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Vol. 9, No. 2, November 2018

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FOCUS AND SCOPE

Jurnal Riset Teknologi Pencegahan Pencemaran Industri (Research Journal of Industrial Pollution Prevention Technology) seeks to promote and disseminate original research as well as review, related to following area:

Environmental Technology : within the area of air pollution technology, wastewater treatment technology, and management of solid waste and harzardous toxic substance.

Process Technology and Simulation : technology and/or simulation in industrial production process aims to minimize waste and environmental degradation.

Design Engineering : device engineering to improve process efficiency, measurement accuracy and to detect pollutant.

Material Fabrication : environmental friendly material fabrication as subtitution material for industry.

Energy Conservation : process engineering/ technology/ conservation of resources for energy generation.

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MAILING ADDRESS

Center of Industrial Pollution Prevention Technology.
Jl. Ki Mangunsarkoro No. 6 Semarang, Jawa Tengah, 50136 Indonesia.
Telp. +62 24 8316315
Fax. +62 24 8414811
e-mail: jurnalrisettppi@kemenperin.go.id
Jam kerja : Senin – Jum'at
07.30 – 16.00 GMT+7

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PREFACE

Alhamdulillah Robbie 'Alamin, Journal of Industrial Pollution Prevention Technology (JRTPPI) again will publish scientific articles, especially in the field of environmental technology for volume 9 no 2. Our high appreciation is directed to the authors, editorial board, structural officials of BBTPPI who have actively participated so as to maintain consistency of quality and punctuality of our periodic publications.

This edition of the issue is continuously from previous editions, where all the articles are published in full-text English. This change is an attempt of the editorial board to improve the author's performance in delivering the results of their researches. Articles in full-text English are more likely to be read by broader audience so that it will increase the number of citations. This policy is also applied in order to actualize our hope of being a globally indexed international journal.

The articles contained in this edition consist of three important groups, namely: the biological treatment for energy production and wastewater, advanced process technology and biotransformation studies of pollutans. The six manuscripts accepted and published in this edition are from LIPI, BBTPPI, and University. The duration of submission, review, and editing of the manuscripts ranged from 4-7 months.

Hopefully, these scientific articles may be new source of knowledge and experience for readers from academic, researcher, industry, and society at large. We realize that nothing is perfect until the improvement of all parties involved is continuously done.

Semarang, November 2018

Chief Editor

Jurnal Riset
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DAFTAR ISI

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ABSTRACT

Published on 26 November 2018

Dwi Agustiyani Muslichah, Maman Rahmansyah (Research Center for Biology, Indonesian Institute of Sciences Cibinong Science Center, West Java, Indonesia)

Initial study of thiocyanate microbial degradation by isolates from polluted soil in gold mining area in Indonesia

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2018, Vol. 9, No. 2, p. 1-10, 3 ill, 3 tab, 25 ref

This study was conducted to clarify the ability of denitrifying bacterial group utilized nitrogen (N) due to their ability to decompose N in thiocyanate structure. Thiocyanate is a chemical substance that categorized as a pollutant in the environment, this chemical mainly generated by some industrial activities. Denitrifying bacterial group obtained from bulk of sludge samples collected from the gold tailing, and some soil samples collected around the gold mining site. The samples were taken to the Microbiology Laboratory, Research Center for Biology, to be investigated. Samples were initially acclimatized by potassium nitrate (KNO_3), acetonitrile, and liquid waste or sludge. The result showed that denitrifying bacteria in the samples could utilize 60 to 90% of NO_3-N (nitrate) in 42 days incubation. Isolation process were then conducted in each samples, and four denitrification bacterial, named as AN, Ea, L7T5, and PETI-7 isolates were obtained. The isolates formerly were cultured in a denitrifying bacterial medium containing KSCN (Potassium Thiocyanate), amended with glucose and sodium acetate as carbon source. Those four isolates performed satisfactory in aerobic and anaerobic cultures medium for denitrifying process, and utilizing glucose and sodium acetate as co-carbon source, but all bacterial isolates were unable to use thiocyanate as a single carbon source. Thiocyanate degradation performed by the isolates through a simultaneous conversion along with denitrification process. This phenomenon turn to open the opportunity on role of application denitrifying bacteria become bioresources material in efforts to decompose thiocyanate.

(Author)

Keywords: denitrifying bacteria, degradation, mining waste, thiocyanate

Aris Mukimin, Kukuh Aryo Wicaksono, Nur Zen, Agus Purwanto, Hanny Vistanty (Center of Industrial Pollution Prevention Technology, Semarang)

Implementation of electrocatalytic reactor as oxidation unit for residual reagent wastewater of testing laboratory

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2018, Vol. 9, No. 2, p. 11-20, 8 ill, 26 ref

The remaining reagent from the sample analysis process become a significant source of hazardous waste of laboratory tasting activities. Methylene blue, phenol and oil are pollutants common in the residual reagent waste. The electrocatalytic reactor is effective oxidation units for these organic pollutants. The reactor was made for a 50 L capacity with cylindrical metal oxide as the anode. The three anodes which 6 cm in diameter and 50 cm in length were paired stainless cathode with a distance of 2.5 cm. The reactor was also equipped with a stirrer that is connected to the motor so that the mass transfer and oxidizing agents is more effective. The reactor application was carried out by feeding the residual reagent waste into the electrocatalytic unit and giving DC potential 5 Volt. Each COD content for reagent waste of detergent: 2864 mg/L, phenol: 838 mg/L and oil: 708 mg/L. The reactor has reduced COD to 2157 mg/L (detergent), 399 mg/L (phenol) and 506 mg/L (oil) for 120 minutes. The high COD content in residual is caused by solvent (chloroform or hexane) that used at extraction step in determining the process of a sample. This compound is tough to oxidize into CO2 by OH radical or hypochlorite acid formed at the anode during the electrolysis process.

(Author)

Keywords: hazardous, waste reagent laboratory, electrocatalytic, phenol, methylene blue, oil

Pujiastuti Peni, Narimo, Roesleini J. Putri (Faculty of Engineering Setia Budi University, Surakarta, Central Java, Indonesia)

Biotransformation studies of agricultural nitrogen pollutans in Keduang watershed

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2018, Vol. 9, No. 2, p. 21-29, 8 ill, 2 tab, 27 ref

The present study seeks to examine nitrogen biotransformation of agricultural wastewater carried out by nitrosomonas and nitrobacter

into Ammonia ($N\text{-}NH_3$), Nitrite ($N\text{-}NO_2$), and Nitrate ($N\text{-}NO_3$) in Keduang watershed. Natural capability of the bacteria is necessary to find out to monitor assimilative capacity of the waterbody towards pollutants. Grab sampling technique was applied in agricultural land and Keduang watershed in reference to Indonesian National Standard (SNI) 6989.59:2008. Meanwhile, analysis of $N\text{-}NO_2$ was based on Indonesian National Standard (SNI) 06-6989.09-2004, $N\text{-}NO_3$ on SNI 6989.79-2011, and $N\text{-}NH_3$ on SNI 06-6989.30-2005. The nitrosomonas and nitrobacter were isolated and identified on NA medium considering methods of Capuccino and Sherman (2005). Afterwards, characterization of colony morphology variants was determined, and both gram stain and biochemical test were conducted. A number of 48.8 nitrosomonas colonies/100 mL were identified in samples of agricultural wastewater, which enable to transform Ammonia ($N\text{-}NH_3$) of 0.1390 mg/L into Nitrite ($N\text{-}NO_2$) of 0.0632 mg/L. Meanwhile, a number of 330 nitrobacter colonies/ 100 mL are capable of transforming Nitrite ($N\text{-}NO_2$) into Nitrate ($N\text{-}NO_3$) of 0.2168 mg/L. In conclusion, there is a positive relationship between nitrosomonas in transforming Ammonia into Nitrite and nitrobacter in converting Nitrite into Nitrate. Nitrogen pollutants of the agricultural wastewater in Keduang watershed are able to be reduced by both nitrosomonas and nitrobacter.

(Author)

Keywords: agricultural wastewater, biotransformation, nitrogen, nitrobacter, nitrosomonas

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Rame, Nilawati, Silvy Djayanti, Novarina Irnaning Handayani, Agus Purwanto, Lisa Ruliaty, Ganang Dwi Harjanto (Center of Industrial Pollution Prevention Technology, Semarang)

Efficient cell-wall disruption of microalgae Chlorella Vulgaris in water by catalytic ozonation over microporous carbon-supported titanium oxide

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2018, Vol. 9, No. 1, p. 30-36, 4 ill, 29 ref

Microalgae *Chlorella vulgaris* are industrially important microorganisms that have been studied for producing valuable bioproducts such as feed, food, cosmetics and pharmacy industries. The cell-walls of *Chlorella vulgaris* probably represent the biggest barrier to target bioproduct extraction. Many cell-wall disruption methods have been reported for microalgae in order to maximize the extraction efficiencies. However, but there has been no industrial scale application related to the high costs and electrical energy. This study investigated several parameters for cell-wall disruption from microalgae *Chlorella vulgaris* during catalytic ozonation over microporous carbon-supported titanium oxide, including flow ozone, catalytic time and reactor capacity. At the same time, the cell-wall disruption yield and an active compound yield such as chlorophyll and carotenoid were evaluated for each pretreatment. Pretreatment with 1 minute at 1 liter per minute in 2 liters produced chlorophyll yield by approximately 59.45% and the carotenoid was reduced to 98.18%. Carbon-supported titanium oxide reduces the required O_3 dose and catalytic time for cell-wall disruption, although the chlorophyll yield does not exceed 75.67%. Catalytic ozonation at 1 minute at 4 liters per minute produced 76.47% cell-wall disruption of *Chlorella vulgaris*, chlorophyll 56.75% and carotenoid 89.09%.

(Author)

Keywords: cell-wall disruption, Chlorella, catalytic ozonation

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Nani Harihastuti, Rame, Silvy Djayanti (Center of Industrial Pollution Prevention Technology, Semarang)

High performance of enzymatic bioprocess for production of biomass-based bioethanol of sago palm fiber waste

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2018, Vol. 9, No. 2, p. 37-45, 4 ill, 5 tab, 20 ref

Biomass waste in the form of fiber dregs contains many components of lignocellulose and hemicellulose. Lignocellulose can be used to produce ethanol through enzymatic biotechnology processes. Sago palm fiber industry is one potential industry producing biomass waste in the form of solid waste of fiber dregs (about 30% from the weight of processed raw materials). Solid fiber waste contains crude fiber and lignocellulose compound consists of cellulose (35-50%), hemicellulose (20-35%) and lignin (12-20%). This study aimed to utilize solid waste of sago palm fiber as a raw material of bioethanol production through enzymatic biotechnology processes of delignification, saccharification and fermentation which was then purified by distillation process to get ethanol. Delignification, saccharification, and fermentation stages are conducted using Phanerochaete chrysosporus Mushroom, Trichoderma viride fungus, and saccharomyces cerevisiae, respectively and then purified by distillation process (one level) to produce ethanol. The process of saccharification and fermentation process were done in an integrated manner (addition of Trichoderma viride and yeast saccharomyces cereviceae fungi done simultaneously). The weight of raw materials of solid waste fiber treated was about 6 kgs. Alcohol content obtained was about ± 4% (distillation). No water, air, and soil pollution inflicted, more added value, and sustainable were the main benefits of biotech process or bioprocess.

(Author)

Keywords: high performance, bio-enzymatic, sago palm fiber waste, bioethanol

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Rustiana Yuliasni, Nanik Indah S, Kukuh Aryo W, Nani Harihastuti (Center of Industrial Pollution Prevention Technology, Semarang)

Influence of operational condition on the performance of halotolerant enriched - activated sludge system for treating medium salinity roasted peanut wastewater

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2018, Vol. 9, No. 2, p. 46-54, 7 ill, 1 tab, 18 ref

iomass waste in the form of fiber dregs contains many components of lignocellulose and hemicellulose. Lignocellulose can be used to produce ethanol through enzymatic biotechnology processes. Sago palm fiber

industry is one potential industry producing biomass waste in the form of solid waste of fiber dregs (about 30% from the weight of processed raw materials). Solid fiber waste contains crude fiber and lignocellulose compound consists of cellulose (35-50%), hemicellulose (20-35%) and lignin (12-20%). This study aimed to utilize solid waste of sago palm fiber as a raw material of bioethanol production through enzymatic biotechnology processes of delignification, saccharification and fermentation which was then purified by distillation process to get ethanol. Delignification, saccharification, and fermentation stages are conducted using Phanerochaete chrysosporus Mushroom, Trichoderma viride fungus, and saccharomyces cerevisiae, respectively and then purified by distillation process (one level) to produce ethanol. The process of saccharification and fermentation process were done in an integrated manner (addition of Trichoderma viride and yeast saccharomyces cereviceae fungi done simultaneously). The weight of raw materials of solid waste fiber treated was about 6 kgs. Alcohol content obtained was about ± 4% (distillation). No water, air, and soil pollution inflicted, more added value, and sustainable were the main benefits of biotech process or bioprocess.

(Author)

Keywords: halotolerant, activated sludge, medium salinity wastewater, peanut roasted wastewater, operational condition

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Efficient cell-wall disruption of microalgae *Chlorella Vulgaris* in water by catalytic ozonation over microporous carbon-supported titanium oxide

Rame[1]*, Nilawati[1], Silvy Djayanti[1], Novarina Irnaning Handayani[1], Agus Purwanto[1], Lisa Ruliaty[2] and Ganang Dwi Harjanto[3]

[1]*Center of Industrial Pollution Prevention Technology, Semarang, Jl. Ki Mangunsarkoro No.6, Karangkidul, Semarang Tengah, Kota Semarang, Jawa Tengah, Indonesia 50136*

[2]*BBPBAP Jepara, Jl. Jalan Cik Lanang, Bulu, Jepara, Kabupaten Jepara, Jawa Tengah, Indonesia 59401*

[3]*Neoalgae Indonesia Makmur, Jl. Slamet Riyadi No. 20, Kabupaten Sukoharjo, Jawa Tengah, Indonesia 57562*

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A B S T R A C T

Microalgae *Chlorella vulgaris* are industrially important microorganisms that have been studied for producing valuable bioproducts such as feed, food, cosmetics and pharmacy industries. The cell-walls of *Chlorella vulgaris* probably represent the biggest barrier to target bioproduct extraction. Many cell-wall disruption methods have been reported for microalgae in order to maximize the extraction efficiencies. However, but there has been no industrial scale application related to the high costs and electrical energy. This study investigated several parameters for cell-wall disruption from microalgae *Chlorella vulgaris* during catalytic ozonation over microporous carbon-supported titanium oxide, including flow ozone, catalytic time and reactor capacity. At the same time, the cell-wall disruption yield and an active compound yield such as chlorophyll and carotenoid were evaluated for each pretreatment. Pretreatment with 1 minute at 1 liter per minute in 2 liters produced chlorophyll yield by approximately 59.45% and the carotenoid was reduced to 98.18%. Carbon-supported titanium oxide reduces the required O_3 dose and catalytic time for cell-wall disruption, although the chlorophyll yield does not exceed 75.67%. Catalytic ozonation at 1 minute at 4 liters per minute produced 76.47% cell-wall disruption of *Chlorella vulgaris*, chlorophyll 56.75% and carotenoid 89.09%.

1. INTRODUCTION

Microalgae is a unique resource of health and nutrient food that cannot be isolated by common technology, it requires specific technology. Unfortunately, from the high destruction of the bioactive compound of microalgae to its intensive use common technology, efficient cell-wall disruption of microalgae is subject to ever-increasing pressures that affect the bioactive quality and efficiency of the process. For over many years research has been devoted to improve bioactive quality/quantity by developing efficient cell-wall disruption of microalgae for all applications involving homogenization (Cheng et al., 2013), ultrasonication (Ciudad et al., 2013), microwave (Daly et al., 2011), solvent (Dong et al., 2015), acid/base (Kim et al., 2015), fenton chemical (Siew et al., 2008), hydrolytic enzyme (Taskova et al., 2006) and supercritical CO_2 ($ScCO_2$) (Wang et al., 2015). Current common technology in cell-wall disruption of microalgae struggles to minimize bioactive destruction and efficiency of the process.

Advanced oxidation processes (AOP), base to their high oxidation potentials, have great potential to be the most efficient solution for the cell-wall disruption of

*Correspondence author. Tel.: +624 8316315
E-mail: rame@kemenperrin.go.id (Rame)

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microalgae. AOP is aqueous phase oxidation methods consisting of highly reactive species used in the oxidative cell-wall disruption of microalgae. AOP creates a more powerful and less selective secondary oxidant, hydroxyl radicals, in the water (Gottschalk et al., 2000; Li et al., 2010; Zaikov and Rakovsky, 2009). The secondary oxidant can cause the oxidation of most cell-wall organic compounds until they are mineralized as carbon dioxide and water. The hydroxyl radical (2.87 eV) has much higher oxidation potential than ozone (2.07 eV) or hydrogen peroxide (1.78 eV) and usually reacts faster, thus leading to smaller contact time and carbon footprint (Barsanti and Gualtieri, 2018; Gracia et al., 2000; Guo et al., 2012; Posten and Chen, 2016). Common AOP systems use or combine ozone, UV, hydrogen peroxide to create hydroxyl radicals. The high cost and potential for contamination due to the use of UV, hydrogen peroxide in the AOP process to produce hydroxyl radicals must be avoided (Rame et al., 2017).

Catalytic ozonation has been demonstrated to be an effective and efficient advanced oxidation processes used in industry and wastewater treatment for removal of colour (Rame et al., 2017), gaseous pollution (Mastan et al., 2012), pesticides, pharmaceuticals (Rame et al., 2018) and pathogen (Wu et al., 2016). However, its usage for microalgae production is limited by the fast release of the hydroxyl radical (•OH) if natural microalgae concentrations are not significant.

State of the art AOP systems bases catalytic ozonation developed by this paper is on the use of microporous carbon-supported titanium oxide and ozone to create hydroxyl radicals, the ultimate oxidant for cell-wall disruption of microalgae. Ozone is produced when oxygen molecules (O_2) are split into atomic oxygen (O) then recombined into ozone (O_3)(Gottschalk et al., 2000; O'Donnell et al., 2012; Zaikov and Rakovsky, 2009). In this research, ozone is produced when a gas containing oxygen is passed through an electrical field separated by two electrodes. When oxygen molecules in the gas interact with the electrical field, they split and recombine forming ozone. This process is the corona discharge ozone generation method. Catalytic ozonation system uses microporous carbon-supported titanium oxide to produce hydroxyl radicals from ozone in water, making it the most effective method for hydroxyl radicals production in industrial applications.

This study investigated several parameters for cell-wall disruption from microalgae *Chlorella vulgaris* during catalytic ozonation over microporous carbon-supported titanium oxide, including flow ozone, catalytic time and reactor capacity. To assess whether the parameter changes the effectiveness of the cell-wall disruption, at the same time, the cell-wall disruption yield and an active compound yield such as chlorophyll and carotenoid were evaluated for each pretreatment. The microalgae *Chlorella vulgaris* was chosen because it is the second largest microalgae used after *Spirulina*.

2. METHODS

2.1. Materials

Chlorella vulgaris was obtained from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. Briefly, Chlorella vulgaris were cultivated with sea water on the open pound (Jepara). Chlorella vulgaris were fed daily with nutrient and aeration periodically. Chlorella vulgaris used in the catalytic ozonation experiment was harvested after 6 days of cultivation.

2.2. Experiments

The experimental set-up for the ozonation was based on a 10 g/h ozone generator from BUMA, Semarang, Indonesia, which was supplied with dry oxygen gas. A diffuser was used to disperse the generated O_3 into a collection bottle.

The experimental design used a central composite design (four levels) with three variables: flow ozone, catalytic time and reactor capacity. The flow ozone set at three different levels: 1, 2, 3, 4 liters per minute, catalytic time of 1,2,3,4 minutes and reactor capacity 500 mL, 2000 mL, 10000 mL.

In this study catalytic ozonation of microporous carbon-supported titanium oxide were used to determine the effect of approach on bioactive destruction and cell-wall disruption of microalgae *Chlorella vulgaris*.

2.3. Analysis

2.3.1. Quantification of ozone concentration

The output O_3 concentration of the ozone generator was measured with a UV-Vis benchtop at λ 260 nm in a quartz cuvette. Specific volumes of the O_3 stock solution were added in each batch bottle to give the desired O_3 concentration. Ozone gas flow is regulated by flow meter. Ozone gas flows for 30 seconds with an ozone flow of 1 liter per minute in each batch bottle.The actual concentration O_3 gas dissolved in water was quantified with the titrimetric method. The O_3 concentration was analyzed by two different methods. Spectroscopy analysis was performed by benchtop O_3 meter for gas phase, while the titrimetric method was used for quantitative analysis for aqueous phase (Gottschalk et al., 2000; Zaikov and Rakovsky, 2009).

2.3.2. Cell-wall disruption

We performed spectroscopy analysis using scanning electron microscope (SEM) and transmission electron microscopy (TEM) to obtain data of cell-wall disruption microalgae *Chlorella vulgaris*.

2.3.3. Bioactive analysis

Determination of bioactive concentration was performed via spectroscopy UV/Vis, which was measured in a UV/Vis detector (based on the method of Tsaloglou, 2016). The carotenoid was detected by the detector at λ 452 nm, chlorophyll was detected by the detector at λ 663 nm and a specific pattern was detected by the detector at λ 490 nm (Cuellar-bermudez et al., 2014; Posten and Chen, 2016; Tsaloglou, 2016).

3. RESULT AND DISCUSSION

Spectroscopy UV/Vis analysis was performed to find out the bioactive concentration from *Chlorella vulgaris*. Analysis visible spectroscopy was done for calculation of chlorophyll concentration, specific pattern and carotenoids, also cell-wall disruption level. The results of the cell-wall disruption of microalgae *Chlorella vulgaris* using spectroscopy UV/Vis is shown in **Figure 1** and **Figure 2**.

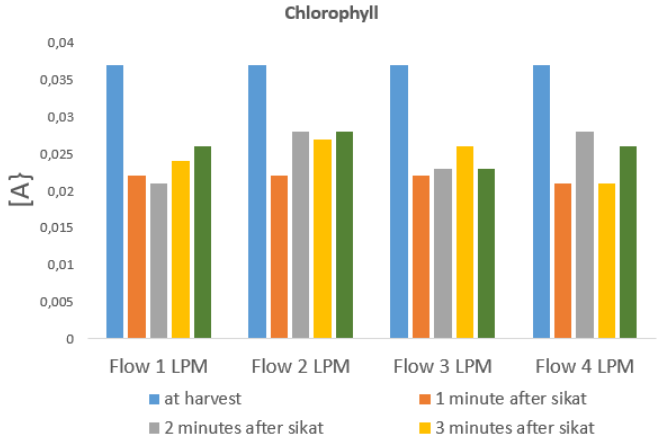

Figure 1. Data characterization of chlorophyll of the Chlorella from the ozonation catalytic from minute 0 to minutes 4 was analyzed on the day of harvesting, after 1 minute of ozonation catalytic and after 2, 3, 4 minutes of further ozonation catalytic

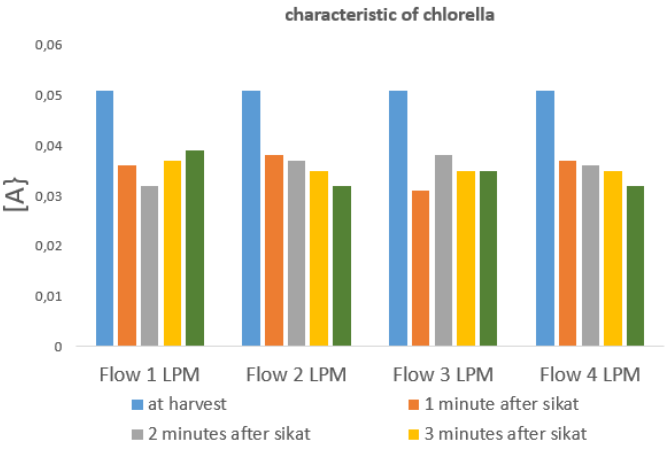

Figure 2. Characterization of the specific pattern of the Chlorella from the catalytic ozonation from 0 to 4 minutes on the day of harvesting

The results of microalgae testing using a scanning electron microscope (SEM) is shown in **Figure 3**.

SEM images of samples microalgae are shown in **Figure 3** : from *Chlorella vulgaris* cells before treatment (pictured left) and prepared from *Chlorella vulgaris* cells after treatment (pictured right). After treatment with catalytic ozonation, *Chlorella vulgaris* split into several pieces. This indicated that cell-wall of microalgae was catalytically disrupted by ozonation. In the sample prepared before treatment, the cell shape is still intact and the cell wall

surrounds the cells evenly with size distribution centered around 3.35 µm was observed.

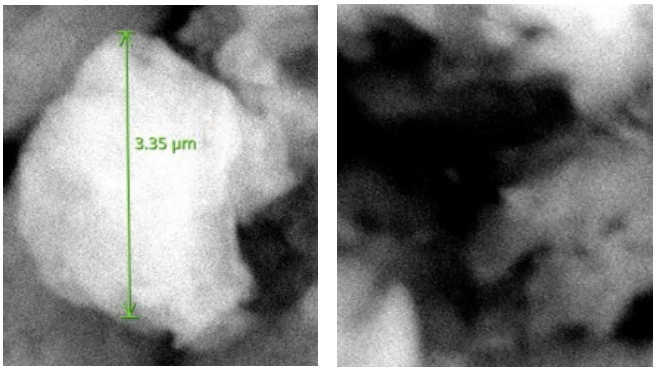

Figure 3. SEM image of Chlorella vulgaris cells after augmented aquades (left) and after catalytic ozonation with MCTO catalyst (right)

Microalgae test results using transmission electron microscopy (TEM) is shown in Figure 4.

