ozonation to indicate solubilisation, as obtained by Zhang *et al*. [1] and Zhang *et al*. [23]. However, Tsuno and Nagare [18] had obtained low VSS/TSS ratio from a sewage treatment facility, which shows the sludge had low solubilisation property because of the high concentration of inorganic materials present.

3.3 SCOD Solubilisation

The amount of solubilised matter due to cell breakage, and damaged EPS can be represented by SCOD and NH3-N values, as shown in Figure 4 below. The solubilisation of organic matters gradually increased with ozonation time. It reached a maximum solubilisation of 71.8%, and then decreased by 4.6% at the end of the experiment. This solubilisation efficiency trend was the same as the trend for SS disintegration. Disintegration of activated sludge floc, follow by cell walls damage and thus release SCOD in the aqueous phase. Longer ozonation period allows the sludge to consume more ozone for disintegration and breakage, and therefore, more solubilisation product released. Tsuno and Nagare [18] agreed that the solubilisation increased with ozone consumption. The combination of soluble organic matter from cells and EPS resulted in a tremendous increase of SCOD concentration. The fall off solubilisation value at 50 minutes of ozonation showed that the easy-soluble organic matters had solubilised into supernatant, and left behind the hard-soluble organic matter [6].

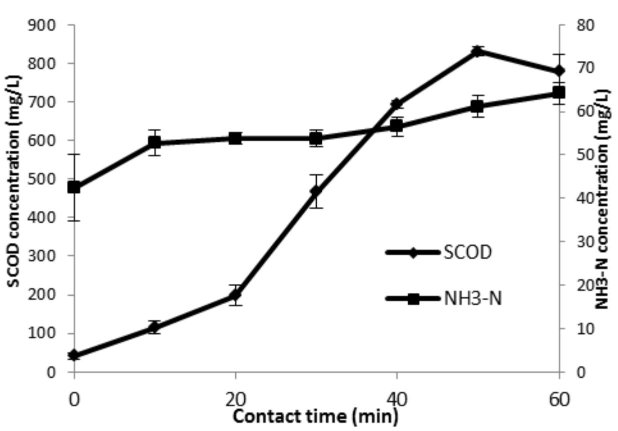


Figure 4 SCOD and NH3-N concentration after ozonation

The nitrogenous compound comes from protein and polysaccharide, mainly in the microbial floc, and EPS was measured by NH3-N concentration, as previously shown in Figure 4. A rapid increase in the early ozonation phase may be caused by the release of NH3-N from destruction of EPS and sludge cells. The solubilisation became slower after 10 minutes ofozonation may be caused by the released of nitrogenous organic from the cell itself when NH3-N from EPS has been released earlier. The solubilised NH3-N throughout the experiment was 34%.

The presence of soluble organic acids, such as acetic acid in the supernatant from microbial cell can act as hydroxyl radical scavenger [27]. This may be the reason why the SCOD and NH3-N solubilisation reduced at the end and slow throughout the process.

3.4 pH Changes

The activated sludge ozonation process also changed the pH of supernatant, and depicted in Figure 5 below. As ozone attacked the EPS, followed by the sludge cells, it broke the walls of these cells, which then released the organic matters included deoxyribonucleic acid (DNA) and other volatile fatty acids. This turned the supernatant a little acidic, therefore, decreased the pH of supernatant from 7.34 to 6.02 in this study. The oxidation of organics into lower molecular weight acids is also a reason for the sludge supernatant turning slightly acidic [1]. Xu *et al*. [34] and Song *et al*. [35] confirmed the present of volatile fatty acid (VFA) e.g. lactic acid, acetic acid, propionic acid and butyric acid in ozonated sludge supernatant. Such reduction also seen in the process conducted by Chu *et al*. [6] for sludge solubilisation using microbubble ozonation, Dytczak *et al.* [36] in improving the denitrification of activated sludge by ozonation and Zhang *et al.* [23] in the study of ozonated sewage sludge properties.

