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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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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 10 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 third series published that in full-text English. This continous policy 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 air, water and solid pollutant treatment, namely: wet scrubber, solidification and wastewater treatment technology. There is an article that explore halofilic bacteria to improve salt production. The five manuscripts accepted and published in this edition are from research institute and University. The duration of submission, review, and editing of the manuscripts ranged from 4-5 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, December 2019

Chief Editor

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DAFTAR ISI

Jurnal Riset
Teknologi Pencegahan Pencemaran Industri
Volume 10 No. 2, November 2019

ABSTRACT

Published on 16 December 2019

Lestari Wevriandini, Erni Martani (Department of Agricultural Microbiology, Faculty of Agriculture, Gadjah Mada University)

Decolorization of Vat Violet 1 Dye from Textile Industrial Wastewater using Biofilm of Fungal and Bacterial Consortium

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2019, Vol. 10, No. 2, p. 1-6, 4 ill, 2 tab, 14 ref

Increasing of textile industries creates a critical need for a proper treatment plan to control and minimize possibilities of contaminants and toxic compounds being released to the environment. Biological approaches by utilizing microorganisms, although because in the rise of practicality and cost-effectiveness, are still flawed and require more analysis and development. One of such approach that is often being researched is the utilization of biofilm for treating industrial waste, and among those is about the use of fungal and bacterial consortium. This research was conducted to examine and acquire a more stable biofilm formed by fungal and bacterial consortium for decolorization process of textile wastewater. Isolates were selected by examining their decolorization capability, antagonistic activity, and mixed culture formation (consortium). The selection continued with biofilm forming in material plastic LLDPE. Superior consortium from bacteria and fungi in the form of biofilm in material plastic LLDPE then was applied for the decolorization of Vat Violet 1 dye from textile industrial wastewater. The selection resulted in two superior fungal isolates coded as strain JYGC1 and K2; and three bacterial isolates were coded as strain ATA6, PK29, and PK65. These isolates were then combined to form biofilm on the surface of material plastic LLDPE and examined for their applicability to decolorize wastewater containing Vat Violet 1 under variation of pH condition of 5, 7, and 8. Biofilm with regular thickness was formed by the fungal bacterial consortium and capable of increasing the rate of decolorization activity. The highest biomass yield before and after application to the wastewater was found to be at pH 7 at about 0.66 g and 0.45 g, respectively. The thickness and biomass corresponds with decolorization activity, which is also the highest on pH 7, with difference of 1.155 between before and after application; much higher than without biofilm application at 0.714.

(Author)

Keywords: decolorization, textile wastewater, vat violet 1 dye, biofilm, fungal, bacterial consortium

Zulkarnaini, Ansiha Nur, Wina Ermaliza (Departement of Environmental Engineering, Universitas Andalas)

Nitrogen Removal in the Anammox Biofilm Reactor using Palm Fiber as Carrier in Tropical Temperature Operation

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2019, Vol. 10, No. 2, p. 7-15, 9 ill, 1 tab, 26 ref

Anaerobic ammonium oxidation (anammox) is the process of converting ammonium directly into nitrogen gas with nitrite as an electron acceptor under anaerobic conditions. This process is more effective than conventional nitrification-denitrification, but this process is very dependent on several parameters, one of which is temperature. The optimum temperature range for the growth of anammox bacteria is 30-40^{0}C. The purpose of this research was to determine the efficiency of nitrogen removal by anammox process using palm fibers in the Up-Flow Anaerobic Sludge Blanket (UASB) reactor in the tropical temperature. The experiment was conducted at a laboratory scale with a variation of Hydraulic Retention Time (HRT) 24 h and 12 h using artificial wastewater. The reactor was inoculated with anammox granule genus Candidatus Brocadia. The concentration of ammonium, nitrite, and nitrate in the influent and effluent was measured using a UV-Vis spectrophotometer based on standard method. Based on the experiment, the ratio ΔNH_4^+-N:ΔNO_2^--N and ΔNO_3^--N:ΔNH_4^+-N similar with stoichiometry of anammox. The maximum Nitrogen removal performance (NRT) achieved 0.11 kg-N/m^3.d at Nitrogen Loading Rate (NLR) 0.14 kg-N/m^3.d and 0.20 kg-N/m^3.d at NLR 0.29 kg-N/m^3.d. The removal efficiency for Ammonium Conversion Efficiency (ACE) and Nitrogen Removal Efficiency (NRE) in HRT 24 h were 79% and 76%, respectively. While decreased in HRT 12 h were 72% and 69%, respectively. Anammox process can be applied in the tropical temperature at a laboratory scale using a UASB reactor with palm fibre as the carrier. The utilization of palm fiber as an anammox biofilm carrier increased the nitrogen removal performance of the reactor.