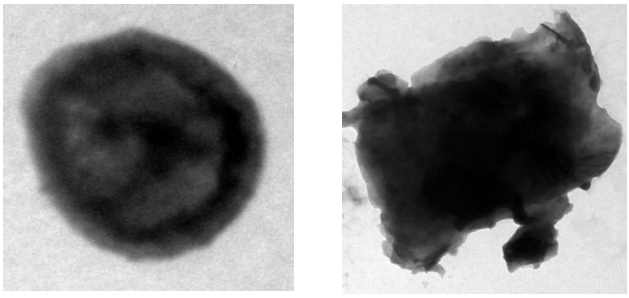

Figure 4. TEM image of Chlorella vulgaris cells without (left) and with catalytic ozonization treatment (right)

The TEM image of the microalgae samples is shown in Fig. 4. In the sample prepared from *Chlorella vulgaris* cells before treatment (pictured left), the cell is still intact, where the cell shape is still intact and the cell wall surrounds the cells evenly were observed. In the sample prepared from *Chlorella vulgaris* cells after treatment (pictured right), only left part of the cell which has changed, where the cell shape is not complete and the cell wall surrounds the cell unevenly were also observed. A similar result has been obtained by (Zheng et al., 2011) that disrupted by microwaves and grinding in liquid nitrogen. This indicated that there has been cell-wall disruption of microalgae *Chlorella vulgaris* by catalytic ozonation.

This paper is covering the cell-wall disruption and characterization of bioactive from *Chlorella vulgaris* using catalytic ozonation. In order to study the method of extraction of bioactive, the biomass from own culture with well-known characteristic is essential. It due to the content of the pigment in the biomass itself determine the efficiency of the method. In this study, we used specific pattern concentration extracted from *Chlorella vulgaris* to assess the cell disruption efficiency. The results showed that specific pattern concentration represented the disruption efficiency. Previous research found the shortest disruption time was 2 minute by grinding in liquid nitrogen and the disruption efficiency of *Chlorella vulgaris* were 100% (71.76% unsaturated and 28.24% saturated fatty acid) respectively (Zheng et al., 2011). But chemical using dimethyl carbonate found disruption efficiency of *Chlorella vulgaris* were 82,90% (Young et al., 2018) and acid treatments were approximately 86% (Chao et al., 2018), also $ScCO_2$ extraction using ethanol (10% v/v) as co-solvent led to the efficiency of 97% (Sara et al., 2018).

Our study found out that catalytic ozonation with an appropriate dosage disrupted the cell wall of *Chlorella vulgaris* which disruption efficiency of *Chlorella vulgaris* was 76.47% in 1 minute. The processing time was a bit fast, and not require a relatively stable reaction temperature or other condition. This cell disruption method is also easy to scale-up. The cell wall of *Chlorella vulgaris* only contains three component, algenan base layer, fibrillar layer and cell membrane (D'Hondt et al., 2018). The lack of components and the thinness of the cell wall of *Chlorella vulgaris* cause the cell wall to be easily destroyed compared to other microalgae. Excess hydroxyl radicals in the catalytic ozonation process can destroy bioactive microalgae compounds. This causes a decrease in cell-wall disruption efficiency. The similar result has been obtained by present study provides the impact of pulsed electric fields and high-pressure homogenization treatments on the disintegration efficiency of *Chlorella vulgaris* were relatively low and did not exceed 5.2% (Daniele et al., 2018).

Evaluation of cell-wall disruption of microalgae *Chlorella vulgaris* in water by catalytic ozonation over microporous carbon-supported titanium oxide (Figure. 1 and 2). (a) A specific pattern of *Chlorella* in the output

decreased until 76.47%, to 0.037 from highest input 0.051 in catalytic ozonation with flow of 4 liters per minute for 1 minutes. (b) chlorophyll decreased from 0.037 to 0.028 and 75.67% in catalytic ozonation with flow of 2 liters per minute for 2 minutes. (c) carotenoids from input decreased from 0.055 to 0.054 on output. Whilst the efficiency 98.18 % in catalytic ozonation with flow 1 liters per minute for 1 minute. Carotenoids were trapped around cell-wall and very difficult to destroy (Posten and Chen, 2016)(d) Also, the chlorophyll from input decreased from 0.037 to 0.021. Whilst the efficiency 56.75 % in catalytic ozonation with flow of 4 liters per minute for 1 minute. (e) The number of chlorophyll from input decreased from 0.037 to 0.022 on output. Whilst the efficiency 59.49% at catalytic ozonation with flow 1 liters per minute for 1 minute. Catalytic ozonation is very reactive to organic such as chlorophyll (Zaikov and Rakovsky, 2009) (f) carotenoids decreased from 0.055 to 0.049 and 89.09% in efficiency at catalytic ozonation with flow of 4 liters per minute for 1 minute. (g) The percentage difference of bioactive concentration from reactor capacity 500 mL, 2000 mL, 10000 mL was no significant difference. These data further indicate that cell-wall disruption of microalgae *Chlorella vulgaris* in water by catalytic ozonation over microporous carbon-supported titanium oxide until 10000 mL are functional.

Altogether, we have presented a promising approach to cell-wall disruption of *Chlorella vulgaris*, with reduced process-time, handling steps and chemical free. This system is easily scalable and can be adapted to automatization and online control. This study has the potential to bring the environmentally friendly, green technology and efficient of manufactured bioactive of *Chlorella* one step closer to reality. The experiment took 1 minute to get 76.47% cell-wall disruption of *Chlorella vulgaris*. No special handling steps are needed for catalytic ozonation processes. Because the harvest of *Chlorella vulgaris* is already in the solution phase. The catalytic ozonation unit only requires oxygen gas as an input ozone generator and microporous carbon-supported titanium oxide as a catalyst classified as environmentally friendly.

4. CONCLUSION

The required delivered flow ozone to achieve 76.47% cell-wall disruption of *Chlorella vulgaris* was 1 minute at 4 liters per minute, which produced chlorophyll 56.75% and carotenoid 89.09%. Microporous carbon-supported titanium oxide reduces the required O_3 dose and catalytic time for cell-wall disruption. However, it limited chlorophyll yield did not exceed 75.67%. Pretreatment with 1 minute at 1 liter per minute in 2 liters produced carotenoid yield by approximately 98.18%, though it reduced chlorophyll to 59.45%. Catalytic ozonation over microporous carbon-supported titanium oxide was found to be very efficient of cell-wall disruption *Chlorella vulgaris* and minimize bioactive destruction.

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REFERENCES

Barsanti, L., & Gualtieri, P. 2018. Is exploitation of microalgae economically and energetically sustainable? *Algal Research*, *31*(October 2017), 107–115. https://doi.org/10.1016/j.algal.2018.02.001

Chao, Z., Xiaohan, T., Xiaoyi, Y., 2018. Overcoming the cell wall recalcitrance of heterotrophic Chlorella to promote the efficiency of lipid extraction. *Journal of Cleaner Production*. 198,1224-1231

Cheng, J., Sun, J., Huang, Y., Feng, J., Zhou, J., & Cen, K. 2013. Dynamic microstructures and fractal characterization of cell wall disruption for microwave irradiation-assisted lipid extraction from wet microalgae. *Bioresource Technology*, *150*, 67–72. https://doi.org/10.1016/j.biortech.2013.09.126

Ciudad, G., Rubilar, O., Azócar, L., Toro, C., Cea, M., Torres, Á., Navia, R. 2013. Performance of an enzymatic extract in Botrycoccus braunii cell wall disruption. *Journal of Bioscience and Bioengineering*, *xx*(xx), 2–7. https://doi.org/10.1016/j.jbiosc.2013.06.012

Cuellar-bermudez, S. P., Aguilar-hernandez, I., Cardenas-chavez, D. L., Ornelas-soto, N., Romero-ogawa, M. A., & Parra-Saldivar, R. 2014. Extraction and purification of high-value metabolites from microalgae : essential lipids, astaxanthin and phycobiliproteins. *Microbial Biotechnology Published*, *8*, 190–209. https://doi.org/10.1111/1751-7915.12167

Daly, K. E., Huang, K. C., Wingreen, N. S., & Mukhopadhyay, R. 2011. Mechanics of membrane bulging during cell-wall disruption in Gram-negative bacteria. *Physical Review*, *41922*, 1–4. https://doi.org/10.1103/PhysRevE.83.041922.

Daniele, C., Biresaw, D. A., Alberto, C., Francesco, D., Patrizia, P., Giovanna, F., Gianpiero, P., 2018. Effect of pulsed electric fields and high pressure homogenization on the aqueous extraction of intracellular compounds from the microalgae Chlorella vulgaris. *Algal Research*, 31, 60-69.

D'Hondt, E.,Martín-Juárez, J., Bolado, J., Kasperoviciene, J., Koreiviene, J., Sulcius, S., Elst, K., Bastiaens, L. 2017. *Microalgae-Based Biofuels and Bioproducts From Feedstock Cultivation to End-products*. Woodhead Publishing.

Dong, J., Gao, K., Wang, K., Xu, X., & Zhang, H. 2015. Cell wall disruption of rape bee pollen treated with combination of protamex hydrolysis and ultrasonication. *Food Research International*. https://doi.org/10.1016/j.foodres.2015.05.039

Gottschalk, C., Libra, J. A., & Saupe, A. 2000. *Ozonation of Water and Waste Water*. Wiley.

Gracia, R., Sarasa, J., Ormad, P., & Ovelleiro, J.L. 2000. catalytic ozonation with supported titanium dioxide. The stability of catalyst in water. *Ozone: Science & Engineering*, *22*, 185–193. https://doi.org/10.1080/01919510008547219

Guo, Y., Yang, L., Cheng, X., & Wang, X. 2012. The application and reaction mechanism of catalytic ozonation in water treatment. *J Environ Anal Toxicol*, *2*(7). https://doi.org/10.4172/2161-0525.1000150

Kim, D.Y., Vijayan, D., Praveenkumar, R., Han, J.I., Lee, K., Park, J.Y., Oh, Y.K. 2015. Cell-wall disruption and lipid/astaxanthin extraction from microalgae: Chlorella and Haematococcus. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2015.08.107

Li, B., Xu, X., Zhu, L., Ding, W., & Mahmood, Q. 2010. Catalytic ozonation of industrial wastewater containing chloro and nitro aromatics using modified diatomaceous porous fillin. *Desalination*, *254*(1–3), 90–98. https://doi.org/10.1016/j.desal.2009.12.009

Mastan, E., Wu, J., & Doan, H. 2012. An investigation into surface modification of polyethylene films for hydrophilicity enhancement by catalytic ozonation. *Journal of Applied Polymer Science*, 1–8. https://doi.org/10.1002/app.38224

O'Donnell, C., Tiwari, B. K., Cullen, P. J., & Rice, R. G. 2012. *Ozone in Food Processing*. John Wiley & Sons.

Posten, C., & Chen, S. F. 2016. *Microalgae Biotechnology*. Springer.

Rame, R., Pranoto, H., K Winahyu, R. K., Sofie, M., Utomo, A. S., & Raharjo, B. H. 2017. High performance approaches on wastewater treatment technologies in hospital and community health centre in Indonesia. In *Proceedings The 7th International Symposium For Sustainable Humanosphere [ISSH]- A Forum of the Humanosphere Science School [HSS] 2017* (pp. 185–191). Research Center for Biomaterials - LIPI.

Rame, Purwanto, A., & Budiarto, A. 2017. Treatment of textile waste water based catalytic ozonation with Iron (III) Oxide (Fe_2O_3) and Aluminum Oxide

(Al_2O_3) Catalysts Using Micro Diffuser. *Research Journal of Industrial Pollution Prevention Technology*, *8*(2), 67–75. https://doi.org/http://dx.doi.org/10.21771/jrtppi.2017.v8.no2.p67-75

Rame, Tridecima, A., Pranoto, H., Moesliem, & Miftahuddin. 2018. FLASH Technology : Full-scale hospital waste water treatments adopted in Aceh. *E3S Web Conf.*, *31*. https://doi.org/https://doi.org/10.1051/e3sconf/20183104005.

Sara, O., Nicholas, B., Séverine, C., Hosni, T., Ali, I., Pierre-Yves, P., 2018. Supercritical carbon dioxide extraction and fractionation of lipids from freeze-dried microalgae Nannochloropsis oculata and *Chlorella vulgaris*. *Algal Research*, 34, 49-56.

Siew, C., Lim, Y., Hong, C., Rosli, R., & Pei, P. 2008. An alternative Candida spp . cell wall disruption method using a basic sorbitol lysis buffer and glass beads. *Journal of Microbiological Methods*, *75*(3), 576–578. https://doi.org/10.1016/j.mimet.2008.07.026

Taskova, R. M., Zorn, H., Krings, U., Bouws, H., & Berger, R. G. 2006. A comparison of cell wall disruption techniques for the isolation of intracellular metabolites from pleurotus and Lepista sp ., 347–350.

Tsaloglou, M.N. 2016. *Microalgae Current Research and Applications*. Caister Academic Press.

Wang, Q., Wei, W., Kingori, G. P., & Sun, J. 2015. Cell wall disruption in low temperature NaOH / urea solution and its potential application in lignocellulose pretreatment. *Cellulose*, *22*(6), 3559–3568. https://doi.org/10.1007/s10570-015-0767-z

Wu, J., Ma, L., Chen, Y., Cheng, Y., Liu, Y., & Zha, X. 2016. Catalytic ozonation of organic pollutants from bio-treated dyeing and finishing wastewater using recycled waste iron shavings as a catalyst : Removal and pathways. *Water Research*, *92*, 140–148. https://doi.org/10.1016/j.watres.2016.01.053.

Young, M. H., H., Lee, C., Lee, J., Kang, J., Ahn, Y., Min, L., Kyu-Young, Kang,.Y., Yoon-E, C., Jae-Jin, K., 2017. An integrative process for obtaining lipids and glucose from *Chlorella vulgaris* biomass with a single treatment of cell disruption. *Algal Research*, 27, 286-294.

Zaikov, G. E., & Rakovsky, S. K. 2009. *Ozonation of Organic & Polymer Compounds*. iSmithers.

Zheng, H., Yin, J., Gao, Z., Huang, H., Ji, X., & Dou, C. 2011. Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: A comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. *Applied Biochemistry and Biotechnology*, 164(7), 1215–1224. doi:10.1007/s12010-011-9207-1.

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Biotransformation studies of agricultural nitrogen pollutans in Keduang watershed

Pujiastuti Peni[1*], Narimo[1], Roesleini J. Putri[1]

[1]*Faculty of Engineering Setia Budi University, Surakarta, Central Java, 57127, Indonesia*

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ABSTRACT

The present study seeks to examine nitrogen biotransformation of agricultural wastewater carried out by nitrosomonas and nitrobacter into Ammonia (N-NH_3), Nitrite (N-NO_2), and Nitrate (N-NO_3) in Keduang watershed. Natural capability of the bacteria is necessary to find out to monitor assimilative capacity of the waterbody towards pollutants. Grab sampling technique was applied in agricultural land and Keduang watershed in reference to Indonesian National Standard (SNI) 6989.59:2008. Meanwhile, analysis of N-NO_2 was based on Indonesian National Standard (SNI) 06-6989.09-2004, N-NO_3 on SNI 6989.79-2011, and N-NH_3 on SNI 06-6989.30-2005. The nitrosomonas and nitrobacter were isolated and identified on NA medium considering methods of Capuccino and Sherman (2005). Afterwards, characterization of colony morphology variants was determined, and both gram stain and biochemical test were conducted. A number of 48.8 nitrosomonas colonies/100 mL were identified in samples of agricultural wastewater, which enable to transform Ammonia (N-NH_3) of 0.1390 mg/L into Nitrite (N-NO_2) of 0.0632 mg/L. Meanwhile, a number of 330 nitrobacter colonies/ 100 mL are capable of transforming Nitrite (N-NO_2) into Nitrate (N-NO_3) of 0.2168 mg/L. In conclusion, there is a positive relationship between nitrosomonas in transforming Ammonia into Nitrite and nitrobacter in converting Nitrite into Nitrate. Nitrogen pollutants of the agricultural wastewater in Keduang watershed are able to be reduced by both nitrosomonas and nitrobacter.

1. INTRODUCTION

Keduang watershed is located in Wonogiri regency, Central Java, Indonesia.The population is 339,074 people and the area covers 40,116.0735 Ha,36.7% of which is used for agricultural area. Agricultural activities that are not environmental-friendly are sources of wastewater, and these belong to non-point sources of pollution (NPS) (EPA-US, 2009). Dominant parameters of agricultural activity wastewater include sediment, nitrogen, phosphorus, pesticide, BOD and heavy metals. Non-environmental-friendly agricultural activities cause land erosion and sedimentation (Anshori, 2008), carbamatepesticideresidue (Manuba, 2009), and pollution source parameters of TSS, N-NO_3, P-PO_4 (KemenLH[1], 2010). According to Casali etal. (2010), agricultural activities produce sediment runoff, nitrate (N-NO_3) and phosphate (P-PO_4) running to river stream, and therefore cause water pollution. Keduang watershed outlet transports sediment run off and dissolved nutrients (N-NO_3, N-NH_3, H_2PO_4 and K) continuously about 32% from Keduang watershed, 18% from forests and 17% from agriculturalareas (Durn et.al., 2012). N-NH_3

[1]Ministry of Environment of the Republic of Indonesia

Correspondence author.
E-mail: peni.usb@gmail.com (Pujiastuti Peni)

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(ammonia) sources in river watery areas are resulted from agricultural activities using urea fertilizer intensively (Agustiningsih et.al, 2012).

Farmers make use of inorganic fertilizers, like urea and phonska (Pujiastuti, 2015), with high nutrient content. Urea fertilizers consist of 45-46% of N (Nitrogen), meaning that every 100 kg of urea contains 45-46 kg of Nitrogen. Phonska fertilizer is manure containing main elements of N, P and K with the proportion of 15% of N (Nitrogen), 15% of P (Phosphorus) and 15% of K_2O (Potassiumdioxide) (Maridi etal., 2012). The uses of fertilizer cause the absorption of nitrogen to environment and agricultural activities accelerate nitrogen transformation to water body (Xia etal., 2011). Only 30% of fertilizer is absorbed by roots of plan, and the remaining 70% of the fertilizer will run to rivers and dams as pollutant (Agustiningsih et.al., 2012).

Nitrogen contained in the body of water transforms into ammonia (N-NH_3), nitrite (N-NO_2), nitrate (N-NO_3) and $N_2$with the help of bacteria*Nitrosomonas sp* bacteria play important roles in transforming nitrite and nitrate (Saha etal., 2013). Ammonia and nitrite contents in water are toxics for fish (Titiresmi et.al, 2006), irritating gills and other tissues (Shen et.al., 2003), while nitrate providesnutrientenrichment of aquaticplants and this triggers eutrophication to happen. Eutrophication ofwater body will reduce dissolved oxygen, and therefore the self-purification ability of ecosystem becomes lower (Titiresmi et.al.,2006). This study aims at discussing mechanism of nitrogen biotransformation from agriculturalwastewater by *Nitrosomonas* and *Nitrobacter* bacteria intoammonia (N-NH_3), nitrite (N-NO_2) and nitrate (N-NO_3) in watershed in Keduang. The natural ability of bacteria needs to be observed to monitor assimilation capacity of water body towards pollutant.

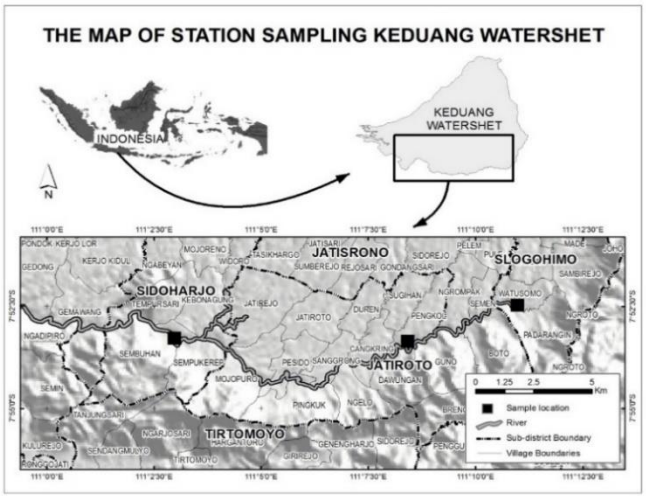

Figure 1. The map of station sampling Keduang watershed

2. METHODS

2.1. Sampling

Samples were taken representatively, based on Indonesian National Standard (SNI) of 06-6989.59:2008, from farming areas in watershed of Keduang, in Slogohimo (station 1), Jatiroto (station 2) and Sidoharjo (station 3) sub-districts, Wonogiri regency, Central Java, Indonesia. Water samples were taken in planting season and 7 days after fertilization. In every area, water samples were taken from 4 points, namely: A) farming outlet, B) farming outlet and river convergence, C) 50 m under farming outlet and river convergence, and D) before farming outlet and Keduang river convergence. Chemical and biological analyses were conducted on water samples in laboratory. The chemical analyses included: 1) pH, 2) Dissolved Oxygen (DO), 3) Ammonia (N-NH_3), 4) Nitrite (N-NO_2) and 5) Nitrate (N-NO_3). Meanwhile, biological analyses were carried out to the existence of *Nitrosomonas* and *Nitrobacter* bacteria.

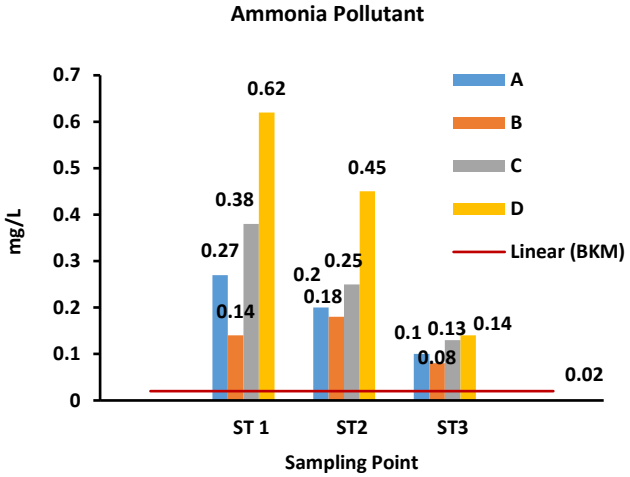

Figure 2. Ammonia pollutans in Keduang watershed from agricultural

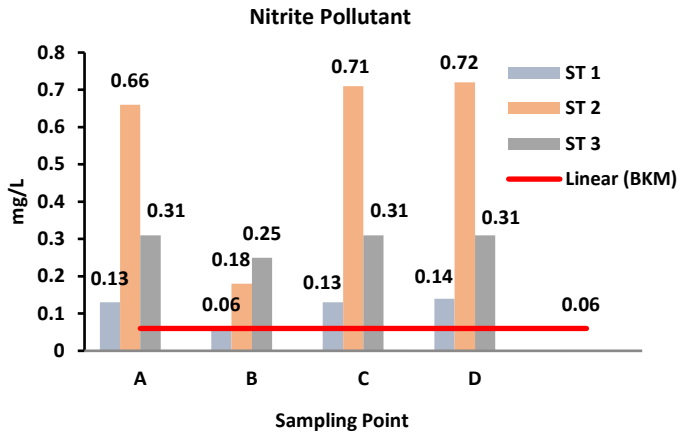

Figure 3. Nitrite pollutans in Keduang watershed

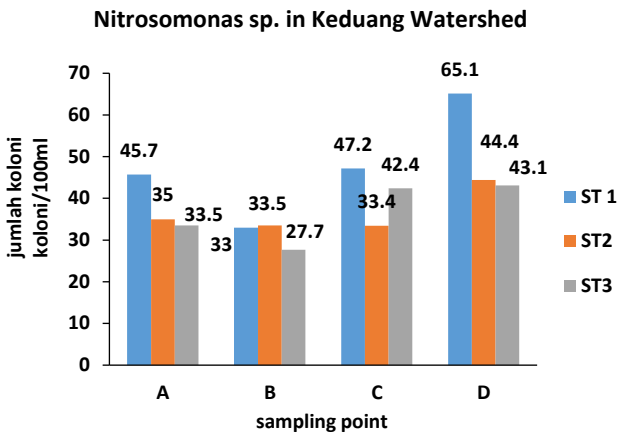

Figure 4. *Nitrosomonas sp.* in wastewater and river water

2.2. Chemical analysis

Analysis of ammonia (N-NH_3) based on Indonesian National Standard (SNI) of 06-6989.30-2005. Analysis of nitrite (N-NO_2) based on Indonesian National Standard (SNI) of 06-6989.09-2004. Analysis of nitrate (N-NO_3) based on Indonesian National Standard (SNI) of 6989.79:2011.

2.3. Biology analysis

The *Nitrosomonas*and *Nitrobacter*were isolated and identified on NA medium considering methods of Capuccino and Sherman (2005). The analysis included qualitative test with gram staining method and enrichment culture method to calculate the number of coloniesas well as qualitative analysis of *Nitrosomonas sp.* and *Nitrobacter sp.* bacteria with gram staining method.

2.4. Correlational analysis of bacteria in transformation process

Correlational analysis of *Nitrosomonas sp.* bacteria for transforming ammonia into nitrite with Chi-Square test using SPSS 17.0 version demonstrated the relationship among *Nitrosomonas sp.* bacteria in the transformation process of ammonia into nitrite. If the value of chi-$square_{count}$ was less than chi-$square_{table}$ (chi-$square_{count}$< chi-$square_{table}$), H_0 was accepted. If the value of chi-$square_{count}$ was greater than chi-$square_{table}$ (chi-$square_{count}$> chi-$square_{table}$), H_0 was rejected. In other words, when probability was greater than 0.05 (probability > 0.05), H_0 was accepted, but when probability was less than 0.05 (probability < 0.05), H_0 was rejected. In which, H_0 meant there was no positive relationship among *Nitrosomonas sp.* bacteria in agricultural water and water in Keduang river when transforming ammonia into nitrite. H_1 meant there was positive relationship among *Nitrosomonas sp.* bacteria in agricultural water and water in Keduang river when transforming ammonia into nitrite.