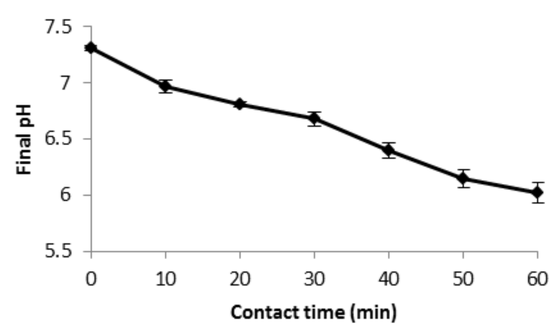


Figure 5 Changes of sludge pH after ozonation

3.5 Correlation of SS Disintegration and SCOD Solubilisation

Solubilisation of organic matter is a significant parameter to show the sludge flocs disintegration. Therefore, a linear graph with strong correlation coefficient (R^2 = 0.970) was obtained, indicating the SCOD solubilisation is directly proportional with SS disintegration, as shown in Figure 6. At lower than 5% sludge disintegration, the solubilisation reached 198.3 mg/L. The solubilisation was increased with SS percentage reduction. At the end of the process, the SCOD solubilisation increased for 95% when the SS disintegration was 17%. Cheng *et al.* [15] found a similar correlation when the SS disintegration efficiency of 12% led to the solubilisation of SCOD by 94% from 50 to 796 mg/L, at ozone dose of 77 mg O3/g TSS in 15 minutes of ozone treatment. Manterola *et al.* [37] confirmed that the organic matter solubilisation is proportional with TSS and VSS reduction efficiency.

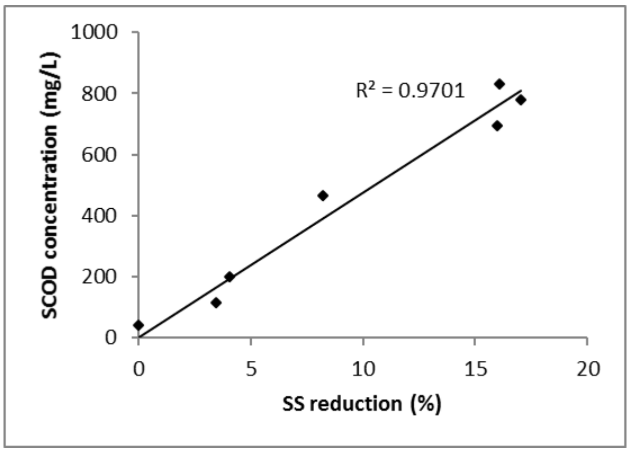


Figure 6 The effect of SCOD solubilisation on SS disintegration

3.6 Correlation of SCOD and NH3-N

The increasing concentration of SCOD and NH3-N were the result of the solubilisation of organic matter due to the attacked of the sludge cell. A power graph with R^2=0.8323 was obtained from the NH_3-N and SCOD solubilisation. The solubilisation of SCOD at less than 200 mg/L had induced NH3-N to rapidly solubilise to 53.7 mg/L, which was at 24.4% of solubilisation. The rapid increment in the early process may be caused by high composition of nitrogenous compound in the EPS. Slower increment of NH3-N concentration in further ozonation suggesting the supply ozone is unable to reach the very inner cell structure containing DNA. Only after 50 minutes of ozonation(47 mgO_3/gTSS), an increment of NH3-N was observed indicating the DNA of cell was destroyed by ozone. Zhang *et al*. [23] confirmed the slow NH3-N solubilisation rate at ozone dose lower than 6.3 mgO_3/gTSS is because ozone has not reached the cells yet. The dose to reach cell is rather low compare to this study because of different sludge sample. Industrial activated sludge (this study) consists of recalcitrant compound and bacteria thus, require higher ozone concentration to react with.

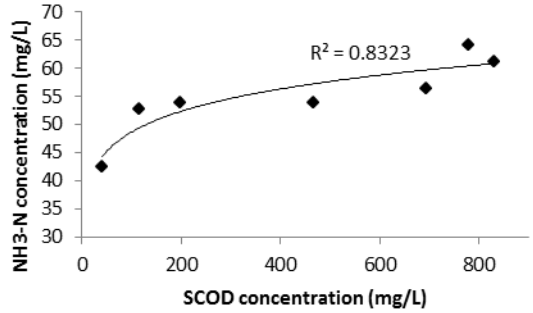


Figure 7 Correlation of NH3-N solubilisation with SCOD solubilisation during ozonation

4.0 CONCLUSION

Sludge ozonation using return activated sludge from a rubber processing industry was studied to evaluate the performance in sludge disintegration and solubilisation. The ozonation was improved the settling properties of sludge, as the SVI reduced from 103 to 79 mL/g. 40 minutes was suggested to be the optimum time for ozonation as it reduced the sludge volume, destructed the organic and inorganic solid the most. At this time, the organic matter solubilisation still increased and reached maximum of 71.8%. Nitrogenous compound (NH_3-N) released from EPS and sludge cell increased up to 34% at the end of treatment. Rapid solubilisation of NH_3-N at initial treatment was due to the release altogether from EPS and cell. As time increased, all NH_3-N from EPS has been released and the further increment was due to solubilisation from the cells. The solubilisation of organic matter also turned the liquid phase into a little acidic due to the present of volatile fatty acid and low molecular weight of acid due to sludge disintegration. Sludge disintegration has positive effect on solubilisation efficiency and confirms the solubilisation of activated sludge from rubber processing industry was due to sludge disintegration.