3. RESULT AND DISCUSSION

3.1. Ammonia pollutans in Keduang watershed from agricultural waste

The activities of non-environmentally friendly watershed, will cause the entry of pollutants into the receiving water body. Sources of ammonia pollutants in river come from agricultural activities that urea fertilizers and domestic activities that contain protein (Agustiningsih etal, 2012). Farmers make use of inorganic fertilizers, like urea and phonska (Pujiastuti, 2015). The ammonia pollutans in the agricultural wastewater and Keduang river at the sampling station are presented in **Figure 2**.

Keduang river water at the sampling point A, at a point before mixing with agricultural waste contains ammonia of 0.27 mg/L at station 1 Slogohimo, 0.20 mg/L at station 2 Jatiroto and 0.1 mg/L at station 3 Sidoharjo.These ammonia pollutants come from residual urea and phonska fertilizers used by farmers, which is not

absorbed by the rice plant.Nitrogen fertilizer in rice field water will be degraded into ammonia. Only 30% of fertilizer is absorbed by roots of plan, and the remaining 70% of the fertilizer will run to rivers and dams as pollutant (Agustiningsih et.al, 2012).The use of fertilizer causes the absorption of nitrogen to environment and agricultural activities accelerate nitrogen transformation to the body of water (Xia et.al., 2011).

Nitrogen in body water transforms into ammonia ($N\text{-}NH_3$). The hydrolysis reaction of urea fertilizer in water is displayed below:

$$\underset{Urea}{CO(NH_2)_2} + H_2O \rightarrow 2\,\underset{ammonia}{NH_3} + CO_2$$

Ammonia nitrogen in watery area is in the form of ammonium ion. The relationship between those two forms is in a balance system below (Titiresmi et.al., 2006):

$$NH_3 + H_2O \rightleftharpoons \underset{ammonium\ ion}{NH_4^+} + OH^-$$

Some of the ammonia in the water will undergo biotransformation into nitrite compounds, so the content of ammonia in water is reduced. Decreased ammonia content in outlet river and estuary, because the role of *Nitrosomonas sp.* bacteria in biotransformation process becomes nitrite compound (Saha et.al., 2013).

Ammonia at C sampling point, the meeting between agricultural wastewater with Keduang river water, has increased when compared with ammonia at the previous sampling point. Ammonia at station 1 was 0.38 mg/L, station 2 was 0.25 mg/L and station 2 was 0.13 mg/L. This is due to a meeting between agricultural wastewater with river water Keduang upstream, so that the ammonia content at that point has increased. However, this ammonia increase is not all ammonia to be an enumerating factor at the next sampling point, since some ammonia undergoes transformation by *Nitrosomonas sp.* bacteria into nitrites. Similarly, ammonia at the sampling point D, 5 m after point C, there is an increase in ammonia rather than the previous point. Ammonia point D at station 1 was 0.38 mg/L, station 2 was 0.45 mg/L and station 3 was 0.14 mg/L. The increasing pattern of ammonia at points C and D versus point A and B occurs in all sampling stations. Not all ammonia is transformed into nitrite compounds by *Nitrosomonas sp.* bacteria.

Table 1. The Characteristics of Cell Morphology Gram Staining

Station	Sampling Point	*Nitrosomonas*		*Nitrobacter*	
		Form	Gram	Form	Gram
1	A	Coccus	Negative	Short rod	Negative
	B	Coccus	Negative	Short rod	Negative
	C	Coccus	Negative	Short rod	Negative
	D	Coccus	Negative	Short rod	Negative
2	A	Coccus	Negative	Short rod	Negative
	B	Coccus	Negative	Short rod	Negative
	C	Coccus	Negative	Short rod	Negative
	D	Coccus	Negative	Short rod	Negative
3	A	Coccus	Negative	Short rod	Negative
	B	Coccus	Negative	Short rod	Negative
	C	Coccus	Negative	Short rod	Negative
	D	Coccus	Negative	Short rod	Negative

Table 2. Number of Colony of Nitrosomonas and Nitrobacter Bacteria

No	Analysis	Unit	Station 1				Station 2				Station 3			
			A	B	C	D	A	B	C	D	A	B	C	D
1	*Nitrosomonas sp*	Colony/100 mL	51.4	48.8	53.6	62.0	49.0	45.0	52.1	57.8	41.3	37.5	50.9	56.6
2	*Nitrobakter sp*		45.7	33.0	47.2	65.1	35.0	33.5	35.4	44.4	33.5	27.7	42.4	43.1

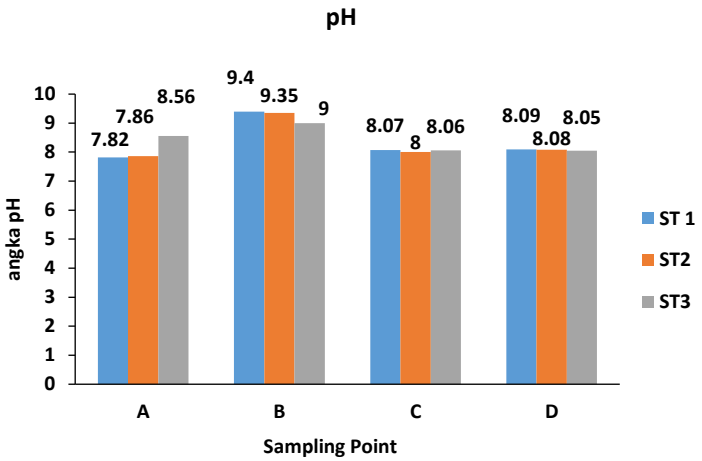

Figure 5. pH in Keduang watershed

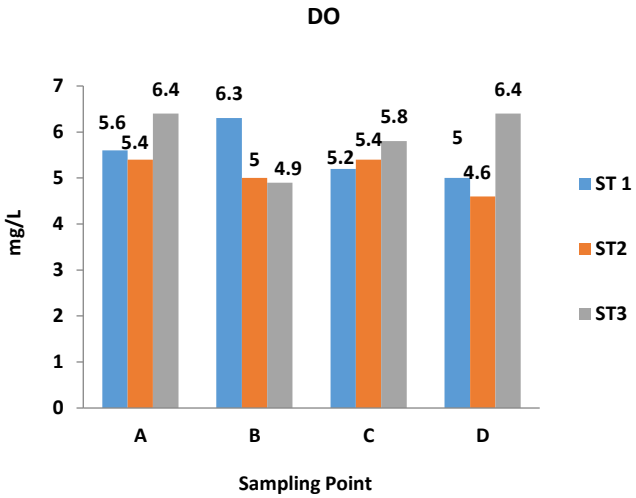

Figure 6. The spreading pattern of DO of theagricultural wastewater and the river water

Ammonia in water can negatively impact aquatic biota, if exceeded water quality standards have been set. Ammonia in water can negatively impact aquatic biota, if exceeded water quality standards have been set. Safe limit for fish to ammonia in water is 0.02 mg/L (Government Regulations No. 82/2001). The concentration of ammonia in the range of 0.2 to 2 mg/L cause toxicity in fish, damage to the gills so that the respiration of fish will be disrupted, and ranges from 0.2 to 9 mg/L of lead toxicity in higher organisms. Ammonia at sampling points C and D on station 1 and station 2 exceeds 0.2 mg/L, it can cause ammonia poisoning effect on the fish. Ammonia in the water of the Keduang river in Slogohimo and Jatiroto areas has the potential to cause ammonia poisoning in fish.

3.2. Biotransformation Amonia (N-NH_3) to Nitrite (N-NO_2) by Nitrosomonas sp

Ammonia is a nutrient for nitrogen bacteria (Aswadi, 2006). The aforementioned ammonia compound is naturally transformed by *Nitrosomonas sp.* bacteria, contained in the river water, into Nitrite (N-NO_2). The first stage of nitrogen biotransformation process is nitrification. It is an oxidation of ammonium ion (NH_4^+) into nitrite ion (NO_2^-) by *Nitrosomonas sp.* bacteria (Saha et.al., 2013). *Nitrosomonas sp.* bacteria need oxygen, the oxidation reaction converts ammonia into nitrite. The chemical reaction is as follows (Aswadi, 2006):

$$NH_4^+ + {}^1/_2O_2 + OH^- \rightarrow NO_2^- + 2H_2O + H^+ + 59.4\ Kcal.$$

The distribution pattern of Nitrite compound on the observed sample is shown in **Figure 3**.

In an aquatic environment with rich amount of ammonia, groups of nitrification bacteria (*Nitrosomonas sp.*) can be found in large number. Through the process of nitrification, the ammonia compound is transformed into nitrite that may be poisonous for the aquatic biota. Nitrite content in the wastewater in watershed of Keduang ranges

from 0.06 to 0.25 mg/L. Based on the result of the laboratory analysis, it can be assumed that there is biotransformation process on the agricultural wastewater from ammonia into nitrite with the help of *Nitrosomonas sp.* On the basis of the observation of morphological characteristics of the cells through gram staining, it is found out that there are coccus gram-negative bacteria identifying the existence of *Nitrosomonas sp.*in all sampling points.

The sample of agricultural wastewater is positive with *Nitrosomonas sp.* bacteria, 37.5 - 48.8 colonies/100 ml. It means that in every 100 mL of the wastewater, there are approximately 37.5 – 48.8 colonies of *Nitrosomonas sp.* Once the agricultural wastewater assembles with river water, the content of nitrite ranges from 0.06 to 0.72 mg/L. Nitrite easily evaporates and is easily oxidized into nitrite compounds. It causes the content of nitrite in water to decrease. Nitrite is a toxic nitrogen compound, found in small concentration in the waters (Marganof, 2007). In waters with sufficient oxygen, nitrite is immediately oxidized to nitrate. Class 2 water quality stadard, based on Indonesia regulation No. 82 in 2001, maximum nitrite 0.06 mg/L. Keduang river waters have nitrite content above quality standards. This can endanger the water biota. Nitrite level of 0.5-5 mg/L can have a negative impact on fish life (Suriadarma, 2011). Nitrite is absorbed by fish, will react with hemoglobin, forming methemoglobin which cannot function as an oxygen transporter. Continuous absorption of nitrite by fish, will cause hypoxia and cyanosis. Blood of fish containing methemoglobin will be brown (Suriadarma, 2011).

3.3. Nitrosomonas sp. and Nitrobacter sp. in Keduang watersheed

The results of identification on the characteristics of cell morphology after gram staining were presented in **Table 1** and **Table 2**.

*Nitrosomonas sp.*are bacteriathat contribute to the oxidation process of ammonia into nitrite in nitrogen cycle. Morphologically, this bacterium has short steam, ellipse cell, motile and non-motile, consortium shape and is in pairs as short chain or individual. It is a gram-negative bacterium that has cyomembrane. The cells grow freely in the medium and form thin matrix (Fatmawati et.al., 2012; Masniawati et.al., 2017). This bacterium may optimally grow at 5 - 30°C and with an optimum pH of 5.8-8.5; and live in sea water, fresh water, and soil (Ramadhani, 2015). The spreading pattern of *Nitrosomonas sp.* in the agricultural wastewater and river water of Keduang is shown in **Figure 4**.

The spreading pattern of *Nitrosomonassp.*follows the spreading patterns of ammonia and nitrite. At station 1, every 100 mL of agricultural wastewater in Slogohimo sub-district contains 48.8 colonies of *Nitrosomonassp.*bacteria. In the Rhizosphere rice plants (*Oryza sativa* L.) have *Nitrosomonas*bacteria, around $14{,}8x10^5$ CFU/mL - $19{,}74x10^5$ CFU/mL (Masniawati et.al., 2017). These bacteria play a role in converting ammonia to nitrite. Every 1 liter of agricultural wastewater in Slogohimo sub-district contains 0.14 mg of ammonia, 0.06 mg of nitrite and 4.48 colonies of *Nitrosomonassp.* After the agricultural wastewater flows to Keduang river and assembles in the riverside, this water mixture increases the number of *Nitrosomonassp.*from 48.8 into 53.6 colonies/100 ml. *Nitrosomonassp.*also increases into 62.0 colonies when it arrives at the middle of the river. It also happens in station 2 and station 3 in which the contents of ammonia at the three stations also increase after converging with the river water. The number of *Nitrosomonassp.*influences the amount of nitrite in all sampling points, i.e. the amount of nitrite is lower than ammonia. It proves that *Nitrosomonassp.*can transform ammonia into nitrite. Indeed, it also proves that there is oxidation from nitrite into ammonia. The function of bacteria in transforming nitrogen compound is influenced by the content of dissolved oxygen in the water body. If the dissolved oxygen (DO) in the water is ≥ 4.0 mg/L, it can fulfill the oxygen needed bybacteria to breed and oxidize nitrite into nitrate. The agricultural wastewater and the river water samples contain 4.60 to 6.40 mg/L of DO.

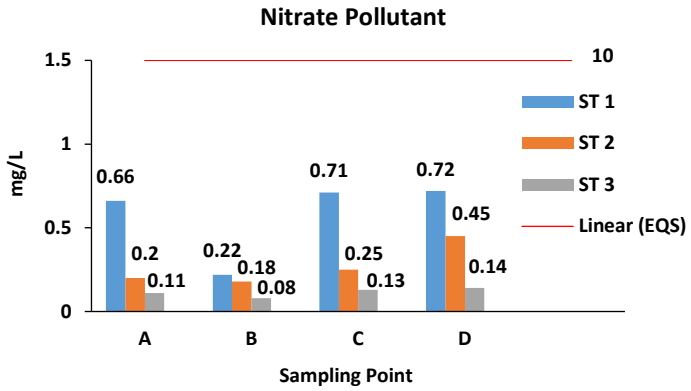

Figure 7. Nitrate in the agricultural wastewater and the river water

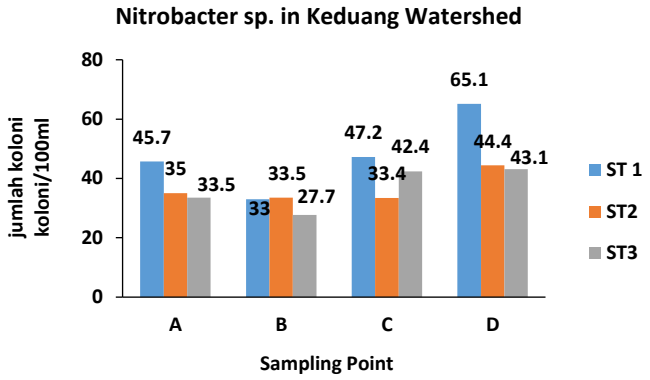

Figure 8. The spreading pattern of Nitrobacter sp. in wastewater and river water

The measurement of pH degree was conducted in the sampling withdrawal process. This step is conducted in order to validate the preciseness of pH values. The pH of the watershed of Keduang ranges from 9.0 to 9.40, higher than the pH of the river water before and after it converges with the agricultural wastewater. In this pH, the processes of nitrification and nitration can occur optimally, i.e. the efficiency rate of the biotransformation process is more than 90% (Shen et.al., 2003). The rate of the nitrification process can decrease at the pH of 6.3 – 6.7 and would be stopped at the pH of 5.0 – 5.5 (Titiresmi et.al., 2006).

The process of nitrogen compounds transformation in water is influenced by the dynamics of the DO (Mayo etal., 2014). DO concentration that is less than 2 mg/L (< 2 mg/L) in water can disturb nitrification process (Titiresmi et.al., 2006). DO samples of the agricultural wastewater and the river water observed range between 4.60 – 6.40 mg/L. It means that every 1 L of agricultural wastewater contains 4.60 – 6.40 mg of dissolved oxygen. The DO spreading pattern of the agricultural wastewater and the river water in three stations is shown in **Figure 6**. It is assumed that the DO values of all sampling points still meet the demand of the optimum DO range for nitrification process.

3.4. Biotransformation Nitrite (N-NO_2) to Nitrate (N-NO_3) by Nitrobacter sp.

Habitat of *Nitrobacter sp.* is spread in fresh water, sea water and soil (Kiding et.al., 2015). Nitrite compounds in water are transformed into nitrate through oxidation and accelerated by the existence of *Acetobacter sp.* bacteria. This biotransformation is called as nitration, the transform of nitrite compound into nitrate by nitrobacteria colonies, such as *Nitrobacter agilis* (Titiresmi et.al., 2006), *Nitrobacter winogradski* (Inamori et.al., 1997 in Titiresmi et.al., 2006), through the following reaction:

$$NO_2^- + 1/_2O_2 \longrightarrow NO_3^- + 18\ Kcal$$

Nitrate (N-NO_3) compounds resulted from nitrification process with *Nitrobacter sp.*are stable. Nitrite compounds in the agricultural wastewater of Keduang watershed range from 0.06 to 0.25 mg/L. They are transformed by *Nitrobacter sp.* into stableNitrate compounds with the contents of 0.08 – 0.22 mg/L. The meeting point area of the agricultural wastewater and the river water contains 0.13 – 0.71 mg/L of nitrate. The sample of the river water in station 3 contains nitrate compounds of 0.14 – 0.72 mg/L. The contents of nitrate in the agricultural wastewater and the river water are still under the second class of the water quality standard, i.e. of a maximum of 10 mg/L. The spreading pattern of *Nitrobacter sp.* in wastewater and river water, is shown in **Figure 8**.

3.5. The correlational analysisofNitrosomonas sp. and Nitrobacter sp. in the biotransformation of nitrogen compounds

Through chi-squaretest using SPSS, it is found out the value of chi-square$_{count}$of 16.661 for nitrification process and 109.904 for nitration process with the significance level

of 0.000. As the significance level is under 0.0005, H_0 is declined which means that there is correlation between *Nitrosomonas sp.* And*Nitrobacter sp.* bacteria in the agricultural wastewater and the river water of Keduang within the process of ammonia biotransformation into nitrite and nitrate. The nitrification is done by *Nitrobacter sp.* bacteria. These bacteria use nitrite as the source of energy and oxygen is usedas the acceptorselectron (Sudarno, 2012). The transformation of nitrite into nitrate by *Nitrobacter sp.* can happen in an aerobic environment, i.e. an environment with the existence of oxygen to survive.

4. CONCLUSION

Nitrosomonas sp. and *Nitrobacter sp.* bacteria play an active role in the biotransformation process of nitrogen pollutans in agricultural wastewater in the Keduang watershed. The ammonia pollutans are transformed into nitrite pollutans by the role of *Nitrosomonas sp.* bacteria, then *Nitrobacter sp.* converted nitrite into nitrate. The samples of the agricultural wastewater and the river water of Keduang show that there are 48.8 – 62.0 colonies/100 mL of *Nitrosomonassp.* bacteria, *Nitrobactersp.* with the number of 33.0–65.1 colonies/100 mL, ammonia ($N\text{-}NH_3$) of 0.08–0.62 mg/L, nitrite ($N\text{-}NO_2$) of 0.06-0.72 mg/L, and Nitrate ($N\text{-}NO_3$) of 0.08 – 0.72 mg/L.Amonia and nitrite levels in the sample, above the class 2 Government Regulations No.82/2001 water quality standard, so that have can an impact on fish and others aquatic biota.

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REFFERENCES

Agustiningsih, D., Sasongko, S.B.& Sudarso. 2012.Analisis Kualitas Air dan Beban Pencemaran Berdasarkan Penggunaan Lahan di Sungai Blukar Kabupaten Kendal.*Proceeding in The National Seminar of "Pengelolaan SDA & Lingkungan"*, Semarang, Indonesia. 11 September2012.

Anshori, Imam, 2008, *Kajian Sistemik Untuk Pemandu Arah Kebijakan Mengurangi Sedimentasi Waduk Wonogiri*, Thesis Program Magister Studi Pembangunan Sekolah Arsitektur, Perencanaan dan Pengembangan Kebijakan, ITB.

Aswadi Muhammad, 2006. Pemodelan Fluktuasi Nitrogen (Nitrit) pada Aliran Sungai Palu. *Jurnal SMARTek*, 4 (2), 112-125.

Cappucino, James G, Natalie Sherman, 2014, Microbiology: a laboratory manual/James G. Cappucino, Natalie Sherman, 10th ed. Pearson, www.pearsonhighered.com

Casali, J. R. Gimenez, J. Diez, J. Álvarez-Mozos, J. D.V. de Lersundi, M. Goni, M.A. Campo, Y. Chahor, R. Gastesi, J. Lopez, 2010. Sediment production and water quality of watersheds with contrasting land use in Navarre (Spain). *Agricultural Water Management* 97 pp. 1683–1694

Durn, Z.V.H., Francia, M.J.R., Gracia, T.I., Rodriques, ap.c.r.,Martinez, R.A. & Cuadros, T.S. 2012. Runoff & Sediment Yield from a Small Watershed in Southeastern Spain (Lanjar n): implication for water quality. *Hidrological Sciences Journal*, 57 (8), 1610-1625.

EPA-US, 2009, 2009 *Hazardous Waste Report*, Instructions and Form, United State Environmental Protection Agency, EPA Form 8700-13 A/B, November 2009.

Kementrian Lingkungan Hidup, 2010, *Status Lingkungan Hidup Indonesia 2010*, ISBN 978-602-8358: 39-2

Kiding Agustina, Khotimah Siti, Linda Riza,.2015, Karakterisasi dan Kepadatan Bakteri Nitrifikasi pada Tingkat Kematangan Tanah Gambut yang Berbeda Di Kawasan Hutan Lindung Gunung

Ambawang Kabupaten Kubu Raya, Jurnal Protobiont, Vol. 4 (1), 2015: 17-21

Manuba, I.B. Putra, 2009, *Cemaran Pestisida Karbamat Dalam Air Danau Buyan Buleleng Bali*, Jurnal Kimia 3(1), ISSN 1907-9850, Januari 2009:47-54

Masniawati, A., As'adi, A. 2017. Isolasi Bakteri Nitrifikasi pada Rhizosfer Tanaman Padi Aromatik Lokal (Oryza sativa L.) di Kabupaten Toraja Sulawesi Selatan. *http:// scholar google.co.id.*

Marganof, 2007. Model Pengendalian Pencemaran Perairan di Danau Maninjau Sumatra Barat, Laporan Hasil Penelitian Sekolah Pascasarjana IPB Bogor.*http://www.damandiri.or.id/ile/marganoipb*

Maridi, Sutarno, Shalihuddin Djalal Tandjung, Ari Handono Ramelan. 2012. *Fighting Through Community Participation Based on Vegetative Conservation Approach of Wonogiri Reservoir Sedimentation Sub - Watershed of Keduang.* Surakarta : Universitas Sebelas Maret. Journal of Environment and Earth Science. ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online). Vol 2, No.7, 2012 0948 (Online)

Mayo W. Aloyce, Hanal E Emmanual, 2014, Dynamics of Nitrogen Transformation and Removal in a Pilot High Rate Pond. Journal of Water Resource and Protetection, 2014, 6, 433-445.

Presiden Republik Indonesia. 2001. "Peraturan Pemerintah Republik Indonesia Nomor 82 Tahun 2001 Tentang Pengelolaan Kualitas Air dan Pengendalian Pencemaran Air". Jakarta

Pujiastuti, P., Sumardiyono, Mutiah, Syahriyati. 2015. Pola Distribusi Pencemar Nitrogen dan Fosfor Non Point Source Sub Das Wuryantoro ke Waduk Gajah Mungkur Wonogiri.*Paper presented in SNaTKII II (National Seminar on Chemical, Industrial and Information Technologies)*, Surakarta, Indonesia.10 October2015.

Ramadhani, Rezky. 2015. "Distribusi Bakteri Nitrifikasi (Nitrosomonas dan Nitrobacter) di Muara Sungai Tallo Kota Makasar". *Skripsi.* Makasar : Universitas Hasanudin

Saha Mousumi, Sarkar Agnisvar and Bandhophadhyay. 2013. *Developmant of Molucular Identification of Nitrifing Bacteria in Water Bodies of East Kolkata Wetland, West Bengal, Journal Bioremidiation & Biodegradation*, 2013, 5;1, http://dx.doi.org/ 10.4172/2155-6199.1000211. ISSN: 2155-6199 JBRBD, *an open access journal.*

Shen, Q.R., Ran, W., Cao, H. 2003.Mechanisms of Nitrite Accumulation Occurring in Soil Niytrification.*Chemosphere Journal*, 50(6), 747-753.

Standar Nasional Indonesia 6989.79:2011 – *Bagian 79: Cara Uji Kadar Nitrat (NO_3-N) dengan Alat Spektrofotometer UV – Visible Secara Reduksi Kadmium.2011:* BSN.

Standar Nasional Indonesia 06-6989.59:2008 tentang Air dan Air Limbah – Bagian 59: Metode pengambilan contoh air limbah. 2008: BSN.

Standar Nasional Indonesia 06-6989.30-2005 tentang Air dan Air Limbah – Bagian 30 : Cara Uji Kadar Amonia dengan Spektrofotometri secara Fenat. 2005 : BSN

Standar Nasional Indonesia 6989.09-2004 tentang Air dan Air Limbah – Bagian 9: Cara Uji Nitrit (NO_2-N) secara Spectrofotometri. 2004: BSN.

Sudarno. 2012. Perkembangan Biofilm Nitrifikasi di Fixed Bed Reactor pada Salinitas Tinggi.*Jurnal Presipitasi*, 9 (1).

Suriadarma Adi, 2011, Dampak Beberapa Parameter Faktor Fisika Kimia Terhadap Kualiitas Lingkungan Perairan Wilayah Pesisir Karawang-Jawa Barat. Jurnal Riset, Geologi dan Pertambangan, Vol/ 21, No. 2, Juni 2011. Puslit Geoteknologi. LIPI.

Titiresmi &Nida, S.2006.Teknologi Biofilter untuk Pengolahan Limbah Ammonia.*Jurnal Teknik Lingkungan PTL-BPPT*, 7(2), 173-179.

Xia Yu, H. Lingguang, Xu Ligang. 2011.Characteristics of Diffuse Source N Pollution in Lean River Catchment.*Procedia Environmental Sciences*, 10, 2437 – 2443

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High performance of enzymatic bioprocess for production of biomass-based bioethanol of sago palm fiber waste

Nani Harihastuti, Rame, Silvy Djayanti

Balai Besar Teknologi Pencegahan Pencemaran Industri, Jl. Ki Mangunsarkoro No.6, Karangkidul, Semarang Tengah, Kota Semarang, Jawa Tengah, Indonesia 50136

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A B S T R A C T

Biomass waste in the form of fiber dregs contains many components of lignocellulose and hemicellulose. Lignocellulose can be used to produce ethanol through enzymatic biotechnology processes. Sago palm fiber industry is one potential industry producing biomass waste in the form of solid waste of fiber dregs (about 30% from the weight of processed raw materials). Solid fiber waste contains crude fiber and lignocellulose compound consists of cellulose (35-50%), hemicellulose (20-35%) and lignin (12-20%). This study aimed to utilize solid waste of sago palm fiber as a raw material of bioethanol production through enzymatic biotechnology processes of delignification, saccharification and fermentation which was then purified by distillation process to get ethanol. Delignification, saccharification, and fermentation stages are conducted using Phanerochaete chrysosporus Mushroom, Trichoderma viride fungus, and saccharomyces cerevisiae, respectively and then purified by distillation process (one level) to produce ethanol. The process of saccharification and fermentation process were done in an integrated manner (addition of Trichoderma viride and yeast saccharomyces cereviceae fungi done simultaneously). The weight of raw materials of solid waste fiber treated was about 6 kgs. Alcohol content obtained was about ± 4% (distillation). No water, air, and soil pollution inflicted, more added value, and sustainable were the main benefits of biotech process or bioprocess.

1. INTRODUCTION

Sago palm fiber industries are spread over places in Central Java, such as in Jepara, Pati, Kendal and Klaten. Identification result has indicated that sago palm fiber industrial centers in Daleman village of Klaten are about 52 of 1320 factories with production capacity and solid waste generated of 660 ton/month and of ± 240 ton, respectively.

Sago palm fiber industry is one of the industries that has the potential to produce solid waste biomass as fiber pulp. Carbohydrate and crude fiber contents in sago palm fiber are 19.69% and 29.28%, respectively (Rame & Nani, 2016). Solid waste biomass as fiber pulp contains many components of lignocellulose and hemicellulose. Palm pulp contains Lignocellulose crude fiber compounds of cellulose (35-50%), hemicellulose (20-35%) and lignin (12-20%) (Mosier et al., 2005). Solid waste used for livestock fodder and mushroom growing media other they throw it in roadside and the side of the river. Problems accumulate increasingly so that it's will disrupt activities and comfortness people in long-term and can cause

*Correspondence author. Tel.: +624 8316315
E-mail: nanisoeharto@yahoo.com (Nani Harihastuti)

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environmental pollution because the sago palm fiber still contains organic substances that will decompose into a simple compound that has a bad odor (Jin, 2015).

The alternative of solid waste management for sago palm fiber is utilized as raw material in bioethanol manufacture. Factors that encourage the research use of lignocellulosic materials into bioethanol to energy source caused 1) energy needs increase every year, 2) energy sources from natural resources such as oil, gas, and coal are decreasing, 3) bioethanol has characteristics that can increase efficiency combustion 4) reduce greenhouse gas emissions and global warming, and 5) lignocellulosic materials are abundant and are not used as foodstuffs, so energy source does not interfere with the food supply.

The aim of this study was to utilize solid waste fiber/ sago palm fiber as the raw material of bioethanol manufacture through biotechnology process with stages were deligninfication, saccharification and fermentation process technology to obtain ethanol by the distillation process.

Biomass is the raw materials of alternative energy sources that possible for a substitute for fossil fuels. Sago fiber biomass generated from the extracting process can be made into bioethanol (Awg-Adeni, Bujang, Hassan, & Abd-Aziz, 2013). Several studies suggested that bioethanol production were not feasible economically and also caused an environmental effect. Ethanol production by integration and separation using solid waste of sago palm fiber fiber as raw material, and *Phanerochaete chrysosporus* in delignification process (Saritha et al., 2012), *Trichoderma viride* in saccharification process (Ko et al., 2009) and *Saccharomyces Cerevisiae* in fermentation process are expected to produce ethanol without causing environmental effect (Mussatto et al., 2010).

Conversion of lignocellulose material into ethanol is basically done through several stages that are stimulated by enzymes according to their activities including delignification, saccharification and fermentation stages (Sun & Cheng, 2002). Previous research executed the delignification process using diluted strong acid, but this method may produce furfural and hydroxymethylfurfural byproducts which can inhibit fermentation process (Harmsen, 2010) and the wastewater will pollute the environment and requires equipment that resistant for acid and high-temperature.

According to (Hermiati et al., 2010), utilization of white fungus in the delignification process has been reported by some researchers and is a better method than chemical because of its selectivity, energy-efficiency, and environmental-friendly. However, enzymatic degradation of cellulose generally does not lead into high conversion, due to the presence of complex cellulose in the lignocellulose structure (biomass), a crystalline structure in cellulose and the strong bond between cellulose and lignin, so that cellulose degradation cannot be easily (Walker & Stewart, 2016). To find out how far the enzymatic bioprocess of lignocellulose into bioethanol as a source of renewable alternative energy from palm sugar fiber bedding, it is necessary to do this research (Nigam & Singh, 2011).

2. METHODS

The material for this research was a solid waste of palm fiber taken from the small industry of starch palm in Daleman, Tulung, Klaten, Central Java Province. Microbes producing Enzymes such as *Phanerochaete chrysosporus*, *Trichoderma viride*, *Saccharomyces Cerevisiae* were obtained from PAU Center for Nutrition Studies and Nutrition- UGM. Potato Dextro agar media and Malt Agar extract, chemicals for microbial nutrition consist of KH_2PO_4; $(NH_4)\ 2SO_4$; $MgSO_4.7H2O$; $CaCl_2.2H_2O$; $FeCl_3.6H_2O$; $ZnSO_4.7H_2O$; Urea; $MnSO_4.H_2O$; $NaNO_3$; HCl and chemicals for carbohydrate, glucose analysis.

The equipment used were a plastic box, distillator, glass equipment, autoclave, incubator, oven, pH meter, thermometer, and blender. The research steps were microbial regeneration; microbes used were a phanerochaete chrypsosporus mushroom (white fungus mushroom) for the delignification process, *Trichoderma viride* fungus for saccharification process, and yeast *Saccharomyces Cerevisiae* for fermentation process. Each type of microbe was regenerated in accordance to the stage of the process to

be done. Preparation of nutrient solution from chemical compounds was dissolved in aquadest up to 5 liters volume.

The sterilization of materials sago palm fiber and nutrient solution were done using autoclave at 121°C for 15 minutes. The container for experiments was sterilized using alcohol 70 %. Raw materials of sago palm fibers were prepared out by drying, cutting, grinding sifting, and weighing. Sago palm fiber was dried until water content ≤ 10%, then cut and ground to the size of ± 0.5 cm to get similar shapes and facilitate fermentation process. The quality of the material was measured based on the analysis of cellulose, hemicellulose and lignin parameter (SNI 0492, 2008) (SNI 2891, 1992). The experiment was started with delignification process using *Phanerochaete chrysosporus* fungus/ white filaments varied of 2 tubes, 4 tubes, 6 tubes, and 8 tubes. The performance tests of the content were lignin, cellulose, hemicellulose. The results of analysis were evaluated and the smallest and biggest lignin and cellulose contents were used as the basic for experiments. The delignification process was done at of 30-35 °C for 25 days.

The microbial cultures preparation for saccharification process by microbial inoculation was done with the addition of *Trichoderma viride* at 30-40 °C and pH 5. The experiments were carried out with ripening of 3 days, 4 days, 5 days, 6 days. The term of saccharification refer to the analysis of glucose level (SNI 2891, 1992) and selected highest glucose content was used as the basic for the next experiments.

The preparation culture of *Saccharomyces Cerevisiae* for fermentation processes.

The microbial inoculation with ripening for 3-7 days to convert glucose to ethanol with addition of *Saccharomyces Cerevisiae* 3 tubes per 1 kg of delignified powder. Fermentation was done at 30 °C with ripening times were 3, 5 and 7 days. The fermentation time was reached, filtrate that was purified by distillation processes to get ethanol as product. The distillation process carried out by one stage and setting temperature was 78°C (Ko et al., 2009). The result of ethanol was analyzed using Gas Chromatography. Flow diagram of bioprocess stages is shown in **Figure 1**.

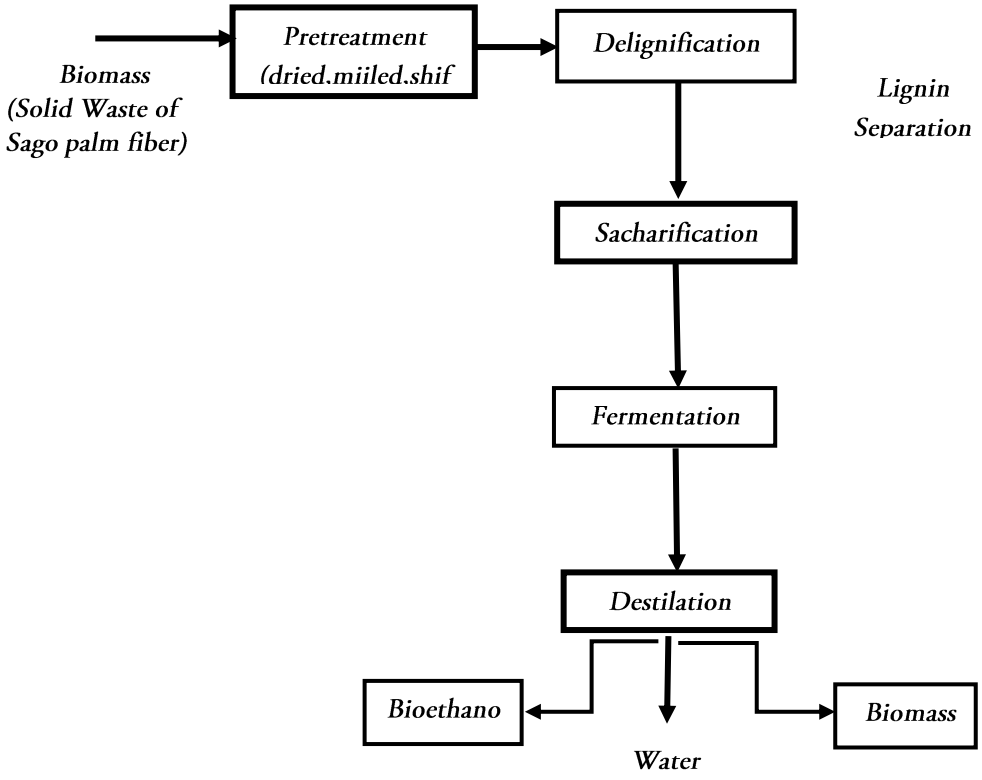

Figure 1. Flow diagram to produce bioethanol with raw material solid waste of sago palm fiber by enzymatic processes

The venue of activity of the Research was conducted at BBTPPI in Biotechnology Laboratory, Jalan Ki Mangunsarkoro No. 6 Semarang and testing laboratory in PAU UGM Jogyakarta.

3. RESULT AND DISCUSSION

3.1. Characterization of the analysis result

Characterization of analysis result from solid waste of sago palm fiber after pretreatment is tabulated in **Table 1**.

From characterization result of raw materials mentioned above, sample number 3 was selected (milled mixture 40 mesh) as the research raw materials based.

3.2. Delignification Process of Results

From **Table 2** obtained the best delignifcation process on DG3 formula, which then used as delignification process based using solid waste 6 kg.

3.3. The result of analysis Saccharification Processes

Saccharification Process of results can be seen in **Table 3**.

Results of the saccharification process on 4 (four) treatments, obtained as in **Table 3** above. The highest glucose level was obtained in the saccharification process by using 1 tube *Trichoderma viride* / 0,5 kg powder delignified tube with 3 days ripening time and 1.95% glucose level was obtained. It is used for reference of the next process.

Results of the saccharification process on 4 (four) treatments, obtained as in table 3 above. The highest glucose level was obtained in the saccharification process by using 1 tube *Trichoderma viride* / 0,5 kg powder delignified tube with 3 days ripening time and 1.95% glucose level was obtained. It was used for reference of the next process.

Table 1. The result of analysis organic solid waste compound of sago palm fiber

No	Sample Code	Lignin (%)	cellulose (%)	HemicelIulosa (%)	Carbohydrate (%)	Water (%)
1.	Dregs of Flour	9,77	19,48	35,94	53,99	15,66
2.	Dregs of Fiber	15,50	43,74	20,77	31,94	11,57
3.	Dregs of Mixture milled 40 mesh	14,04	30,72	33,23	50,15	9,5

Table 2. Result of analysis delignification processes

Sample Code	Lignin (%)	Cellulose (%)	Hemicellulosa (%)	Carbohydrate (%)	Water (%)
DG 1	6,89	22,80	33,94	55,23	9,54
DG 2	7,75	28,05	33,28	54,42	9,54
DG 3	6,32	30,49	31,67	56,75	9,12
DG 4	9,22	27,98	27,86	53,63	9,35

Where :

DG 1 =Delignification Using *Phanerochaete Chrys* 1 Tube/0,5 kg solid waste

DG 2 =Delignification Using *Phanerochaete Chrys* 2 Tube/0,5 kg solid waste

DG 3 =Delignification Using *Phanerochaete Chrys* 3 Tube/0,5 kg solid waste

DG 4 =Delignification Using *Phanerochaete Chrys* 4 tabung/0,5 kg solid waste

Table 3. The result of glucose analysis at Saccharification processes

Saccharification	Glucose Content in Ripening Time				
	3 days (%)	4 days (%)	5 days (%)	6 days (%)	7 days (%)
S 1	1,95	1,50	O,90	0,31	0,53
S 2	0,94	1,07	0,67	0,41	0,55
S 3	0,70	0,78	0,74	0,33	0,38
S 4	1,10	1,04	0,72	0,39	0,48

Where :

S 1 = Saccharification using 1 tube *Trichoderma viride* / 0,5 kg powder delignified

S 2 = Saccharification using 2 tube *Trichoderma viride* / 0,5 kg powder delignified

S 3 = Saccharification using 3 tube *Trichoderma viride* / 0,5 kg powder delignified

S 4 = Saccharification using 4 tube *Trichoderma viride* / 0,5 kg powder delignified

3.4. Fermentation Process Results

Fermentation process followed by the purification process with distillation obtained the results as in table 4 below:

Table 4. Ethanol Results of Fermentation Processes at Variation Times

Number	Code Process	Content of Ethanol (%)
1	Fermentation 3 days	8,92
2	Fermentation 5 days	1,48
3	Fermentation 7 days	0,13

Regarding **Table 4** above on 3 days fermentation, the highest ethanol contain was 8,92 %. Sago palm fiber Solid waste is a by-product resulting from the extraction of sugar sago palm fiber. Solid waste used obtained from industrial centers of sago palm fiber in Klaten.

Based on the test results, waste contains cellulose 19,48%; hemicellulose 35.94%, and carbohydrates 53.94%. While the waste fiber form has a content of cellulose 43.74%; hemicellulose 20.77%, and carbohydrates 31.94. Since the cellulose content is relatively high then the solid waste of sago palm fiber is feasible to be used as biomass for bioethanol bioprocess. Visualization of solid waste of sago palm fiber in **Figure 2** as below:

Figure 2. Solid Waste of Sago palm fiber dregs

Figure 3. Solid Waste after pretreatment

Pretreatment on solid waste of sago palm fiber by grinding was done to increase the surface area of waste so that microbe may easily penetrate into waste powder of sago palm fiber. Solid waste was not directly delegated because of the high water content of > 60%. Waste was aerated to dry to avoid damage to carbohydrate structure. Dried waste was mashed up to 40 mesh and powder-shaped to enlarge the

surface area. It was expected to enlarge the field of waste contact with microorganisms. Next, waste that has been smoothed, then added water and nutrients, and put into heat-resistant plastic and sterilized.

Solid waste samples were initially delignified. This process is important because it can affect the bioethanol rendement. Lignocellulose is an organic component in nature that consists of cellulose, hemicellulose, lignin, extractive, and ash (Louren, 2015). The delignification process is intended to decompose lignin from waste using *Phanerochaete chrysosporus* to obtain free cellulose used saccharification process (Saritha et al., 2012). Waste that still contains lignin can inhibit the fermentation process. Lignin was separated by adding *Phanerochaete chrysosporus* from the medium of PDA (stock) and inoculated into the waste then incubated for 25 days at room temperature (27° C) until the *Phanerochaete chrysosporus* mycelium enveloped it as shown in Figure 3, after which it was dried in the sun.

Delignification results above show that the smallest lignin content with cellulose was relatively high using 3 tubes / 0.5 kg of waste. The next experiment using 6 tubes/ kg of Starch palm solid waste. Probably, *Phanerochaete chrysosporus* fungus 3 tubes / 0.5 kg of waste, peroxidase enzyme activity is maximum so that lignin decomposes higher (You & Percival Zhang, 2017).

Figure 4. Solid waste delignification by Phanerochaete chrysosporus

Delignification of research results was significantly decreased, the waste weight of 47.8% of 500 grams of palm sugar solid waste into 261 grams. However, the decomposed lignin was reduced by 55% from 14.04% to 6.32%. This may be due to the presence of lignin and its toxic derivatives. The scope of the research was only focusing on bioprocess, so the lignin separation was not done.

Delignification results were used as inserts on the saccharification process by using *Trichoderma viride* fungus. Common bioprocess biomass research into ethanol tends using cellulase enzymes (Maki et al., 2013). But economically the use of off-site cellulase enzymes (cellulase enzymes produced in different places) is not feasible because of the high price of pure cellulase enzymes.

In this study, saccharification was done with an on-site cellulase enzyme where the enzyme was produced by *Trichoderma viride* with an output substrate of the delignification process. It was expected that *Trichoderma viride* can produce sufficient cellulase enzymes to break down cellulose. Saccharification with the on-site cellulase enzyme was carried out at room temperature, making it more energy-efficient than saccharification with an off-site cellulase enzyme that requires about 48°C warming.

Glucose testing result at the stage of saccharification with *Trichoderma viride* showed that the highest glucose content was saccharification with 1 tube *Trichoderma viride* at 3 days curing at 1.95% / 0,5kg of delignification powder so that for subsequent experiments using 2 tubes Trichoderma / 1kg powder delignification. See **Table 3**).

Fermentation is divided into liquid and solid fermentation. In this research, liquid fermentation was carried out. Liquid fermentation is divided into two types: subsurface fermentation (batch process, fed-batch, and continuous process) and surface fermentation. The batch process is a fermentation once downloaded, the media and the inoculum are inserted simultaneously into a fermentation device and the product is taken at the end of the fermentation. Fed-batch is a combination of batch and continuous systems, a constant concentration of nutrients is fed into a fermentation device with a certain volume until the product is obtained close to the maximum. While continuous process, drainage of substrate and product taking were done continuously. Fermentation conducted in this research was a batch process. Before fermentation, all tools, media, and materials were sterilized beforehand.

Glassware sterilized oven at 160°C for 2 hours (Dion & Parker, 2013). While the media and hydrolyzate were sterilized using autoclave at 121°C and 2 atm for 15 minutes (Jin, 2015).

Fermentation was done by using *Saccharomyces Cerevisiae* microorganisms. *Saccharomyces Cerevisiae* is purified by scratch graft method and then incubated in the media to tilt as a stock culture. It can then be used by activating it in the media for italics as shown in the figure in the documentation attachment of research activities. The agar medium used was YMA (yeast malt agar) consisting of: yeast extract (3 g / L), malt extract (3 g / L), peptone (5 g / L), glucose (10 g / L), and agar (15 g / L).

The fermentation raw materials used were derived from saccharification products. The fermentation process was stopped by heating the tube containing the fermented samples in a water bath 60 ° C for 10 minutes (Mussatto et al., 2010). This heating aimed to stop the activity of *Saccharomyces Cerevisiae* without destroying existing ethanol. After that the sample was distilled at 80 ° C for 3 hours and the alcohol content was then analyzed (Ko et al., 2009). **Table 5** shows some data displaying of changing period of solid waste.

The first experiment the addition of mushrooms and yeasts was done gradually in accordance with the stages of each process. In the process of saccharification of 3-7 days, time shows the results were still fluctuating. Highest glucose concentration was obtained at 1.95% after 3 days. These results form the basis of the further fermentation process. The fermentation process was carried out with time variables of 3, 5 and 7 days at fermentation temperature of 30-35°C. The initial pH of the fermentation process of 4-5 showed relatively more stable results until the fermentation time is over. The purification process is then carried out by one level distillation at the boiling point of alcohol (78°C). Test results showed the obtained alcohol content was about 8.92%.

The second experiment of saccharification and fermentation were done in integratively (addition of *Trichoderma viride* fungus and yeast *Saccharomyces Cerevisiae* done simultaneously) (Rame et al., 2017). The operating conditions were all done similar to the first experiment with the weight of processed the raw material was about 6 kg. After the purification process, ethanol content obtained was about 4% (distillation).

The ethanol content itself represents the enzymatic efficiency of *Trichoderma viride* and Saccharomyces cerevisiae. In the presence of *Trichoderma viride* and Saccharomyces cerevisiae, glucose obtained was higher and will be used in the growth of *Trichoderma viride* and Saccharomyces cerevisiae. So at the end of the saccharification process, the resulting glucose was smaller and will reduce the efficiency of the saccharification process.

Trichoderma viride's optimal growth at the saccharification stage will increase the cellulase enzyme during saccharification (Posts et al., 2016). *Trichoderma viride*, however, tends to use glucose in its growth compared to the existing cellulose in solid waste of sago palm fiber. Though glucose is a product of the process of saccharification will then be used as a substrate in the fermentation process to become bioethanol, so it is necessary to develop *Trichoderma viride* optimally without reducing the resulting glucose product.

Table 5. The content of Material Analysis Result at Process stages

No	Data	Solid waste	Delignification	Saccharification	Fermentation
1	Weight	6 kgs	3,5 kgs	3 kgs	1,8 kgs
2	Cellulosa	31,61 %	30,49 %	-	-
3	Lignin	12,63 %	6,32 %	-	-
4	Glucose	-	-	1,95 %	-
5	Ethanol	-	-	-	8,92%

Judging from the results of ethanol content obtained, the gradual process of saccharification and fermentation is a better process than an integrated process. The availability of abundant waste of palm sugar, makes it possible for the production of pure cellulase enzyme with a solid waste substrate of sago palm fiber so that the saccharification efficiency will increase to convert cellulose into glucose.

4. CONCLUSION

The pretreatment and bioprocess of the solid waste fiber of pulp mill industries has the potential as a raw material for bioethanol production that is sustainable and renewable. The addition of *Trichoderma viride* and yeast Saccharomyces cereviceae gradually obtained alcohol content ± 8.92%. While the integrated process of saccharification and fermentation (addition of *Trichoderma viride* fungus and yeast Saccharomyces cereviceae done simultaneously) obtains alcohol content of about 4%. Judging from the results of ethanol content obtained, the process of saccharification and fermentation in stages is a better process than the integrated process. The operating conditions of bioprocess did not reach optimum, because of the presence of inhibitors that inhibit enzyme activity in each bioprocess stages. A simple distillation process (one level) is also one of the factors causing low levels of bioethanol obtained.

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REFERENCES

Awg-Adeni, D. S., Bujang, K. B., Hassan, M. A., Abd-Aziz, S .2013. Recovery of glucose from residual starch of sago hampas for bioethanol production. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/935852

Dion, M., Parker, W. 2013. Steam Sterilization Principles. *Pharmaceutical Engineering*, *33*(6), 1–8.

Hermiati, E., Mangunwidjaja, D., Sunarti, T. C., Suparno, O. 2010. Pemanfaatan biomassa lignoselulosa ampas tebu untuk produksi bioetanol, *29*(4), 121–130.

Jin, X. 2015. Breaking Down Cellulose Enzymatic Hydrolysis of Cellulose Cost of Enzymatic Hydrolysis. *Physics 240*, *1*(1), 1–2.

Ko, J. K., Bak, J. S., Jung, M. W., Lee, H. J., Choi, I. G., Kim, T. H., Kim, K. H. 2009. Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresource Technology*, *100*(19), 4374–4380. https://doi.org/10.1016/j.biortech.2009.04.026

Louren, A. 2015. The influence of heartwood on kraft delignification of Eucalyptus globulus wood The influence of heartwood on kraft delignification of Eucalyptus globulus wood Ana Carina dos Santos Lourenço, (October).

Maki, M., Leung, K. T., Qin, W. 2013. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass Page 2 sur 8. *International Journal of Biological Sciences*, *5*(5), 1–8. https://doi.org/10.7150/ijbs.5.500

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, *96*(6),673–686.https://doi.org/10.1016/j.biortech.2004.06.025

Mussatto, S. I., Dragone, G., Guimarães, P. M. R., Silva, J. P. A., Carneiro, L. M., Roberto, I. C., Teixeira, J. A. 2010. Technological trends, global market, and challenges of bio-ethanol production. *Biotechnology Advances*, *28*(6), 817–830. https://doi.org/10.1016/j.biotechadv.2010.07.001

Nigam, P. S., & Singh, A. 2011. Production of liquid biofuels from renewable resources. *Progress in*

Energy and Combustion Science, *37*(1), 52–68. https://doi.org/10.1016/j.pecs.2010.01.003

P. F. H. Harmsen, W. H. 2010. *Literature review of physical and chemical pretreatment processes for lignocellulosic biomass*. Retrieved from http://www.researchgate.net/publication/254853217_Literature_review_of_physical_and_chemical_pretreatment_processes_for_lignocellulosic_biomass

Posts, Emerald Biology, A., Archi, B. 2016. Fuels for Biofuels part 5 : free cellulases and cellulose hydrolysis, 2–4.