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References

[1] Zhang, G., Yang, G., Liu, H., and Zhang, J. 2009. Sludge Ozonation: Disintegration, Supernatant Changes and Mechanisms. *Bioresour. Technol.* 100: 1505-1509.

[2] Guo, W. Q., Yang, S. S., Xiang, W. S., Wang, X. J, and Ren, N. Q. 2013. Minimization of Excess Sludge Production by In-Situ Activated Sludge Treatment Processes-A Comprehensive Review. *Biotechnol. Adv.* 31: 1386-1396.

[3] Y. Liu, Y. 2003 Chemically Reduced Excess Sludge Production in the Activated Sludge Process. *Chemosphere*. 50: 1-7.

[4] Pérez-Elvira, S. I., Nieto Diez, P., and Fdz-Polanco, F. 2006. Sludge Minimisation Technologies. *Rev. Environ. Sci. Biotechnol*. 5: 375-398.

[5] Campos, J. L., Otero, L., Franco, A., Mosquera-Corral, A., and Roca, E. 2009. Ozonation Strategies to Reduce Sludge Production of a Seafood Industry WWTP. *Bioresour. Technol.* 100(3): 1069-1073,

[6] Chu, L. B., Yan, S. T., Xing, X. H., Yu, A. F., Sun, X. L., and Jurcik, B. 2008. Enhanced Sludge Solubilization by Microbubble Ozonation. *Chemosphere*. 72: 205-212.

[7] Moussavi, G., Asilian, H., and Jamal, A. 2008. Effect of Ozonation on Reduction of Volume and Mass of Waste Activated Sludge. *J. Appl. Sci.* 4(2): 122-127.

[8] Tokumura, M., Sekine, M., Yoshinari M., Znad, H. T.,and Kawase, Y. 2007. Photo-Fenton Process for Excess Sludge Disintegration. *Process Biochem*. 42: 627-633.

[9] Kim, T. H., Lee, S. R., Nam, Y. K., Yang, J., Park, C., and Lee, M. 2009. Disintegration of Excess Activated Sludge by Hydrogen Peroxide Oxidation. *Desalination.* 246(1-3): 275-284.

[10] Chu, L., Wang, J., Wang, B., Xing, X, Yan, S., Sun, X., and Jurcik, B. 2009. Changes in Biomass Activity and Characteristics of Activated Sludge Exposed to Low Ozone Dose. *Chemosphere.* 77: 269-272.

[11] Wu, C., Zhang, G., Zhang, P., and Chang, C. C. 2014. Disintegration of Excess Activated Sludge with Potassium Permanganate: Feasibility, Mechanisms and Parameter Optimization. *Chem. Eng. J.* 240: 420-425.

[12] Zhang, P., Zhang, G., and Wang, W. 2007. Ultrasonic Treatment of Biological Sludge: Floc Disintegration, Cell Lysis and Inactivation. *Bioresour. Technol*. 98(1): 207-210.

[13] Tiehm, A., Nickel, K., Zellhorn, M., and Neis, U. 2001. Ultrasonic Waste Activated Sludge Disintegration for Improving Anaerobic Stabilization. *Water Res.* 35(8): 2003-2009.

[14] Appels, L., Degreve, J., Van der Bruggen, B., Van Impe, J., and Dewil, R. 2010. Influence of Low Temperature Thermal Pre-treatment in Sludge Solubilisation, Heavy Metal Release and Anaerobic Digestion. *Bioresour. Technol*. 101: 5743-5748.

[15] Cheng, C. J., Andy Hong, P. K., and Lin, C. F. 2012. Improved Solubilization of Activated Sludge by Ozonation in Pressure Cycles. *Chemosphere.* 87(6): 637-643.

[16] Muller, J., A. 2000. Pretreatment Process for the Recycling and Reuse of Sewage Sludge. *Water Sci. Technlogy.* 42(9): 167-173.

[17] Richard O. Mines, J., Lackey, L. W., and Tribble, D. 2008. Bench-Scale Ozonation of Waste Activated Sludge. *World Environmental and Water Resources Congress 2008 Ahupua'a.* 2002. 1–8.

[18] Tsuno H. and Nagare, H. 2006. Reduction of Excess Sludge in Sewage Treatment by Ozone Application. *International Conference Ozone and UV*. 2006. 35-40.