Rame, Harihastuti Nani, D. S. 2016. Optimalisasi Proses Sakarifikasi untuk Produksi Bioethanol Dari Limbah Padat Industri Pati Aren Menggunakan *Trichoderma viride* Berbasis Enzim Selulase On Site. In Proceeding Semnas Hasil Hasil Penelitian Pasca Sarjana (pp. 153–157). FKM UNDIP

Rame, Harihastuti, N., Djayanti, S. 2017. Integration Of Fermentation Sacharification Bioprocess In Optimizing Of Bioetanol Based Of Biomass Starch Aren Lignoselulosa Solid Waste. *Proceeding Seminar Nasional Teknologi Industri Hijau 2*, 263–266.

Saritha, M., Arora, A., & Lata. 2012. Biological Pretreatment of Lignocellulosic Substrates for Enhanced Delignification and Enzymatic Digestibility. *Indian Journal of Microbiology*, *52*(2), 122–130. https://doi.org/10.1007/s12088-011-0199-x

PRESS.SNI 0492. 2008. SNI 0492:2008 Pulp dan kayu - Cara uji kadar lignin - Metode Klason.

SNI 2891. 1992. sni-01-2891-1992-cara-uji-makanan-dan-minuman.pdf.

Sun, Y., Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production : a review q. *Bioresource Technology*, *83*(1), 1–11. https://doi.org/10.1016/S0960-8524(01)00212-7

Walker, G., Stewart, G. 2016. *Saccharomyces Cerevisiae* in the Production of Fermented Beverages. *Beverages*, *2*(4),30.https://doi.org/10.3390/beverages2040030

You, C., Percival Zhang, Y. H. 2017. Biomanufacturing by in vitro biosystems containing complex enzyme mixtures. *Process Biochemistry*, *52*, 106–114. https://doi.org/10.1016/j.procbio.2016.09.025

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Initial study of thiocyanate microbial degradation by isolates from polluted soil in gold mining area in Indonesia

Dwi Agustiyani Muslichah, Maman Rahmansyah*

Research Center for Biology, Indonesian Institute of Sciences Cibinong Science Center, Jl. Jakarta-Bogor, km 46 Cibinong 16911, West Java, Indonesia

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A B S T R A C T

This study was conducted to clarify the ability of denitrifying bacterial group utilized nitrogen (N) due to their ability to decompose N in thiocyanate structure. Thiocyanate is a chemical substance that categorized as a pollutant in the environment, this chemical mainly generated by some industrial activities. Denitrifying bacterial group obtained from bulk of sludge samples collected from the gold tailing, and some soil samples collected around the gold mining site. The samples were taken to the Microbiology Laboratory, Research Center for Biology, to be investigated. Samples were initially acclimatized by potassium nitrate (KNO_3), acetonitrile, and liquid waste or sludge. The result showed that denitrifying bacteria in the samples could utilize 60 to 90% of NO_3-N (nitrate) in 42 days incubation. Isolation process were then conducted in each samples, and four denitrification bacterial, named as AN, Ea, L7T5, and PETI-7 isolates were obtained. The isolates formerly were cultured in a denitrifying bacterial medium containing KSCN (Potassium Thiocyanate), amended with glucose and sodium acetate as carbon source. Those four isolates performed satisfactory in aerobic and anaerobic cultures medium for denitrifying process, and utilizing glucose and sodium acetate as co-carbon source, but all bacterial isolates were unable to use thiocyanate as a single carbon source. Thiocyanate degradation performed by the isolates through a simultaneous conversion along with denitrification process. This phenomenon turn to open the opportunity on role of application denitrifying bacteria become bioresources material in efforts to decompose thiocyanate.

1. INTRODUCTION

Thiocyanate ($N{\equiv}C{-}S^-$), is a chemical compound consist of sulfur and single carbon element (C) which is banded with nitrogen (N), and naturally is a simple nitrile compound. Thiocyanate certainly formed through a cyanide detoxification process. On the other hand, thiocyanate also produced in the metal processing industry activities and the soda beverage manufacturer (Wood, 1975; Kelly & Baker, 1990). Thiocyanate is less toxic than cyanide but more stable and thus more difficult to be degraded. Various research has been successfully done using chemical and biological technologies. Furthermore, the research should be improved to understand thiocyanate metabolism and scale up technologies for degradation mode from laboratory to full-scale mode (Gould et al., 2012). Challenge arise in environmental issues because about 80 percent of gold production activities used cyanide to

*Correspondence author. Tel.: +6221 87907604, 87907636
E-mail: maman02rahmansyah@gmail.com (M. Rahmansyah)

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produce 2500 tons of gold per year to fulfill the world market need (KSDEA, 2000).

Thiocyanate is an intermediate compound in the cyanide biodegradation process, restraint by bacterial action. *Neutrophilic thiobacilli* bacteria known to use thiocyanate as electron donor for hovering energy and CO_2 fixation process (Youatt, 1954; De Kruyff et al., 1957; Happold et al., 1954; Happold et al.,1958; Katayama and Kuraishi, 1978; Smith and Kelly, 1988). Based on research done by Sorokin et al (2001) stated that oxidizing thiocyanate bacteria can grow chemolitotrophycly in high acidic and alkaline media. In the further study, that organism group comprised to genus *Thialkalivibrio*, which play role as the sulfur oxidizing bacteria and able to degrade thiocyanate (Sorokin et al., 2002).

Research in thiocyanate waste management based on oxidizing thiocyanate bacterial utilization has not been completed. In the early publication, de Kruyff et al (1957) described that *Thiobacillus denitrificans* able to grow in aerobic and anaerobic culture to exploit thiocyanate due to nitrate function as electron acceptor, and completely undertake nitrate reduction to become N_2. Some of research work also inform that *thiocyanate-dependent denitrification* happen because of multispecies bacterial population (*consortium*) commonly used in sewage treatment systems (Andreoni et al., 1988). In the other study, bacterial species of *Thiobacillus thioparus* only reduce nitrate to nitrite because of thiocyanate present in the media at aerobic process. Thiocyanate as liquid waste was usually reduced through activated-sludge process that have content of certain microbes to degrade thiocyanate as source of nitrogen or sulfur for their metabolic action. Broman et al (2017) worked to remediate wastewater containing metal sulfide ore, and found microbial consortium populations aligning within *Flavobacterium*, *Thiobacillus*, and *Comamonadaceae* lineages.

Considering to the fact that thiocyanate function is not only a source of nitrogen and sulfur, but also has role as electron donor for some certain bacterial group, so in this physiological study, we focused on denitrifying bacteria potential to metabolize thiocyanate. Four denitrifying bacteria were successfully isolated from sludge sample, and soil sample collected from traditional gold mining area. Bacterial growth and its denitrifying progress in associated to thiocyanate decomposing capacity were verified in this study. Mostly, gold mining activities produce thiocyanate because of cyanide application. Face the fact in the thiocyanate waste, the purpose of this research might become affordable to utilize denitrifying bacteria as bioresources agent for thiocyanate removal.

2. METHODS

2.1 Materials

Denitrifying bacterial isolates were taken out from 1.) Sludge sample collected from tailing pond in PT ANTAM, Pongkor, Bogor District, West-Java, Indonesia; 2.) Soil sample around tailing pond; 3.) A soil sample from traditional mining area in Cikotok, Banten Province. All samples were analyzed in Ecology Laboratory, Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences. The isolation processes obtained four bacterial numbers and used in this study (Table 1).

2.2 Procedure

Liquid media for bacteria culture were prepared with 5 g Na_2CO_3; 2.5 g $NaHCO_3$; 1.25 g NaCl; 1 g K_2HPO_4; 100 mg $MgCl_2.6H_2O$; and 1.0 ml *trace element*. All the materials dissolved in 1000 mL aquadest, with pH 9.9. The media was autoclaved, in the temperature 121^{O}C, at 1 atm pressure, for 60 minutes. Augmented media such as Potassium Thiocyanate (KSCN), Potassium Nitrate (KNO_3), and Glucose were added at 500 -1000 mg, 1000 mg, and 2500 mg per liter media, respectively.

Denitrifying activities determined in liquid culture bacterial growth media by measuring NO_3^-N (nitrate) reduction, NO_2-N (nitrite) increase, KSCN (Potassium Thiocyanate) decline, and NH_4-N (ammonium) raise. Bacterial population measured with 436 nm optical density by spectrophotometric instrument (Carvalhal et al. 1991). Concentrations of ammonium, nitrate, nitrite, and thiocyanate were determined by Greenberg et al. (1992)

modified method. Chemical compounds were assessed through supernatant of liquid culture preparations.

Selected isolates (AN, Ea, L7E5, dan PETI-7) were tested in the followed media: **Media-1** contained liquid denitrifying bacteria and glucose; **Media-2** contained liquid denitrifying bacteria and thiocyanate (KSCN); **Media-3** contain liquid denitrifying bacteria and added with glucose and thiocyanate. One mL of isolates culture added with 9 mL media and placed in 300 mL- Erlenmeyer flask. Nitrogen (N_2) gas was flowed into the flask, 30 minutes, and the flask sealed tightly to make it anaerobic. The culture flask was shaken in the shaker with 100rpm and 7 days of incubation. The growth of denitrifying bacteria was checked daily by spectrophotometer instrument. Aerobic denitrification work was set at the same process, but without gas streamed into the flask.

Further verification of AN and Ea isolates was done to confirm the role of bacteria in degrading thiocyanates. Modified denitrifying media with KSCN, glucose, and sodium acetate (CH_3COONa) as the C source augmentation was intended to stimulate the denitrification process by bacteria. The incubation period was conducted at 20 days observation. Biological-cyanide Removal Efficiency (BRE) by bacterial isolates was calculated according to Mekuto et al (2016).

3. RESULT AND DISCUSSION

3.1 Acclimatization process

Dealing with denitrifying bacteria which is being capable to utilize thiocyanate as carbon sources (electron donors) (**Table 1**), therefore in the first step bacterial acclimatization was needed for cultures. Microbes were grown in liquid denitrification media, enriched with some organic carbon sources such as acetonitrile, acetic acid, and wastewater that were collected from tailing pond in gold mining activity. Through 42 days incubation, there was nitrate decreasing of nitrate content in the media culture with had varied decline grades at each sample source.

Decreasing of nitrate in media culture indicates that the existence of the denitrifying process was intent by microbes. Among each acclimatized culture of all samples, four denitrifying bacteria were obtained namely AN, Ea, L7T5, and PETI-7. The four bacterial isolates were tested for denitrification activity with carbon source of glucose and thiocyanate inserted in the media. The results showed that glucose renders as the carbon source and able to stimulate bacterial population (AN, Ea, L7T5, and PETI-7 Isolates) in liquid culture. Growth and metabolic processes in Media-1 presented the same pattern as those fully-fledged in Media-3, even though thiocyanate was present in Media-3 (**Figure 1**). Metabolite product of each bacterial isolate produced ammonium and nitrate which tend to be the same result during the incubation process.

Thiocyanate was inoculated into culture medium (Media-2) and leads to lowest bacterial growth because it had not contain glucose. These results designated that denitrifying bacteria in this study has very low potential to use KSCN without co-carbon source. Each isolate showed the same growth patterns on their metabolic activity when cultured with the media contain thiocyanate and lack of glucose for microbial carbon source.

Table 1. Denitrifying bacteria isolated from sludge and soil samples

Sample Source	NO_3^-N decrease in the sample for 42 days incubation, acclimated with:	Denitrifying Verification:		Isolates Code
		Growth in the media containing KSCN	Gas Produce	
Gold tailing pond in PT ANTAM	Acetonitrile	AN Isolate	+	AN
	Sludge	Ea Isolate	+	Ea
Cikotok Soil	Sludge	L7T5 Isolate	+	L7T5
Pongkor Soil	Sludge	PETI-7 Isolate	+	PETI-7

3.2 Thiocyanate verification

Based on the individual growth, Ea Isolate behaves differently in ammonium change along its metabolic activity. On the other side, AN isolate performed the highest growth in media enriched by glucose. Referring the above mentioned, therefore further study to AN and Ea Isolates was continued. Both isolates were then tested by using sodium acetate (CH_3COONa) as carbon sources to proof their growth and denitrification activity, as well as its ability in thiocyanate degradation. Results of the studies were listed in **Table 2**. Those bacteria isolates were able to grow and utilize CH_3COONa as the carbon source. Increasing of microbial population and decreasing nitrate concentration in the culture media become indicator of denitrifying process and simultaneously due to thiocyanate metabolism by bacteria. Ea isolate has more absorption compared to AN isolate, this is proof that Thiocyanate was more reduced by Ea isolates, especially with CH_3COONa present in the culture. The data below confirm that denitrifying bacteria become less for its capability to use thiocyanate due to single carbon source in the media without co-carbon source.

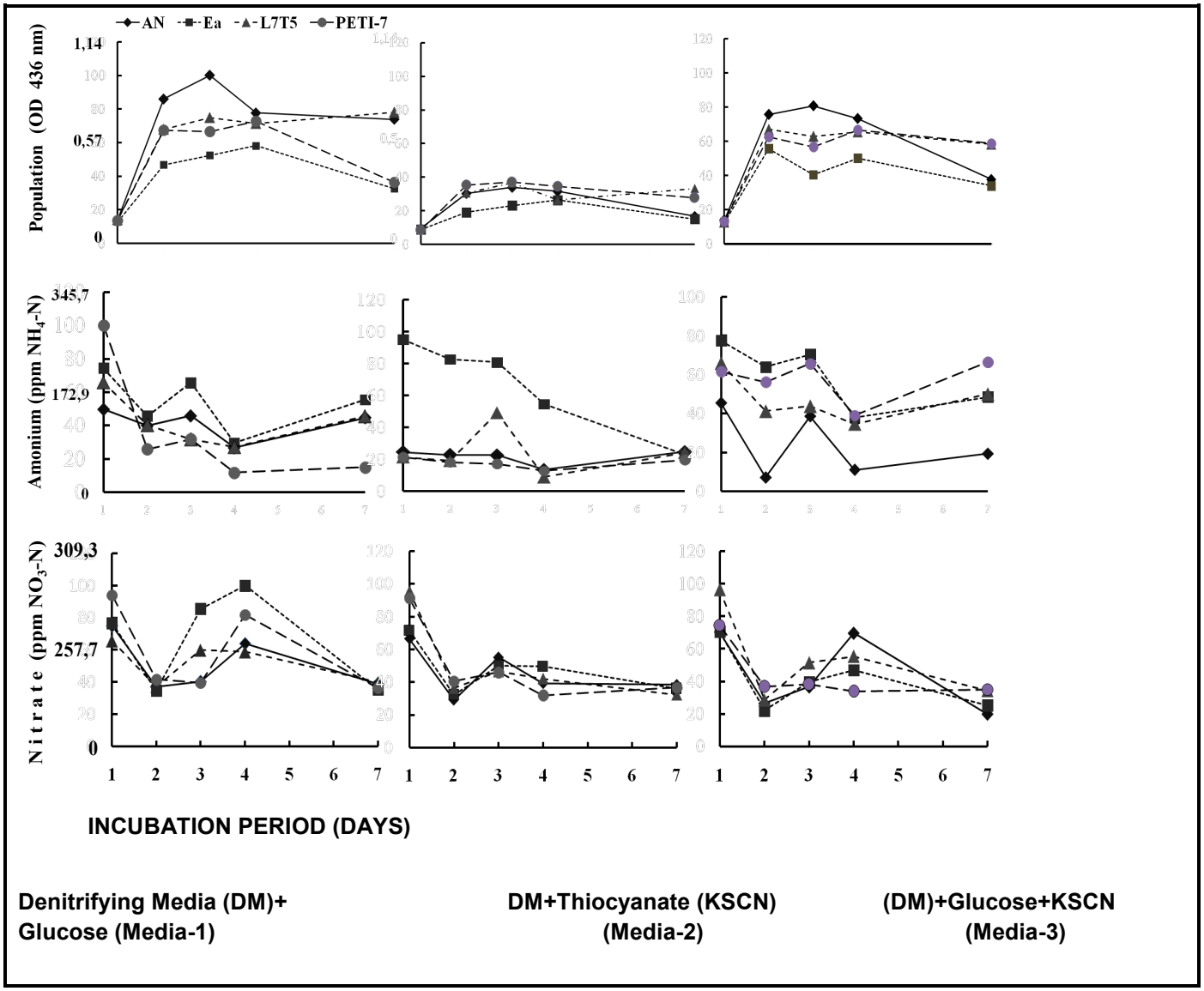

Figure 1. Growth, performance and N metabolic activity in anaerobic condition

Table 2. AN and Ea Isolates in denitrifying liquid media augmented by KSCN and Sodium Acetic Acid (CH3COONa) at twenty days incubation period

Parameters & incubation period		AN Isolate growth in: KSCN+CH_3COONa	Ea Isolate growth in: KSCN+CH_3COONa	Ea Isolate growth in: KSCN
Population (OD 436 nm)	3	0,066	0,160	0,072
	10	0,298	0,166	0,237
	20	1,432	0,585	0,366
Thiocyanate (ppm KSCN)	3	713,07	751,66	667,77
	10	708,75	639,12	642,44
	20	705,65	635,65	640,22
Ammonium (ppm NH_4-N)	3	5,80	10,34	7,80
	10	15,34	18,78	8,60
	20	23,95	0,00	26,75
Nitrate (ppm NO_3-N)	3	32,93	31,98	28,30
	10	5,67	1,60	24,60
	20	5,34	0,42	3,00
Nitrite (ppm NO_2-N)	3	0,551	0,949	0,130
	10	0,287	12,545	1,493
	20	1,135	14,106	3,455

As the complement of this study, denitrifying bacteria also assessed in an aerobic situation. Other studies had reported that denitrifying bacteria can grow both in anaerobically and aerobically (de Kruff et al., 1957: Andreoni et al., 1988). The results of aerobic culture were shown in **Figure 2**, which are indicating that bacterial growth utilizes thiocyanate.

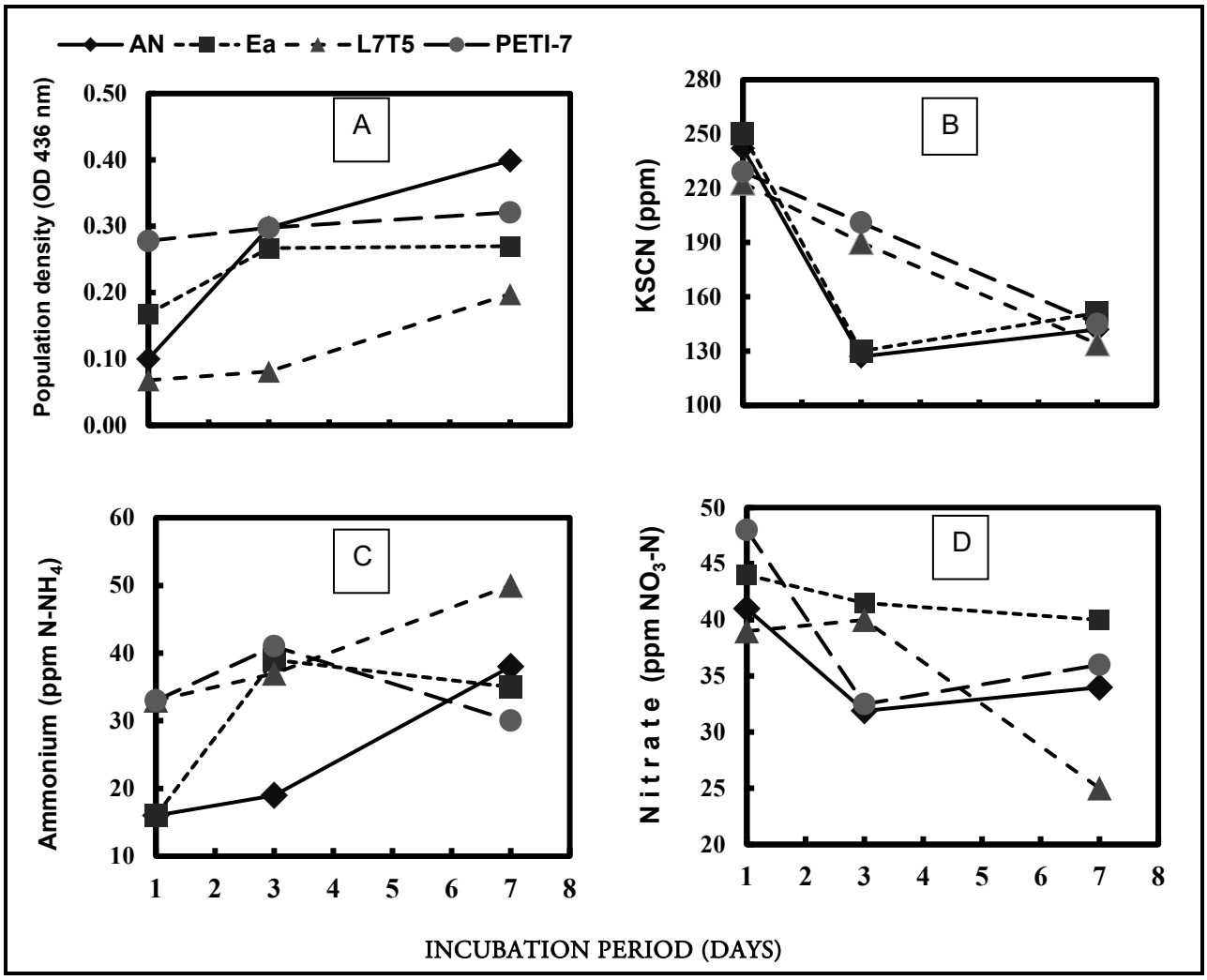

Figure 2. Growth pattern and metabolic activity of Isolates (AN, Ea, L7T5, and PETI-7) in an aerobic situation

Even if the growth tends to escalate in the 7-day incubation period (Figure 2A), denitrification activity was also had appropriate balance among nitrate decrease (Figure 2D) to ammonium increase (Figure 2C). Refer to the result shown that increasing of the bacterial population has opposite fact to nitrate and thiocyanate decline (Figure 2B) in the media. This phenomenon was designated strongly confirm that denitrifying bacteria had performed denitrification process simultaneously with thiocyanate degradation.

Correlation among parameters has been valued in **Table 3** and showed that AN and Ea Isolates are denitrifying bacteria which is capable to degrade thiocyanate. Bacterial population increased which is analogously become turns down of nitrate and thiocyanate content in the media. It means that the change of nitrate and thiocyanate might become strong indication that isolates are denitrifying bacteria which can reduce thiocyanate.

The presence of Ammonium (NH_4) accumulation was also strong signal of thiocyanate degradation due to denitrifying exertion. It has been reported that cyanate degradation reactions as due to microbial metabolism process is as follows: SCN- + 2 H_2O + 2½ O_2 ---> SO_4^{2-} + HCO_3^- + NH_3.

Denitrifying bacteria are heterotrophic and requiring organic carbon for an energy source, which play as an electron donor to perform denitrification reactions. In this situation, results of the study informed that four bacterial isolates growth and denitrification activity are inhibited

when the thiocyanate (KSCN) was used as a single carbon source, without the addition of glucose or sodium acetate. Growth inhibition occurred and it is signed to low growth in bacterial population (OD) when compared with glucose or sodium acetate added into media as carbon source (**Figure 1**).

Inhibition in the denitrification process was indicated by nitrate (NO_3) decrement, but denitrification more slothful due to sodium acetate absence in the media culture (**Table 2**). From this data could be concluded that the thiocyanate decrease was not because of used as energy source for bacterial growth, it was likely to be converted into other chemical compounds, and Budaev et al (2015) were mention in research exertion. Thiocyanate degradation was not observed in this study, but ammonium formation in media culture become virtuous evidence of degradation.

AN Isolate was increased in the population rate and correlated to ammonium accumulation (-Avs.C-) in the culture medium. The value among thiocyanate disappears to ammonium arise (-Bvs.C-), and also to nitrate decrease (-Bvs.D-) in the media were strongly correlated. Based on the data above, there was affirmation for denitrifying bacteria in the study which are able to degrade thiocyanate. Ea Isolates had almost the same metabolic pathway compared to AN performance, but Ea Isolate had tendentiously stronger to degrade thiocyanate. However, all of four isolates actually had almost similar ability to degrade thiocyanate around 3 to 8 percent in Biological Removal Efficiency (BRE) per 24 hour degradation (**Figure 3**).

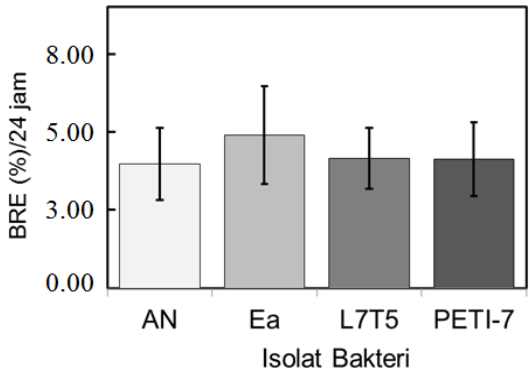

Figure 3. Biological Removal Efficiency (BRE) of Denitrifying Bacteria in the media contain 500 mg/L KSCN

Refer to other other research, thiocyanate commonly becomes energy source for chemolithotrophic sulfuric oxidizing bacteria. Thiocyanate was degraded through two different pathways response. The first reaction works by breaking C-S bonds to cyanate intermediate form ($N{\equiv}C{-}O^{-}$), and if any bicarbonate present then it turns into ammonia and CO_2 by cyanase enzyme response (Youatt, 1954; Happold et al., 1958). The second reaction was hydrolysis process of the nitrile bond ($N{\equiv}C$) which are forms of carbonyl sulfite (S=C=O) and ammonia (Katayama et al., 1992; Katayama et al., 1993; Katayama et al., 1998). Carbonyl sulfite hydrolyzed further into sulfites and CO_2. This sulfite can be utilized as a source of energy and electron donor by autotrophic bacteria for growth.

Table 3. Correlation among parameters based on AN and Ea Isolates in denitrifying liquid media containing KSCN and Sodium Acetic Acid (CH_3COONa)

Parameters (n-1= 2; $p_{0,10}$ & $p_{0,02}$ **r =** 0,900 & 0,980)	AN Isolate growth in: [KSCN+CH_3COONa]			Ea Isolate growth in: [KSCN+CH_3COONa]		[KSCN]
	-Avs^C-	-BvsC-	-BvsD-	-AvsC-	-BvsD-	-AvsB-
Population density - A -						
KSCN degradation - B -	0.923*	0.914*	0.914*	0.982**	0.998**	-0.929*
Ammonium increases - C -						
Nitrate decreases - D -						

* correlated; ** strongly correlated; ^vs. = versus

Three thiocyanate-oxidizing bacterial strains, *Burkholderia* sp., *Chryseobacterium* sp., and *Ralstonia* sp. were isolated by Huang et al (2013) from the activated sludge inside coke wastewater treatment plant. Bacterial biodegradation proceeded individually with the highest rate and utilize thiocyanate as sole carbon source at pH 7.7 and 35^{O}C. This study may provide design for large scale operation of aerobic bioreactor for thiocyanate treatment.

Thiocyanate is also established to become nitrogen sources for algae to produce lipid. Two cultivation methods were developed by Ryu et al (2014) throughout the consortium mode based on algae and bacteria. There are a lithoautotrophic and a photoautotrophic mode. Thiocyanate hydrolysis and a nitrification was occurred under the first (lithoautotrophic) condition, while the oxidized forms of nitrogen were assimilated by the photoautotrophic consortium, and lipids were produced under the second condition.

Usage of microbes for requiring biological degradation of thiocyanate from contaminated wastewater had been successful at the laboratory investigation by van Zyl et al (2015). In the further analysis revealed that the presence of solids could decrease microbial diversity; but it showed that many organisms have genes for denitrification and sulfur oxidation, otherwise the only one genus of *Thiobacillus* sp. present in the culture growth (Rahman et al., 2016). Nitrifying bacteria achieved from this work could be improved as above mode technique to improve application in bioremediation.

4. CONCLUSION

Results of this study showed that four denitrifying bacterial isolates can be alive for aerobic and anaerobic denitrification process. Those bacteria could use glucose and sodium acetate as trigger to convert thiocyanate but were unable to use thiocyanate as the single carbon source. Aerobic or anaerobic denitrification activities on both alive cultures could metabolize organic carbon sources of glucose and sodium acetate but were unable to exploit thiocyanate as the only single carbon source. Decreasing of thiocyanate in the media culture mostly performed by denitrifying bacterial growth through the process of degradation or conversion into certain compounds and ammonium. Denitrification process and thiocyanate degradation can occur simultaneously, both in aerobically and anaerobically conditions. Bacterial capacity to degrade thiocyanate is almost the same in all four bacterial isolates. Handling of thiocyanate waste can be manipulated by utilizing the denitrifying bacteria with carbon source induction. Manipulation of carbon sources can be pursued through the utilization of organic carbon sources that are widely available in nature such as cellulose, hemicellulose, lignocellulose and other sources as agricultural waste, and further research is needed.

REFERENCES

Andreoni V., Ferrari A., Pagani A., Sorlini C., Tandoi V., Treccani V., 1988, Thiocyanate degradation by denitrifying mixed cultures of bacteria, Ann Microbiol Enzimol 38:193-200.

Broman E., Jawad A., Wu X., Christel S., Ni G., Lopez-Fernandez M., Sundkvist JE., Dopson M., 2017, Low temperature, autotrophic microbial denitrification using thiosulfate or thiocyanate as electron donor, Biodegradation 28:287-301.

Budaev SL., Batoeva AA., Tsybikova BA., 2015, Degradation of thiocyanate in aqueous solution by persulfate activated ferric ion, Minerals Engineering 81: 88–95.

Carvalhal MLC., Oliveira MS., Alterthum F., 1991, An economical and time saving alternative to the most-probable-number method for the enumeration of microorganisms, Journal of Microbiological Methods 14:165-170.

Greenberg AE., Clesceri LS., Eaton AD., 1992, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington DC.

Gould WD., King M., Mohapatra BR., Cameron RA., Kapoor A., Koren DW., 2012, A critical review on

destruction of thiocyanate in mining effluents, Minerals Engineering 34:38-47.

Happold FC., Johnstone KI., Roger HS., Youatt JB., 1954, The isolation and characteristics of an organisms oxidizing thiocyanate, J Gen Microbiol 10:261-266.

Happold FC., Jones GL., Pratt DB., 1958, Utilization of thiocyanate by Thiobacillus thioparus and T. thiocyano-oxidans, Nature 182:266-267.

Huang H., Feng C., Pan X., Wu H., Yuan R., Wu C., Wei C., 2013, Thiocyanate oxidation by coculture from a coke wastewater treatment plant, Journal of Biomaterials and Nano-biotechnology 4:37-46. http://dx.doi.org/10.4236/jbnb. 2013.42A005

Katayama Y., Kuraishi H., 1978, Characteristics of Thiobacillus thioparus and its thiocyanate assimilation, Can J Microbiol 24:804-810.

Katayama Y., Kanagawa T., Kuraishi H., 1993, Emission of carbonyl sulfide by Thiobacillus thioparus grown with thiocyanate in pure and mixed cultures, FEMS Microbiol Lett 114:223-228.

Katayama Y., Matsushita Y., Kaneko M., Kondo M., Mizuno T., Nyunoya H., 1998, Cloning of genes coding for the subunits of thiocyanate hydrolase of Thiobacillus thioparus THI 115 and their evolutionary relationships to nitrile hydratase, J Bacteriol 180:2583-2589.

Katayama Y., Narahara Y., Inoue Y., Amano F., Kanagawa T, Kuraishi H., 1992. A thiocyanate hydrolase of Thiobacillus thioparus. A novel enzyme catalyzing the formation of carbonyl sulfide from thiocyanate, J Biol Chem 267:9170-9175.

Kelly DP., Baker SC., 1990. The organosulphur cycle: aerobic and anaerobic processes leading to turnover of C1– sulfur compounds, FEMS Microbiol Rev 87:241-246.

de Kruyff CD., van der Walt JI., Schwartz, 1957, The utilization of thiocyanate and nitrate by Thiobacilli, Antonie van Leeuwenhoek 23:305-316.

KSDEA (Kementrian Sumberdaya dan Energi Australia), 2008, Pengelolaan Sianida. Seri Buku Praktek Kerja Unggulan Program Pembangunan Berkelanjutan untuk Industri Pertambangan. Pemerintahan Persemakmur-an Australia. 99 h. www.ret.gov.au/sdmining, Diakses 12 Juni 2017.

Mekuto L., Ntwampe, SKO., Kena M, Golela MT., Amodu OS., 2016, Free cyanide and thiocyanate biodegradation by Pseudomonas aeruginosa STK 03 capable of heterotrophic nitrification under alkaline conditions, Short Reports, 3 Biotech (2016) 6:6. DOI 10.1007/s13205-015-0317-2.

Rahman SF., Kantor RS., Huddy R., Thomas BC., van Zyl AW., Harrison STL., Banfield JF., 2016, Genome resolved metagenomics of a bioremediation system for degradation of thiocyanate in mine water containing suspended solid tailings, Microbiology Open, 2017;6:e446. https://doi.org/10.1002/mbo3.446

Ryu BG., Kim J., Yoo G., Lim JT., Kim W., Han JI., Yang JW., 2014, Microalgae-mediated simultaneous treatment of toxic thiocyanate and production of biodiesel. Bioresource Technol 158:166–173. doi: 10.1016/j.biortech.2014.01.128 Cross Ref Google Scholar.

Smith NA., Kelly DP., 1988, Oxidation of carbon disulfide as the sole source of energy for the autotrophic growth of Thiobacillus thioparus strain TK-m, J Gen Microbiol 134: 3041-3048.

Sorokin DY., Lysenko AM., Mityushina LL., Tourova TP., Jones BE., Rainey FA., Robertson LA., Kuenen JG., 2001, Thioalkalimicrobium sibiricum, Thioalkali-microbium aerophilum gen. nov., sp. and Thialkalivibrio versutus, Thialkalivi-brionitratis, Thialkalivibrio denitrificans gen. nov., sp. nov., new obligately alkaliphilic and obligately chemolithoautotrophic sulfur-oxidizing bacteria from soda lakes, Int J Syst Evol Microbiol 5:565-580.

Sorokin DY., Tourova TP., Lysenko AM., Mityushina LL., Kuenen JG., 2002, Thialkalivibrio thiocyanoxidans sp. nov. and Thialkalivibrio paradoxus sp. nov., novel alkaliphilic, obligately autotrophic, sulfur-oxidizing bacteria from soda lakes capable of growth on thiocyanate, Int J Syst Evol Microbiol 52:657-664.

Wood JL., 1975, Biochemistry: Thiocyanic Acid and its Derivaties, pp. 156-252. Edited by AA. Newman, London, New York, San Francisco: Academic Press.

Youatt JB., 1954, Studies on the metabolism of Thiobacillus thiocyanooxidans, J Gen Microbiol 11:139-149.

van Zyl AW., Huddy R., Harrison STL., van Hille RP., 2015, Characterization of the complex microbial community associated with the ASTER™ thiocyanate biodegradation system, Minerals Engineering 76:65–71.

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Influence of operational condition on the performance of halotolerant enriched - activated sludge system for treating medium salinity roasted peanut wastewater

Rustiana Yuliasni, Nanik Indah S, Kukuh Aryo W, Nani Harihastuti

Balai Besar Teknologi Pencegahan Pencemaran Industri, Jl. Ki Mangunsarkoro No.6, Karangkidul, Semarang Tengah, Kota Semarang, Jawa Tengah, Indonesia 50136

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A B S T R A C T

This research aimed to investigate the influence of operational condition on the performance of halotolerant enriched - activated sludge system for treating high organic wastewater with medium salinity from roasted peanut industry. Roasted peanut wastewater with VLR ranged from 0.268 to 4.7 kg COD/m^3.day and Chloride concentration ranged between 1582 - 4392 mg/L was treated continuously for almost 77 days. Two identical reactors with Volume 25 L, namely R1 a conventional Activated Sludge (AS) System and R2, a halotolerant enriched-AS. Both reactors were running with the operational condition: HRT (9 h to 46 h) and MLSS (1000-6000 mg/L). Compared to conventional AS system, Halotolerant enriched Activated sludge system could remove an average of 86.7% COD, compared with conventional AS which was 85.7%. Average COD effluent of Halotolerant Enriched-Activated Sludge was also considerably lower, which was 127 mg/L, than conventional AS which was 150 mg/L. Halotolerant enriched-activated sludge also produced less sludge, giving a high F/M ratio (4.9) compared with conventional AS (3.5). In order to make effluent fulfilled stream standard regulation (at central java region COD was<150 mg/L), the favorable operational condition for both reactors would be at VLR 0.268 to 2.03 kg COD, HRT was 25 hours HRT, with MLSS was 2584 – 3956 mg/L and maximum chloride concentration 1920 mg/L

1. INTRODUCTION

Food and Beverage industry falls under one of Indonesia's priority industry, and its development toward the green industry is one of the main concern of the Ministry of Industry, The Republic of Indonesia. Sustainable industrial technology in the Food and Beverage industry has been developed to support its growth. One aspect to be done toward the application of sustainable industrial technology was action related to pollution prevention within the process as well as after the process in order to minimize industrial pollution. Peanut-roasted industry specifically has difficulties to implement green industry technology especially for its wastewater treatment to fulfill stream standard regulation. Peanut-roasted industry wastewater's characterized with high organic but the moderate saline content and the problem related to it is that how to treat the wastewater using high rate technology so it can fulfil the stream standard regulation.

For that matters, high-rate technology, such as activated sludge technology, is preferable because of the

*Correspondence author. Tel.: +624 8316315
E-mail: ***rustianay@yahoo.com*** (Rustiana Yuliasni)

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space limitation for building wastewater treatment plant (WWTP). The more high-rate technology is, the less space needed. Peanut roasted has high organic content, up to 9000 mg/L COD, but this organic content is still not feasible to be treated using anaerobic technology. Anaerobic technology, such as UASB (Marlena et al., 2018) and immobilized UAF (Handayani et al. 2016) , need high COD inlet, long start up and retention time to achieve robust performance. Apart from that ,The combination of both anaerobic-aerobic can also be an option (Yuliasni et al., 2017) to treat more complex wastewater. However, despite its intense energy demand and massive sludge production, AS technology is still the most chosen technology for industrial WWTP, whether used as single technology or combination with other technology (Lefebvre & Moletta, 2006).

For successful full scale application, important parameters such as Mean Residence Time (SRT) , HRT , F/M ratio, MLSS/ MLVSS and DO, should be determined (Durai & Rajasimman, 2011). Hence, the objective of this research is to investigate the effect of operational condition in the performance of halotolerant enriched - activated sludge system, compare with conventional activated sludge for treating moderate salinity peanut roasted wastewater.

2. MATERIAL AND METHODS

2.1. MATERIAL

2.1.1. Activated Sludge Reactor

Two identical activated sludge (AS) reactors made of aluminum were set up, namely R1 and R2. R1 was an activated sludge system without the addition of halotolerant inoculum and R2 was an AS reactor with the addition of halotolerant inoculum. Activated sludge system consists of a feeding tank (V: 50 L), a continuous ditch oval shaped activated sludge reactor which design refers to Oxidation Ditch (V: 25 L), and a clarifier tank (V: 25 L). An aeration unit with diffusers inserted inside the AS reactor.

The activated sludge reactor set up is presented in figure 1 below.

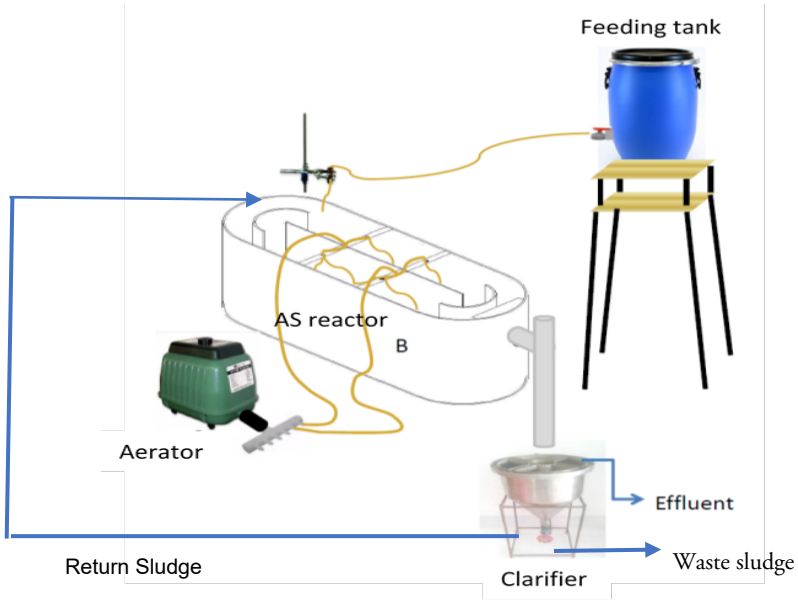

Figure 1. Activated Sludge Reactor Set Up (Two identical reactors called R1 and R2)

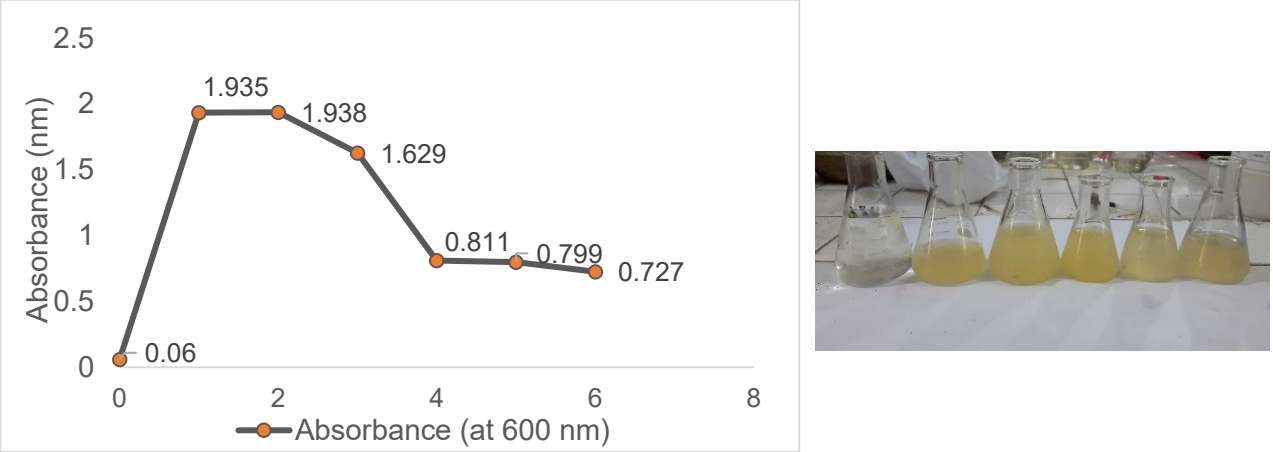

Figure 2. Optical Density (OD) measurement (left) and visual halotolerant inoculum (right)

Table 1. Wastewater typical composition

No	Parameter	Unit	Concentration
1	COD	mg/L	3,587 - 1,180
2	BOD_5	mg/L	164 - 539
3	TSS	mg/L	1,288 – 1,384
4	DHL	mS/cm	5.56 - 9,210
5	Total Nitrogen	mg/L	61.74 - 79.8
6	Total Phosphate	mg/L	0.486 – 1.476
7	H_2S	mg/L	0.152 - 0.373
8	Chloride	mg/L	1,840 – 3,375
9	Phenol	mg/L	0.004 - 0.778

2.1.2. Sludge seed and halotolerant microbial community

Sludge seed was derived from an already established activated sludge WWTP from bakery industry, with initial MLSS concentration was > 5000 mg/L.

The halotolerant inoculum was derived from a dormant seed frozen at 4^0 C, taken from the salt pond in Pati, Central Java. This seed was then inoculated in halotolerant media (refers to ATCC 1097 media) to reach a volume of 5 L, while constantly aerated prior its addition to the R2 reactor. Microbial growth was monitored qualitatively using Optical Density (OD) measurement at wavelength 600 nm, as presented in figure 1 (left). The inoculum used in this experiment was inoculum that inoculated for 24 hours, at the highest value of absorbance at 1.935. Highest absorbance value was an indication of microbial maximum growth (μ_{max})(Melanie et al., 2018).

2.1.3. High organic wastewater with medium salinity

Wastewater used for this experiment was untreated roasted peanut industry wastewater, with the composition shown in table 1.

2.2. METHODS

2.2.1. Experimental Method

8 liter seed AS sludge was added initially added to both of R1 and R2 reactors then adjust with water until volume reached 25 L, while constantly aerated. 100 mg/L sugar was initially added as a carbon source, as well as urea and buffer phosphate, with composition C: N: P = 100:5:1. Microbial growth was monitored with Sludge Volume 30% (SV_{30}) and Mixed Liquor Suspended Solid (MLSS) analysis.

The sludge was ready to be used when SV_{30} was 30% and MLSS was > 2000 mg/L. After sludge was ready, in the R2 reactor, 2.5 L AS sludge withdrawn then refilled with halotolerant inoculum. At day 33, at R2, 1 L halotolerant bacteria was added.

Wastewater was initially stored in the feeding tank, ready to be used. Feeding tank flowed to the AS reactor by gravitation. The treated water overflowed from AS reactor to the clarifier and then to the effluent. To maintain a constant flow, the feeding rate was manually adjusted every day during the experiment to achieve the desired HRT variable. Wastewater feed concentration was also adjusted to reach the desirable COD concentration in the range of 1000 – 3000 mg/L. Inlet and outlet were periodically sampled and analyzed, for parameter: Chemical Oxygen Demand (COD), pH and chloride. SV_{30} and MLSS were also periodically measured. Dissolved Oxygen (DO) was maintained at a minimum of 2.0 mg/L with adjustment of aeration flowrate. pH inlet was 5.8 – 7.5, while outlet pH was 7.1 - 8.5. The experiment was carried out in ambient temperature ± 32^0 C.

COD, DO, chloride and MLVSS was analyzed using the procedure in Standard Methods (SM) for the Examination of Water and Wastewater. Parameter collected on-site such as pH was measured using a pH meter (Krisbow KW06-744).

3. RESULT AND DISCUSSION

3.1. Overall Performance of R1 and R2 (halotolerant enriched)

Activated Sludge system was applied to treat real roasted-peanut wastewater that contains high organic with moderate salt content. The R1 reactor was a conventional activated sludge system, whereas R2 was an activated sludge enriched with halotolerant inoculum. Both R1 and R2 (halotolerant enriched) reactor was running for 77 days. To determine the overall performance of reactors and the effect of halotolerant inoculum addition in AS system, different operation condition was tested such as chloride content, Volumetric Loading Rate (VLR), and Hydraulic Retention Time (HRT). Chloride concentration ranged between 1500 – 4000 mg/L (0.1 -0.4%) , Volumetric Loading Rate ranged between 0.3 – 4.7 kg/m3.day, and HRT was initially set at 45 hours then shortened to ± 9 hours. These operational conditions were maintained at low strength during the initial stage (day 0 to 33 day), then raised gradually (from day 34 to 77). The performance of the reactor is calculated based on COD removal efficiency (%) and COD effluent concentration profile.

Figure 2 depicts the effect of chloride concentration on both R1 and R2 reactors performance. Initially, wastewater with a chloride concentration of 2300 - 2500 mg/L was fed to the reactor for 5 days, then at day 7 to 33, chloride concentrations were decreased to 1500 – 2000 mg/L. After day 33 to day 77, chloride concentrations were raised to 4000 – 4400 mg/L. Throughout the experiment period, both reactors showed a robust performance. The performance of both R1 and the R2 reactor was almost similar, with R2 has a slightly higher performance by 1%, and were uninhibited by chloride concentration < 4000 mg/L (0.4%). The average removal efficiency of R1 was 85.7% and R2 was 86.7%. Similar to (Kargi & Dinçer, 1998), (Wang et al., 2005), (Lefebvre & Moletta, 2006) and (Kargi & Uygur, 1996), that found out that NaCl concentration start to affect the Activated sludge performance when the concentration was above 1%, because of high salt concentration (>1% salt) causes plasmolysis or loss of activity of cells (Dinçer & Kargi, 2001). Furthermore, at medium chloride concentration, the addition of 1L halotolerant at day 33 did not improve the performance of the R2 reactor in terms of COD removal efficiency.

Figure 3 shows the effect of Volumetric Loading Rate (VLR) on R1 and R2 reactors performance. Volumetric loading rate was initially maintained at 0.3 – 1 kg COD/m^3.day at day 0 to 33 then later gradually increased at 2 – 4.7 kg COD /m^3.day until day 77. Figure 3 shows that COD removal efficiency of both reactors was almost similar. The maximum removal (94.2%) achieved when VLR was 2.98 kg COD/m^3.day. At VLR 4.7 kg COD/m^3.day, COD removal was still considerably high (90.6%). This result was higher, when compares with

conventional AS system, that typically has maximum VLR < 2 kg COD/m^3.day. Other study proved that AS system able to treat up to 5.9 kg COD /m^3 (Petruccioli et al., 2002) with 90% COD removal. VLR can be higher for other aerobic technology such as rotating biological ontactor; > 12 kg COD /m^3.day) (Dinçer & Kargi, 2001), aerobic granule; up to 9 kg COD/m^3.day (Moy, Tay, Toh, Liu, & Tay, 2002) or membrane bioreactor (Trussell et al., 2006).

To identify the ability of both reactors fulfilled minimum COD effluent discharge standard regulation, COD effluent were compared with the effluent standard (150 mg/L, according to Central Java regulation) (Figure 4). At day 14 to day 48, when the system was stable and VLR started to increase from 0.268 to 2.03 kg COD, COD effluent concentrations were below the threshold, which means very favorable to discharge the effluent to the environment. However, when VLR raised from 2.03 to 4.7 kg COD/m^3.day, COD effluents were exceeded the threshold. Throughout the experiment, the average COD effluent of R1 was 150 mg/L, whereas R2 was 127 mg/L.