[19] Sheng, G. P., Yu, H. Q., and Li, X. Y. 2010. Extracellular Polymeric Substances (EPS) of Microbial Aggregates in Biological Wastewater Treatment Systems: A Review. *Biotechnol. Adv.* 28: 882-894.

[20] Wingender, J., Neu, T. R., and Flemming, H. C. 1999. *What are Bacterial Extrcellular Polymeric Substances? Microbial Extracellular Polymeric Substances: Characterization, Structures and Function*. Springer-Verlag Berlin Heidelberg. 1-18.

[21] Sutherland, I. W. 2001. Biofilm Exopolysaccharides: A Strong and Sticky Framework. *Microbiology*. 147: 3-9.

[22] Liu, Y., Liu, Y., and Tay, J. 2004. The Effects of Extracellular Polymeric Substances on the Formation and Stability of Biogranules. *Appl. Microbiol. Biotechnol.* 65: 143-148.

[23] Zhang, J., Zhang, J., Tian, Y., Li, N., Kong, L., Sun, L., Yu, M., and Zuo, W. 2016. Changes of Physicochemical Properties of Sewage Sludge during Ozonation Treatment: Correlation to Sludge Dewaterability. *Chem. Eng. J.* 301: 238-248.

[24] Mines, R. O., Northenor, C. B., and Murchison, M. 2008. Oxidation and Ozonation of Waste Activated Sludge. *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng*. 43(6): 610-618.

[25] APHA. 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st ed. Washington, DC: American Public Health Association.

[26] Nagare, H., Tsuno, H., Saktaywin, W., and Soyama, T. 2008. Sludge Ozonation and its Application to a New Advanced Wastewater Treatment Process with Sludge Disintegration. *Ozone Sci. Eng*. 30(2): 136-144.

[27] Yan, S. T., Chu, L. B., Xing, S. H., Yu, A. F., Sun, X. L., and Jurcik, B. 2009. Analysis of the Mechanism of Sludge Ozonation by a Combination of Biological and Chemical Approaches. *Water Res.* 43(1): 195-203.

[28] n.a. Activated sludge process. [Online]. Available: http://web.deu.edu.tr/atiksu/ana52/acti4.html. [Accessed: 12-Oct-2016].

[29] Wolff, S. and Hurren, D. 2006. Reduction of Excess Sludge by Up to 80% with Ozone Injection-practical Experience. *11th European Biosolids and Organic Resources Conference, 2006.*

[30] Grady Jr. L., Daigger, C. P. T., Love, N. G. G., and M. Filipe, C. D. M. 2011. *Design and Evaluation Suspended. Biological Wastewater Treatment*. Third Edition: CRC Press, 2011: 393-394.

[31] Saktaywin, W., Tsuno, H., Nagare, H., Soyama, T, and Weerapakkaroon, J. 2005. Advanced Sewage Treatment Process with Excess Sludge Reduction and Phosphorus Recovery. *Water Res*. 39: 902-910.

[32] Demir, O. and Filibeli, A. 2012. Fate of Return Activated Sludge after Ozonation: An Optimization Study for Sludge Disintegration. *Environ. Technol.* 33(16-18): 1869-1878.

[33] Rizzo, L., Meric, S., Guida, M., Kassinos, D., & Belgiorno, V. 2009. Heterogenous Photocatalytic Degradation Kinetics and Detoxification of an Urban Wastewater Treatment Plant Effluent Contaminated with Pharmaceuticals. *Water Research.* 43: 4070-4078.

[34] Xu, G., Chen, S., Shi, J., Wang, S., and Zhu, G. 2010. Combination Treatment of Ultrasound and Ozone for Improving Solubilization and Anaerobic Biodegradability of Waste Activated Sludge. *J. Hazard. Mater.* 180: 340-346.

[35] Song, K. G., Choung, Y. K., Ahn, K. H., Choo, J. and Yun, H. 2003. Performance of Membrane Bioreactor System with Sludge Ozonation Process for Minimization of Excess Sludge Production. *Desalination*. 157: 353-359.

[36] Dytczak, M. A., Londry, K. L., Siegrist, H. and Oleszkiewicz, J. A. 2007. Ozonation Reduces Sludge Production and Improves Denitrification. *Water Res.* 41(3): 543-550.

[37] Manterola, G., Uriarte, I. and Sancho, L. 2008. The Effect of Operational Parameters of the Process of Sludge Ozonation on the Solubilisation of Organic and Nitrogenous Compounds. *Water Res.* 42: 3191-3197.