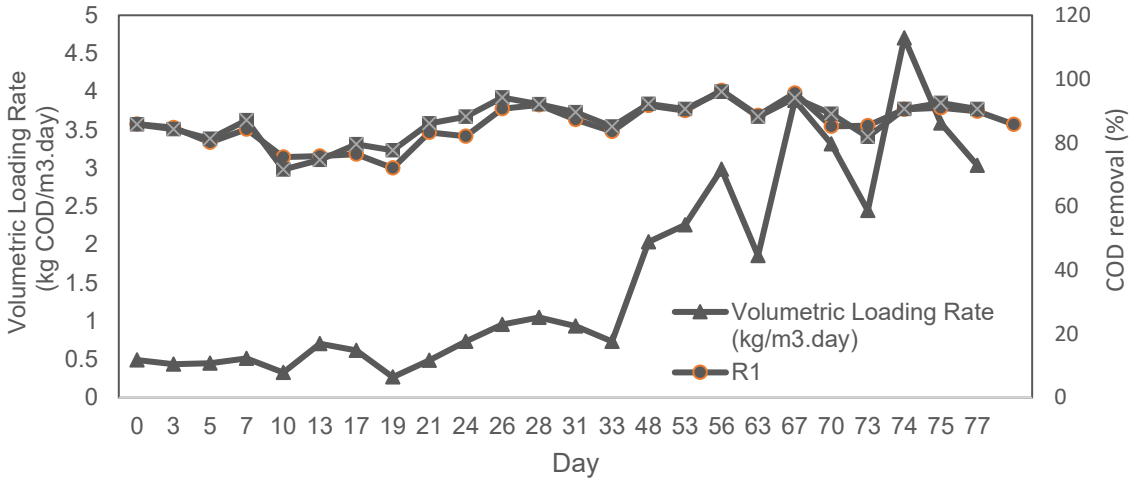

Figure 3. Effect of Volumetric Loading Rate on the COD removal of R1 and R2

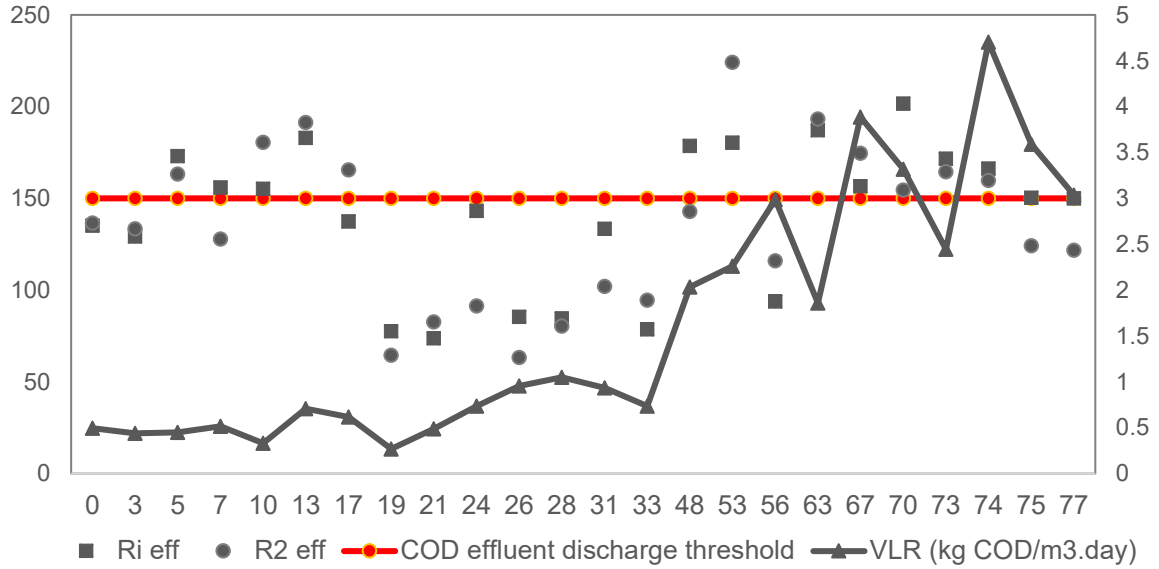

Figure 4. Effect of Volumetric Loading Rate on R1 and R2 effluent concentration

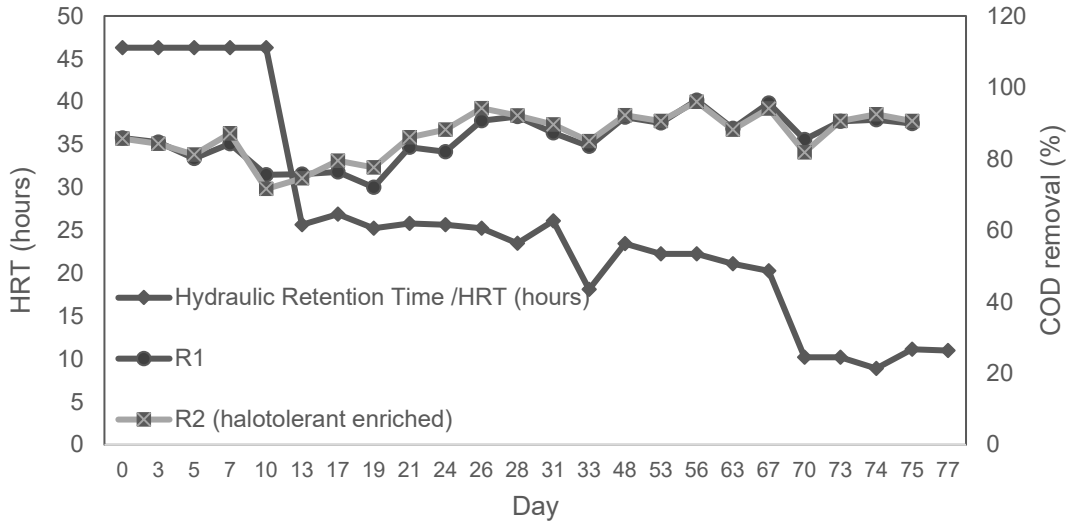

Figure 5. Effect of Hydraulic Retention Time on the COD removal of R1 and R2

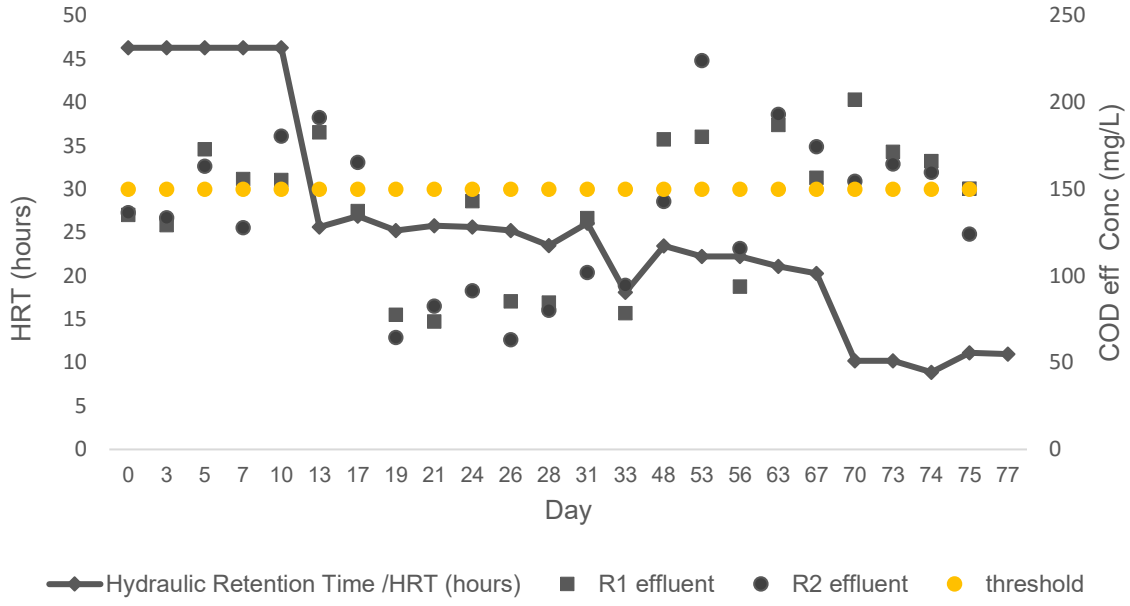

Figure 6. Effect of Hydraulic Retention Time on COD effluent concentration of R1 and R2

Figure 5 shows how hydraulic retention time (HRT) affects the performance of R1 and R2 reactor. The graph shows that in terms of COD removal efficiency, both reactors shows robust performance and did not show different results, even when HRT was shortened from 45 hours to 9 hours. This result when compared to other studies, such as Pala & Tokat (2002), González et al (2007), or Kim et al (2005) was considerably better. However, when the results were compared to the standard effluent threshold, 25 hours of HRT was favorable, with MLSS was maintained between 2584 – 3956 mg/L. At 25 hours, COD removal efficiency of both reactors were >90% and COD effluent could achieve the minimum threshold below 150 mg/L COD (Figure 6).5 shows how hydraulic retention

time (HRT) affects the performance of R1 and R2 reactor. The graph shows that in terms of COD removal efficiency, both reactors shows robust performance and did not show different results, even when HRT was shortened from 45 hours to 9 hours. This result when compared to other studies, such as Pala & Tokat (2002), González et al (2007), or Kim et al (2005) was considerably better. However, when the results were compared to the standard effluent threshold, 25 hours of HRT was favorable, with MLSS was maintained between 2584 – 3956 mg/L. At 25 hours, COD removal efficiency of both reactors were >90% and COD effluent could achieve the minimum threshold below 150 mg/L COD (Figure 6).

3.2. Sludge characteristic in R1 and R2 reactors

Reactor R1 and R2 have pretty similar performance in terms of COD removal and COD effluent concentration profile. However, when it comes to microbial quantity and quality, R1 and R2 have a different characteristic in F/M ratio. F/M ratio is very important to evaluate performance in the aerobic system. Normally for best practice, F/M ratio ranges between 0.2 < F/M < 0.5 for conventional AS, for Completely mixed (CSTR) ranges between 0.2 < F/M < 1.0 and for High rate, range between 0.4 < F/M < 1.5 (Water Resources Division, 2017). The higher F/M ratio value, the better performance of AS, because to remove a certain level of COD will need less microorganisms and subsequently reduce Waste Activated Sludge (WAS) quantity and reduce the cost of solid handling as well.

In this study, F/M ratio was calculated based on amount COD inlet (kg/m^3) per MLVSS concentration in the reactor (kg/m^3) (as shown in figure 7). At day 0 to day 28, when 10% halotolerant was added to the R2 reactor, F/M of both reactors were similar. After the addition of 1 L halotolerant in R2 (14% v/v), R2 has a higher F/M ratio than R2, almost 1.5: 1. R1 has the highest F/M ratio at 3.9, and R2 at 4.7.

Feeding with similar loading rate, chloride concentration and operated with similar HRT, both reactor R1 and R2 have similar COD removal and COD effluent profile, except that R2 need fewer microorganisms than R1. This result was against Sivaprakasam et al (2008) and Kargi & Uygur (1996) that claimed that to treat saline wastewater a lower F/M should be maintained in order to achieve > 90% COD removal. Having said that the salt content in this experiment was < 0.4%, it was likely that at salt content <0.4%, F/M ratio was not affecting the reactor's COD removal but only affecting sludge density.

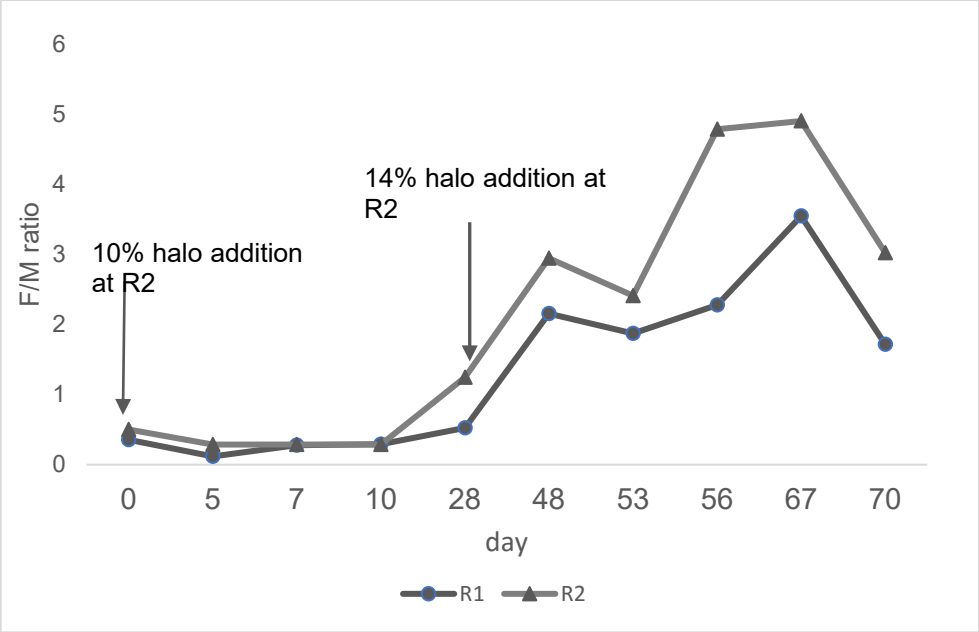

Figure 7. Comparison of the F/M ratio of R1 and R2

4. CONCLUSION

The addition of halotolerant Inoculum in conventional activated sludge system to treat high organic wastewater with medium salt content gives effect on the performance of activated sludge (AS) system. Roasted peanut wastewater with VLR ranges from 0.268 to 4.7 kg COD/m3.day and Chloride concentration ranges between 1582 - 4392 mg/L was treated efficiently until COD removal reaches a maximum of 94.6%. Compared to conventional AS system, Halotolerant enriched activated sludge system could remove an average of 86.7% COD, compared with conventional AS which was 85.7%. Average COD effluent of Halotolerant enriched-Activated sludge was also considerably lower, which was 127 mg/L, than conventional AS which was 150 mg/L. Halotolerant enriched-Activated sludge also produced less sludge, giving a high F/M ratio (4.9) compared with conventional AS (3.5).

For practical use, for both conventional and halotolerant-enriched AS system, in order to make effluent fulfilled stream standard regulation (at central java region COD was < 250 mg/L), the favorable operational condition would be VLR 0.268 to 2.03 kg COD, HRT was 25 hours HRT; with MLSS was 2584 – 3956 mg/L and maximum chloride concentration was 1920 mg/L.

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REFERENCES

Dinçer, A. R., Kargi, F. (2001). Performance of rotating biological disc system treating saline wastewater. *Process Biochemistry*, *36*(8–9), 901–906. https://doi.org/10.1016/S0032-9592(00)00287-9

Durai, G. (Annamalai U., & Rajasimman, M. (nnamalai U. (2011). Biological Treatment of Tanery Wastewater- A Review. *Journal of Environmental Science and Technology*, *4*, 1.

González, S., Petrovic, M., Barceló, D. (2007). Removal of a broad range of surfactants from municipal wastewater - Comparison between membrane bioreactor and conventional activated sludge treatment. *Chemosphere*, *67*(2), 335–343. https://doi.org/10.1016/j.chemosphere.2006.09.056

Handayani, N. I., Moenir, M., Setianingsih, N. I., Malik, R. A. (2016). Isolation of Anaerobic Heterotrophic Bacteria in Textile Industry Waste Water Treatment. *Jurnal Riset Teknologi Pencegahan Pencemaran Industri*, *7*(1), 39–46.

Kargi, F., Dinçer, A. R. (1998). Saline wastewater treatment by halophile-supplemented activated sludge culture in an aerated rotating biodisc contactor. *Enzyme and Microbial Technology*, *22*(6), 427–433. https://doi.org/10.1016/S0141-0229(97)00215-9

Kargi, F., Uygur, A. (1996). Biological treatment of saline wastewater in an aerated percolator unit utilizing halophilic bacteria. *Environmental Technology*, *17*(3), 325–330. https://doi.org/Doi 10.1080/09593331708616391

Kim, S., Eichhorn, P., Jensen, J. N., Weber, A. S., Aga, D. S. (2005). Removal of antibiotics in wastewater: Effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science and Technology*, *39*(15), 5816–5823. https://doi.org/10.1021/es050006u

Lefebvre, O., Moletta, R. (2006). Treatment of organic pollution in industrial saline wastewater: A literature review. *Water Research*, *40*(20), 3671–3682. https://doi.org/10.1016/j.watres.2006.08.027

Marlena, B., Yuliasni, R., Budiarto, A., Arum, S., Moenir, M., Syahroni, C. (2018). Jurnal Riset Teknologi Pencegahan Pencemaran Industri Removal of ammonia on catfish processing wastewater using horizontal sub-surface flow constructed wetland (HSSFCW), *9*(1), 15–21.

Melanie, S., Winterburn, J. B., Devianto, H. (2018). Production of Biopolymer Polyhydroxyalkanoates (PHA) by Extreme Halophilic Marine Archaea Haloferax mediterranei in Medium with Varying Phosphorus Concentration, *50*(2), 255–271. https://doi.org/10.5614/j.eng.technol.sci.2017.50.2.7

Moy, B. Y. P., Tay, J. H., Toh, S. K., Liu, Y., Tay, S. T. L. (2002). High organic loading influences the physical characteristics of aerobic sludge granules. *Letters in Applied Microbiology*, *34*(6), 407–412. https://doi.org/10.1046/j.1472-765X.2002.01108.x

Pala, A., Tokat, E. (2002). Color removal from cotton textile industry wastewater in an activated sludge system with various additives. *Water Research*, *36*(11),2920–2925. https://doi.org/10.1016/S0043-1354(01)00529-2

Petruccioli, M., Cardoso Duarte, J., Eusebio, A., Federici, F. (2002). Aerobic treatment of winery wastewater using a jet-loop activated sludge reactor. *Process Biochemistry*, *37*(8), 821–829. https://doi.org/10.1016/S0032-9592(01)00280-1

Sivaprakasam, S., Mahadevan, S., Sekar, S., Rajakumar, S. (2008). Biological treatment of tannery wastewater by using salt-tolerant bacterial strains. *Microbial Cell Factories*, *7* (May 2014). https://doi.org/10.1186/1475-2859-7-15

Trussell, R. S., Merlo, R. P., Hermanowicz, S. W., Jenkins, D. (2006). The effect of organic loading on process performance and membrane fouling in a submerged membrane bioreactor treating municipal wastewater. *Water Research*, *40*(14), 2675–2683. https://doi.org/10.1016/j.watres.2006.04.020

Wang, J.L., Zhan, X. M., Feng, Y. C., Qian, Y. (2005). Effect of salinity variations on the performance of activated sludge system. *Biomedical and Environmental Sciences : BES*, *18*, 5–8.

Water Resources Division. (2017). Activated Sludge Process Control: Training Manual for Wastewater Treatment Plant Operators.

Yuliasni, R., Setyaningsih, N. I., Handayani, N. I., Budiarto, A. (2017). The performance of combined technology Upflow anaerobic reactor (UAR)-activated sludge (AS) for treating batik wastewater. *Advanced Science Letters*, *23*(3). https://doi.org/10.1166/asl.2017.8725

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Implementation of electrocatalytic reactor as oxidation unit for residual reagent wastewater of testing laboratory

Aris Mukimin, Kukuh Aryo Wicaksono, Nur Zen, Agus Purwanto, Hanny Vistanty

Center of Industrial Pollution Prevention Technology, Jl. Ki Mangunsarkoro No.6, Semarang, Jawa Tengah, Indonesia 50136

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A B S T R A C T

The remaining reagent from the sample analysis process become a significant source of hazardous waste of laboratory tasting activities. Methylene blue, phenol and oil are pollutants common in the residual reagent waste. The electrocatalytic reactor is effective oxidation units for these organic pollutants. The reactor was made for a 50 L capacity with cylindrical metal oxide as the anode. The three anodes which 6 cm in diameter and 50 cm in length were paired stainless cathode with a distance of 2.5 cm. The reactor was also equipped with a stirrer that is connected to the motor so that the mass transfer and oxidizing agents is more effective. The reactor application was carried out by feeding the residual reagent waste into the electrocatalytic unit and giving DC potential 5 Volt. Each COD content for reagent waste of detergent: 2864 mg/L, phenol: 838 mg/L and oil: 708 mg/L. The reactor has reduced COD to 2157 mg/L (detergent), 399 mg/L (phenol) and 506 mg/L (oil) for 120 minutes. The high COD content in residual is caused by solvent (chloroform or hexane) that used at extraction step in determining the process of a sample. This compound is tough to oxidize into CO_2 by OH radical or hypochlorite acid formed at the anode during the electrolysis process.

1. INTRODUCTION

Besides producing a data for analysis the laboratory testing also release waste in form of water, gas and solid. Based on the nature of the waste, there are also those included in the category of hazardous waste (B3), especially waste derived from the remaining of reagent analysis. Some analysis that has the potential to produce residual reagents must be managed in B3. They are detergent, phenol and oil testing (Benatti et al., 2006)

The BBTPPI Semarang testing laboratory is one of the laboratories with high activity. The great number of samples each month causes the volume of the wastewater from the analysis process to be large. The type of analysis includes physical, chemical and biological parameters. Analysis of chemical parameters such as measurement of detergent, phenol and oil contents always produce waste in the form of residual reagents. Based on the characteristic, this wastewater is classified as B3 waste because of its toxicity (Kementerian Lingkungan Hidup, 2014). While, BBTPPI in the waste management of the residual reagents submit to third parties so that it requires a high cost. In addition, the presence of residual waste reagents requires extensive management in accordance with regulations and temporary storage. Based on this, the wastewater treatment of residual reagents is an alternative solution that will have a positive impact on BBTPPI including: (1) reducing the budget for managing B3 waste to third parties, (2) saving temporary

*Correspondence author. Tel.: +624 8316315
E-mail: arismukimin@gmail.com (Aris Mukimin)

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storage space (TPS) of B3 waste and (3) eliminating the handling of B3 waste such as packaging and distribution from the laboratory to the TPS.

An advanced oxidation process is one of the appropriate methods to treat recalcitrant and toxic wastewater (Ikehata, K., and El-Din, 2010; Ledakowicz et al., 2001; Oller et al., 2011) as a residual reagent analysis. Mechanism of degradation of pollutants is through the oxidizing promotion activities such as O_3 (Azbar et al., 2004), H_2O_2 (Masoumbeigi & Rezaee, 2015), HClO (Mukimin et al., 2016; Mukimin et al., 2012) and OH^o (Brillas et al., 2009; Mousset et al., 2018). This oxidation method has also made in an electrochemical system know as EAOP (Electrochemical advanced oxidation process). Some EAOP applications include: textile wastewater (Kaur & Kushwaha, 2018; Mukimin et al., 2015), olive processing, pharmaceutical, hospital (Ganzenko et al., 2014) and batik proccessing (Aris Mukimin et al., 2017).

The ability of EAOP method is determined by the type of anode used. Ti/RuO_2 is a potential electrode material that is applied because it is mechanically stable, effectively producing strong oxidizing agents (Cl_2, ClOH, ClO^-), high overpotential oxygen ≈ 2 V (Kaur et al., 2017). The Ti/RuO_2 surface anode also takes H_2O adsorption to be oxidized produce OH^o which also function as a strongest oxidizer against (Kaur & Kushwaha, 2018). The stability of these metal oxide will be further enlarged by doping iridium (A Mukimin & Purwanto, 2018).

The advantages of this technology include: powerful oxidation process, easy operation, can be automated, no producing sludge and electrodegradation process such as on-off switches and can made portable. The propose of this article is to investigate the ability of EAOP reactors to treat hazardous wastewater, especially residual reagent analyzing detergents, oils, and phenols. Electrocatalytic tube reactor is arranged using $Ti/RuIrO_2$ cylindrical anodes and applied to laboratory wastewater from the residual reagent with a batch system.

2. METHODS

2.1. Material and equipment

Electrocatalytic reactor is made from $Ti/RuIrO_2$ metal oxide anodes (ø 6 cm, ℓ 50 cm) and stainless cathodes (ø 2 cm, ℓ 50 cm). The electrode pair is arranged so that have a distance of 2 cm and placed in the PVC tube reactor. The reactor configuration as in **Figure 1**.

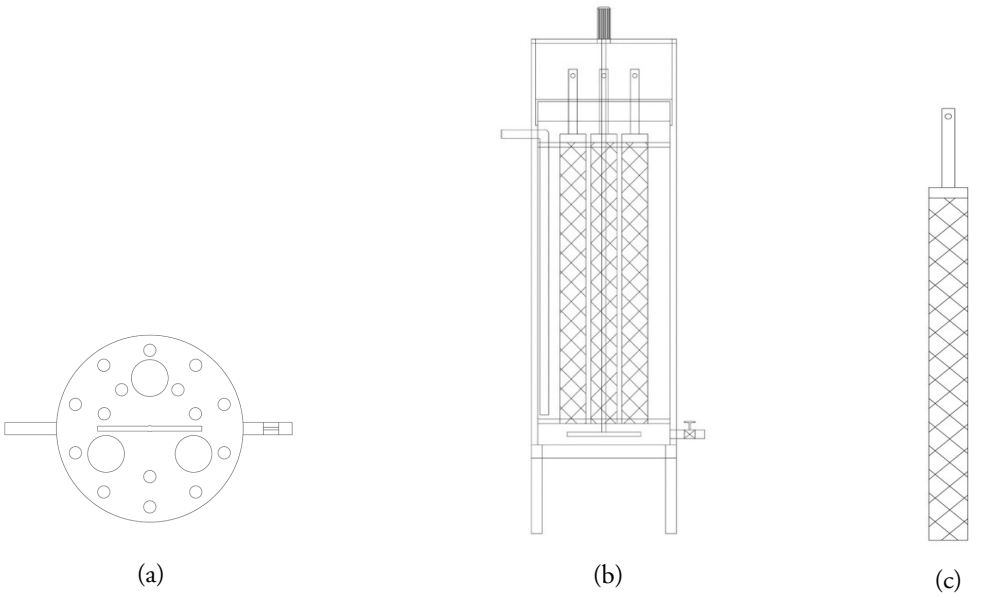


Figure 1. Design of electrocatalytic reactor include: electrode frame (a), main reactor unit (b) and anode (c)

The reactor is also equipped with a supporting unit such as feeding tanks, pumps, pipes, supports, mechanical mixer and DC power supply.

The reactor performance test uses wastewater from the residual reagent of detergent, phenol and oil analysis obtained from the BBTPPI testing laboratory. Determining of COD content using pro analytical chemicals made by Meck without treatment. Testing of the surface image anode and the contents using SEM-EDX from the PHENOM PRO-X DESKTOP type. Measurement of peak absorpt reduction before and after the process is determined by UV-VIS spectrophotometer (SHIMADZU A11454907330).

2.2. Procedure

The reactor application was carried out for residual reagent wastewater from detergents, phenol and oil analysis. The stages of this process were as follows: a 50 L residual reagent of detergent analysis was added with salt to obtain a concentration 2000 mg/L. Feed the wastewater into the reactor using an installed pump, as soon as the reactor was fully charged, the power supply was turned on and the potential is set at 5 V. Turn on the mixer by pressing the switch button on. The reactor performance was is determined by taking samples after 15, 30, 45, 60, 75, 90, 105, and 120 minutes. The COD content was measured using the reflux method with stages according to in APHA-AWWA. The change in absorbance of the sample was measured by UV-Vis scan at a wavelength of 700 – 200 nm. The same procedure was applied to the residual reagent of phenol and oil analysis.

3. RESULT AND DISCUSSION

3.1. Configuration and characterization of electrode

The design of the reactor and the arrangement of electrodes as **Figure 1** have been made and the visuals are as shown in Figure 2.

The anode-cathode pairs have ensured that the two electrodes will not be connected and fix at a distance of 2 cm. The current response which is 5 V indicates that there is no direct connection between the anode and cathode, otherwise, this cell arrangement has produced an electrolyte reaction on the surface of the anode or cathode.

Figure 2. Anode Ti/RuIrO2 pair configuration and stainless cathode installed in an electrocatalytic reactor

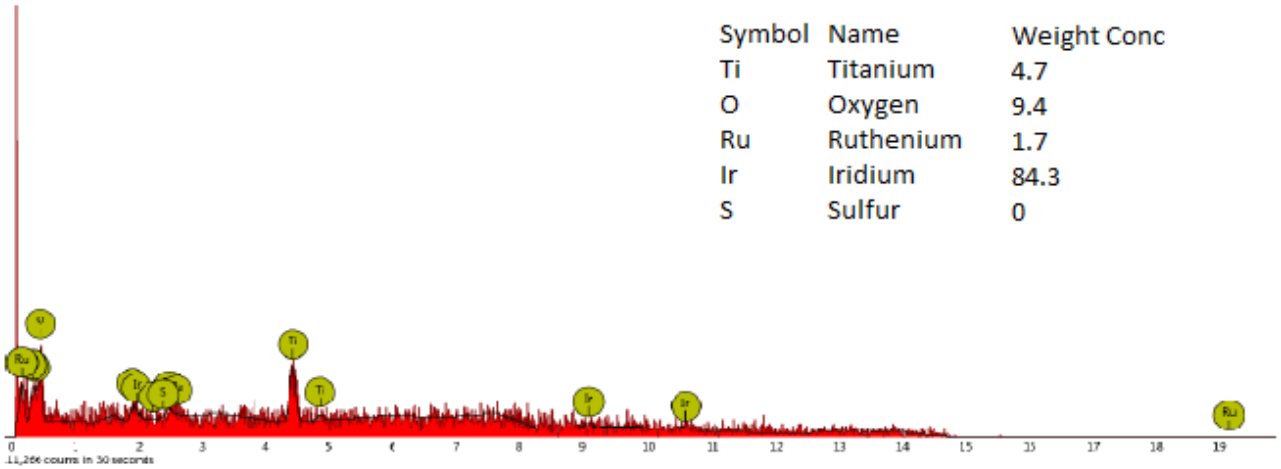


Figure 3. EDX spectra of metal oxide material on the surface of the anode

Electrolyte reaction can be indicated by two things, the formation of fine bubbles and the current response that is not too high. On the surface anode, bubbles are formed for a given potential between 4-5 V. The quantity of bubbles increases if the voltage is raised. This fact indicates that there has been an oxidation of water on the $RuIrO_2$ surface with the help of electrical. Bard & Faulkner (2001) and Mukimin et al (2018) have stated the equation of the reaction that is :

$$M + H_2O \rightarrow M(OH^o) + H^+ + e \quad (1)$$

$$M(OH^o) \rightarrow M + ½\ O_2 + H^+ + e \quad (2)$$

The continuity of this reaction can also be proven from the non-extreme current response. The display of power supply shows a normal range value ie 9 A for three pairs of electrodes with an anode surface area of about 2826 cm^2 or a current density value of 3 mA/cm^2.

The oxidation reaction of water identified from the formation of oxygen bubble and also the current response represent the performance of the anode material. $RuIrO_2$ metal oxide has great electroactive properties as stated by several previous studies using this material (Aris Mukimin et al., 2018). The reactivity of this material is determined by the content of ruthenium which composited on the metal oxide material (Kaur et al., 2017; Mukimin & Purwanto, 2018). Characterization of the anode is installed in an electrocatalytic reactor as in **Figure 3**.

EDX analysis has shown Ru content in anode material in addition to other elements such as oxygen and iridium. The titanium is also measured in this material is made possible by the surface morphology of the anode which is not all closed. Surface image using SEM as **Figure 4** has strengthened the detection of Ti.

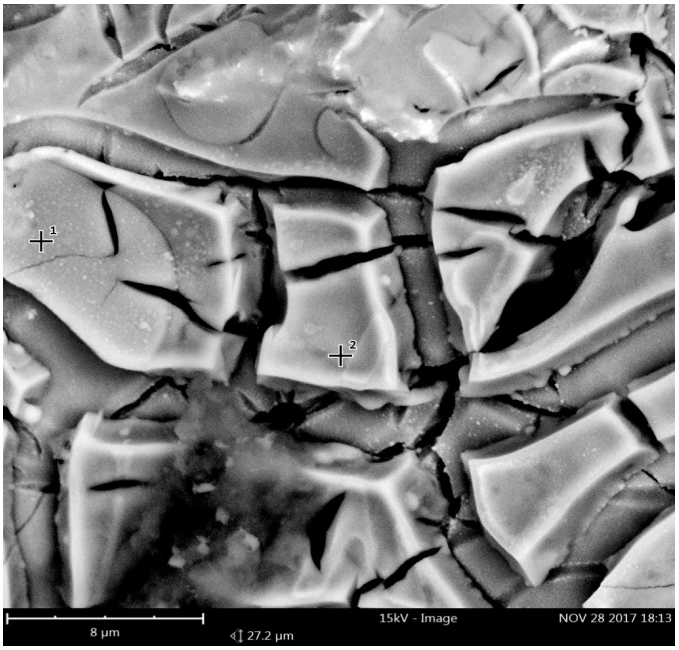


Figure 4. Surface morphology of electrode on magnification 10000x

Metal oxide deposited on the titanium plate as buffer or substrate form an uneven layer. These metal oxide composites tend to accumulate in small areas at the size

ranging from 4-6 µm with 4 µm long thickly striped slits. The gap formed causes the substrate constituent element to be measured in EDX analysis.

3.2. Performance of reactor in removal pollutant from residual reagent of detergent analysis

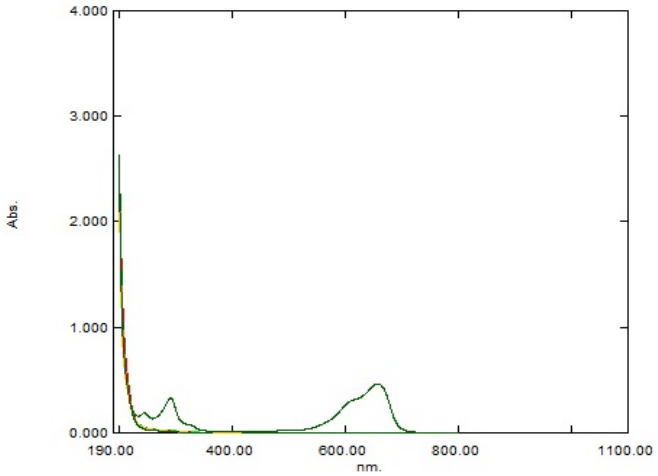
Electrocatalytic reactor using an anode as shown in **Figure 2** have been applied to treat a residual reagent wastewater of detergent analysis. Visualization of the processing results as shown in **Figure 5**.

The residual reagent waste of detergent analysis is visually blue, which is caused by the use of methylene blue as a quantitative indicator of surfactant content. The electrochemical degradation of this pollutant is very strongly recognized by a change in the color of the wastewater between before and after the process. Special pollutant from methylene blue has been rapidly degraded because the blue color that appears immediately drops only in during the process of 15 minutes as **Figure 5**b. This decrease caused by the chromophore structure which is easily oxidized by agents which are generated during electrolysis process such as HClO and OH radical (Katheresan et al., 2018; Kaur, et al., 2017; Aris Mukimin et al., 2018). The degradation pollutant also takes place in the basic structure, namely three aromatic rings formed based on the decrease in UV absorption at a wavelength of 300 nm as shown in **Figure 5**c. (Mousset et al., 2016) states that OH radical are strong oxidizing agents which will quickly break the double bond C=C with high reaction constant (108-1010 M-1 s-1)

The degradation process of pollutant in the residual reagent of detergent analysis is strengthened by the measurement of COD content. **Figure 6** shows a decrease in COD during electrolysis ie from 2864 mg/L to 2157 mg/L for a 120-minute process.

(a)

(b)



(c)

Figure 5. Electrocatalytic reactor to treat a residual reagent wastewater of detergent analysis (a), visual of sample is treated (b) and their UV-VIS spectra (c)

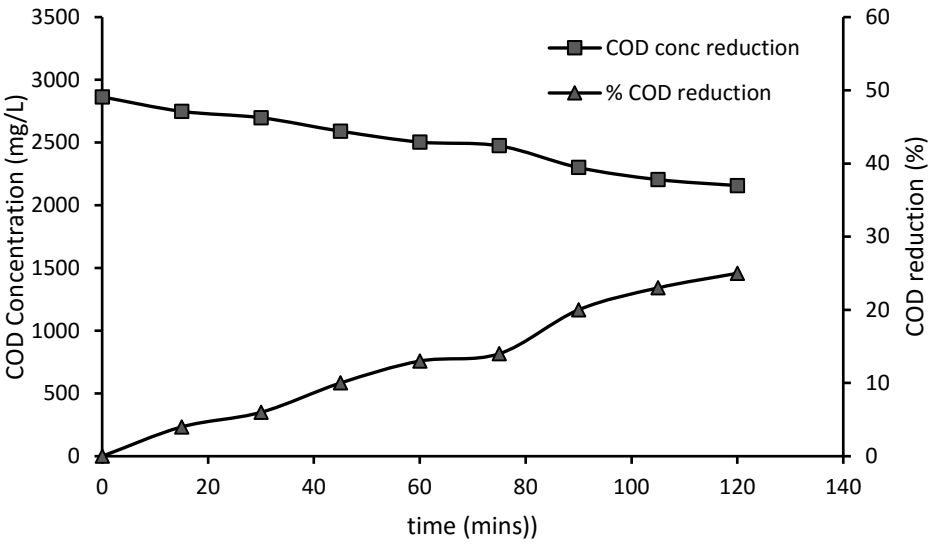


Figure 6. Trend COD reduction during the electrocatalytic process for a residual reagent of detergent analysis at potential 5 V and salt 2000 mg/L

The electrocatalytic reactor has reduced COD ie 707 mg/L in the 2 h duration. This mechanism of degradation through both indirect oxidation and direct at surface anode material. The anode pole which is given a high DC potential will cause the orbital energy level to drop so that the electrons from the pollutant compound will be able to move to the orbital. This direct degradation has also been raised by (Rajeshwar, K. and Ibanez, 1997).

The degradation efficiency of the electrocatalytic reactor in percentage is still low, only about 25% as can be seen from Figure 7. This low value is caused by two things, namely high initial COD concentration (2864 mg/L) and the dominant types of pollutants come from solvents. Electrodegradation process take place on the surface anode, especially by direct mechanism. This phenomenon will limit the amount of pollutant that can be in contact with surface anode. This limitation causes a slowing degradation process. High COD content which is 3000 mg/L requires a long time of degradation with the day order as much biological treatment. The long duration process is not suitable for electrocatalytic technology specification because the cost operating will be high due to electricity consumption. The degradation efficiency is also strongly influenced by the type of pollutant, where the compound that is difficult to be degraded such as solvent will cause processing to not take place.

The residual reagent of detergent analysis has a chloroform which is obtained at extraction stape. This compound is difficult to be degraded because it has a stable structure ($CHCl_3$). (Rossberg et al., 2006) mentions that chloroform will be oxidized by chlorine to form carbon tetrachloride (CCl_4) and chloride acid (HCl). Meanwhile, the electrolysis process in the reactor has produced HClO which can balance with chlorine (Cl_2). This reaction has negative effects on the performance of pollutant degradation, especially solvents which can not be reduced to CO_2 but instead the formation of a new organic compound (CCl_4).

$$CHCl_3 + Cl_2 \rightarrow CCl_4 + HCl \quad (3)$$

This phenomenon is the main source of high COD content in processed wastewater (2157 mg/L). The presence of chloroform compound in the wastewater is also confirmed from the distillation when it is evaporated. At a temperature of 62 °c this wastewater evaporates to form a distillate up to 10%.

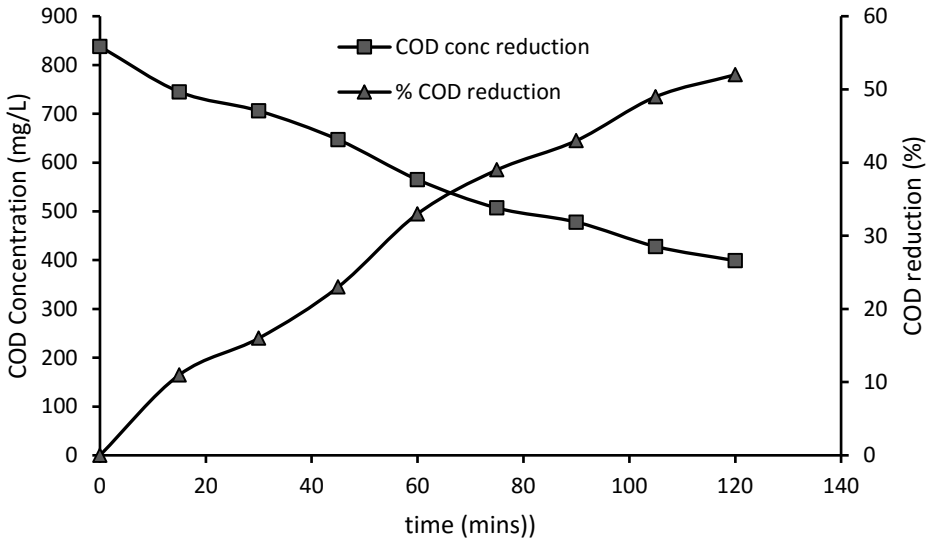


Figure 7.Trend COD reduction during the electrocatalytic process for a phenol analysis reagent waste at potential 5 V and salt 2000 mg/L

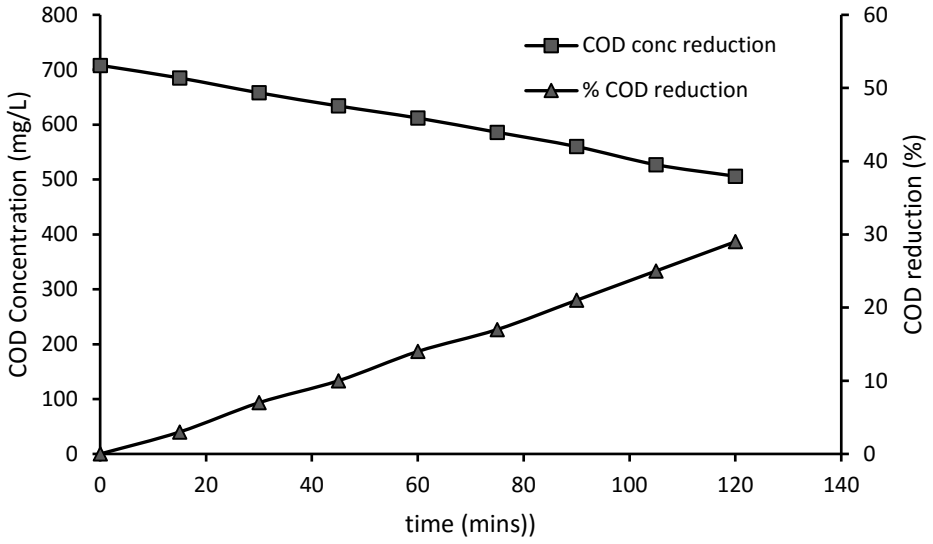


Figure 8. Trend COD reduction during electrocatalytic process for an oil analysis reagent waste at potential 5 V and salt 2000 mg/L

3.3. Performance of reactor in removal pollutant from the residual reagent of phenol analysis

Residual waste from phenol analysis contains several compounds such as phenol, 4-aminoantipirin, $K_4Fe(CN)_6$, NH_4OH, $PO_4^{3-}$and $CHCl_3$. The wastewater is yellowish color formed from the reaction between amino antipyrine and potassium ferricyanide. Degradation of these pollutants by electrolysis has been carried out based on COD content as shown in **Figure 7**.

The efficiency of COD removal was 52 % or reduction from 838 mg/L to 399 mg/L. The remaining of COD is still high due to the solvent pollutants used in this phenol analysis. As with detergent analysis, the method of determining phenol also uses chloroform as solvent extraction. It was previously explained that chloroform is not able to be oxidized to CO_2 but by chlorine, it will form a carbon tetrachloride solvent. The final COD concentration in treated wastewater from the remaining reagent phenol (399 mg/L) is smaller than the detergent (2157 mg/L) which is affected by the volume of chloroform used when extraction.

Pollutant phenol can be degraded through oxidation reaction from OH radical or HClO which are formed during electrolysis. This indirect degradation mechanism will produce chlorophenol as an intermediate compound before it becomes organic acid (Feng & Li, 2003; Aris Mukimin et al., 2015). The mechanism of the direct degradation of phenol pollutant is also possible as mentioned by Marhiyasu et al (Mathiyarasu et al., 2004). Besides phenol, degradation of organic compounds also takes place for aminoantipirene. The double bond in the aromatic ring of this pollutant can be cut off by strong OH oxidizing agent produced on the surface of the Ru anode (Kaur et al., 2017).

The decreasing of potassium ferricyanide takes place through an oxidation by chlorine which is formed during electrolysis. This reaction will produce potassium ferrocyanide with the equation:

$$K4[Fe(CN)] + Cl2 \rightarrow 2\ K3[Fe(CN)6] + KCl \quad (4)$$

The formed potassium ferrocyanide will come out of the solution to settle. This is in accordance with the facts of the study, where the resulting sediment in wastewater is processed.

3.4. Performance of reactor in removal pollutant from residual reagent of oil analysis

The residual reagent of oil analysis obtained pollutant comes from the substance in the sample that is not extracted. COD content of this wastewater before being treated with a concentration of 708 mg/L indicates chemical pollutants including an organic compound. The electrolysis process has reduced the COD content to 506 mg/L as shown in **Figure 8**. The mechanism degradation of pollutants takes place indirectly and directly as mentioned in the residual reagent of detergent or phenol analysis.

Low efficiency of COD reduction (29%) indicates that wastewater contains compounds that are difficult to be oxidized. Base on the identification of the sample, the use of n-hexane solvent at the time extraction is the causative sources. The structure of a compound that is like a spine with six linear carbon becomes stable so it is difficult to be degraded. However, Koch and Waaf (1973) mention that carbon-hydrogen bonds have the opportunity to reach with some radicals. The slow trend of COD reduction as Figure 9 indicates the n-hexane oxidation process by OH radicals formed in the reactor.

4. CONCLUSION

The electrocatalytic reactor has been able to reduce pollutants found in the residual reagent of detergent, phenol and oil analysis. The performance of COD removal for each wastewater was 25% (2846 mg/L to 2157 mg/L), 52% (838 mg/L to 399 mg/L) and 29% (708 mg/L to 506 mg/L) for 120 minutes processing. The relatively large COD content was caused by solvent chloroform in the detergent and phenol analysis and n-hexane in the oil. This reason is base on analysis procedure used and evaporation result of the waste sample. The stable structure of the solvents makes it difficult to be degraded either by oxidizing agent formed during electrolysis or the direct transfer of electrons to the anode.

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REFERENCES

Azbar, N., Yonar, T., & Kestioglu, K. (2004). Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent. *Chemosphere*, *55*(1), 35–43. https://doi.org/10.1016/j.chemosphere.2003.10.046

Bard, A. J., & Faulkner, L. R. (2001). *Electrochemical Methods: Fundamentals and Applications* (second). John Wiley & Sons, Inc.

Benatti, claudia telles, Tavares, celia regina granhen, & Guedes, terezinha aparecida. (2006). Optimization of Fenton ' s oxidation of chemical laboratory wastewaters using the response surface methodology. *Journal of Environmental Management*, *80*, 66–74. https://doi.org/10.1016/j.jenvman.2005.08.014

Brillas, E., Sirés, I., & Oturan, M. A. (2009). Electro-Fenton process and related electrochem- ical technologies based on Fenton's reaction chemistry. *Chem. Rev.*, *109*, 6570–6631. https://doi.org/10.1021/cr900136g

Emmanuel Mousset, Giovanni, F., Hullebusch, E. D. Van, Oturan, N., & Oturan, M. A. (2016). A complete phenol oxidation pathway obtained during electro-Fenton treatment and validated by a kinetic model study. *Applied Catalys B: Environmental*, *180*, 189–198. https://doi.org/10.1016/j.apcatb.2015.06.014

Feng, Y. J., & Li, X. Y. (2003). Electro-catalytic oxidation of phenol on several metal-oxide electrodes in aqueous solution $, *37*, 2399–2407. https://doi.org/10.1016/S0043-1354(03)00026-5

Ganzenko, O., Huguenot, D., van Hullebusch, E. D., Esposito, G., & Oturan, M. A. (2014). Electrochemical advanced oxidation and biological processes for wastewater treatment: A review of the combined approaches. *Environmental Science and Pollution Research*, *21*(14), 8493–8524. https://doi.org/10.1007/s11356-014-2770-6

Ikehata, K., and El-Din, M. . (2010). Degradation of Recalcitrant Surfactants in Wastewater by Ozonation and Advanced Oxidation Processes: A Review. *The Journal of the International Ozone Association*, *26*(May 2013), 327–343. https://doi.org/10.1080/01919510490482160

Katheresan, V., Kansedo, J., & Lau, S. Y. (2018). Efficiency of Various Recent Wastewater Dye Removal Methods: A Review. *Biochemical Pharmacology*. https://doi.org/10.1016/j.jece.2018.06.060

Kaur, J., Kushwaha, J.P., Sangal, V. . (2017). Evaluation and disposability study of actual textile wastewater treatment by electro-oxidation method using Ti/RuO2 anode. *Process Safety and Environmental Protection*, *111*, 13–22.

Kaur, P., Kushwaha, J.P., S. V. K. (2018). Electrocatalytic oxidative treatment of real textile wastewater in continuous reactor: Degradation pathway and disposability study. *Journal of Hazardous Materials*, *346*, 242–252.

Kementerian Lingkungan Hidup. (2014). Peraturan pemerintah: pengelolaan limbah bahan beracun berbahaya. Republik Indonesia.

Koch, H., Haaf, W., Prichard, W. W., & McKusick, B. C. (1973). of Reliable Methods for the Preparation of Organic Compounds Working with Hazardous Chemicals. *Organic Syntheses*, *44*(September), 1–5. https://doi.org/10.15227/orgsyn.044.0001

Ledakowicz, S., Solecka, M., & Zylla, R. (2001). Biodegradation , decolourisation and detoxification of textile wastewater enhanced by advanced oxidation processes, *89*, 175–184.

Masoumbeigi, H., & Rezaee, A. (2015). Removal of methylene Blue dye from synthetic wastewater using UV/H2O2 advanced oxidation process. *Journal of Health Policy and Sustainable Health*, *2*(1), 160–166.

Mathiyarasu, J., Joseph, J., Phani, K. L. N., & Yegnaraman, V. (2004). Electrochemical detection of phenol in aqueous solutions, *11*(November), 797–803.

Mousset, E., Oturan, N., Oturan, M. A., Mousset, E., Oturan, N., & Oturan, M. A. (2018). An unprecedented route of • OH radical reactivity : ipso-substitution with perhalogenocarbon compounds To cite this version : HAL Id : hal-01712279 electrocatalytical process : ipso-substitution with Paper submitted to Applied Catalysis B - Environment fo. *Applied Catalys B: Environmental*, *226*, 135–146.

Mukimin, A., & Purwanto, A. (2018). Removal Efficiency of Nitrite and Sulfide Pollutants by

Electrochemical Process by Using Ti / RuIrO 2 Anode. *Indo. J. Chem.*, *18*(2), 286–293. https://doi.org/10.22146/ijc.26609

Mukimin, A., Vistanty, H., & Zen, N. (2015). Oxidation of textile wastewater using cylinder Ti / b -PbO 2 electrode in electrocatalytic tube reactor. *CHEMICAL ENGINEERING JOURNAL*, *259*, 430–437. https://doi.org/10.1016/j.cej.2014.08.020

Mukimin, A., Vistanty, H., Zen, N., Purwanto, A., & Wicaksono, K. A. (2018). Performance of bioequalization-electrocatalytic integrated method for pollutants removal of hand-drawn batik wastewater. *Journal of Water Process Engineering*, *21* (July 2017), 77–88. https://doi.org/10.1016/j.jwpe.2017.12.004

Mukimin, A., Wijaya, K., & Kuncaka, A. (2012). Oxidation of remazol brilliant blue r (RB . 19) with in situ electro-generated active chlorine using Ti / PbO 2 electrode. *Science of the Total Environment, The*, *95*, 1–9. https://doi.org/10.1016/j.seppur.2012.04.015

Mukimin, A., Wijaya, K., & Yuliastuti, R. (2016). Reaktor tabung elektrokatalitik dan sistem pengolahan air limbah industri pewarnaan yang menggunakan reaktor tersebut.

Mukimin, A., Zen, N., Purwanto, A., Wicaksono, K. A., Vistanty, H., & Alfauzi, A. S. (2017). Application of a full-scale electrocatalytic reactor as real batik printing wastewater treatment by indirect oxidation process. *Biochemical Pharmacology*. https://doi.org/10.1016/j.jece.2017.09.053

Oller, I., Malato, S., & Sánchez-pérez, J. A. (2011). Science of the Total Environment Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination — A review. *Science of the Total Environment, The*, *409*(20),4141–4166. https://doi.org/10.1016/j.scitotenv.2010. 08. 061

Rajeshwar, K. and Ibanez, J. G. (1997). *Environmental Electrochemistry: Fundamentals and Applications in Pollution Abatement*. Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-576260-1.X5000-1

Rossberg, M., Aktiengesellschaft, H., Main, F., Republic, F., & Adolf, T. (2006). *Chlorinated Hydrocarbons*. Wiley-VCH Verlag GmbH&Co.KGaA.Weinheim. https://doi.org/10.1002/14356007.a06_233.pub2


9 772503 501001


9 772087 096504