(Author)

Keywords: anammox, UASB, palm fiber, tropical temperature, nitrogen

Rizal Awaludin Malik, Nilawati, Novarina Irnaning Handayani, Rame, Silvy Djayanti, Ningsih Ika Pratiwi, Nanik Indah Setianingsih, Nasuka (Center of Industrial Pollution Prevention Technology, Semarang, Central Java, Indonesia)

Low Energy Bacteria Preservation of Extremely Halophilic Archaea Haloferax Lucentense and Haloferax Chudinovii Immobilized using Natural Zeolite

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2019, Vol. 10, No. 2, p. 16-28, 4 ill, 2 tab, 47 ref

The methods of microbial cells preservation were already known by liquid drying, freeze-drying, and freezing. Those methods could preserve bacteria cells in a long period of time but its survivability was relatively low and used relatively high energy during preservation. Immobilization was known as entrapping, attaching or encapsulating bacterial cells in a suitable matrix. This research was conducted to know the suitability of zeolite as immobilization carrier and also as preservation matrix of two halophilic archaea *Haloferax chudinovii* and *Haloferax lucentense*. Variable of this research was the type of the carrier which was raw zeolite, 110°C and 300°C heat-activated zeolite carrier, parameters measured in this study was physical and chemical of zeolite such as chemical content, Si/Al ratio, surface area and pore volume, and biochemical assay, bacterial cells numbers after immobilization and bacterial cells after preservation as bacterial response to the immobilization and preservation. Heat activation was significantly affecting the zeolite chemical composition, carrier surface area, and pore volume. Compared to other pretreated zeolite, highest quality zeolite was obtained in 110°C pretreated zeolite which has which has 65,625 m^2/gr for surface area 0,071 cc/gr pore volume and 5,13 Si/Al ratio. . The bacterial cells obtained after immobilization process was 1,8x10^7 cfu/g, 3,0 x 10^7 cfu/g, and 2,1x10^7 for raw zeolite, 110°C pretreated zeolite and 300°C zeolite respectively. After 4 months preservation, the slight reduction of the bacterial cells was observed. Immobilization halophilic archaeae using zeolite as carrier was proven as low cost and effective preservation method due to relatively simple process and unspecific preservation temperature requirements.

(Author)

Keywords: immobilization, zeolite, Halophilic archaea, low cost preservation

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Waste to Product : Bisolum-Bricks, Incorporating of WWTP Sludge of Textile Industry into Bricks for Wall Pairs

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2019, Vol. 10, No. 2, p. 29-35, 2 ill, 9 tab, 16 ref

The disposal of WWTP sludge is one of problems in textiles industry, which requires serious attention to find a way out. Utilization of sludge from the textile industry wastewater treatment, according to the Republic of Indonesia Goverment Regulation No.110 year 2014, can be used as a mixture of brick raw materials, must consider the availability of technology, meet environmental quality standards and meet technical requirements for use. Environmental feasibility refers to Goverment Regulation No.110 year 2014, carried out with TCLP toxicity tests on raw materials for soil, WWTP sludge and brick products. Acute toxicity test LD 50, carried out on brick products in which using a mixture of WWTP Sludge. Technical feasibility is carried out by testing the quality of brick products in accordance with solid brick Nasional Indonesian Standard (SNI) for wall pairs. Research results prove the toxicity test on raw materials and brick products with a mixture of up to 60% of waste, still meets the requirements of TCLP and LD.50 according to IGR No.110 year 2014. Test the quality of bricks at the use of 40% and 60% mixture of sludge still meet Nasional Indonesian Standard (SNI 15-2094-2000) solid red brick for wall pairs.

(Author)

Keywords: bisolum, bricks, textiles, WWTP sludge, TCLP, LD-50

Ikha Rasti Julia Sari, Januar Arif Fatkhurahman, Bekti Marlena, Nani Harihastuti, Farida Crisnaningtyas, Yose Andriani, Nasuka (Center of Industrial Pollution Prevention Technology, Semarang, Central Java, Indonesia)

Wet Scrubber Performance Optimization Application Assisted with Electrochemical-Based Ammonia Sensors

Jurnal Riset Teknologi Pencegahan Pencemaran Industri, November 2019, Vol. 10, No. 2, p. 36-42, 5 ill, 19 ref

Crumb rubber is one of Indonesia's agroindustry export commodities. This industry faces environmental problems due to their wastes, both liquid and air. The source of air pollution is commonly from drying process that emitted odor from its evaporation and heating phenomena. Industry uses wet scrubber technology as air pollution control from emitted odor from drying process. Preliminary identification in noncontrolled wet scrubber shown that wet scrubber efficiency around 47%. Low efficiency wet scrubbing process causes rain drop of water vapor around drying process. This research used electrochemical based sensor MICS 5524 as ammonia monitoring instrument, assisted with arduino as microcontroller to regulate water discharge through valve controlling scrubbing process. This electrochemical based sensor reads ammonia based on voltage reads by Arduino microcontroller. Ammonia reading then control scrubbing process by adjusting valve opening for spray water distribution. Wet scrubber efficiency increases to 66,96% due to water scrubbing control, also can save water utilization as high as 61,90%, followed by absence of rain drop contains ammonia around

drying process area.

(Author)

Keywords: crumb rubber, ammonia, wet scrubber, sensor electrochemical

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Decolorization of Vat Violet 1 Dye from Textile Industrial Wastewater using Biofilm of Fungal and Bacterial Consortium

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ABSTRACT

Increasing of textile industries creates a critical need for a proper treatment plan to control and minimize possibilities of contaminants and toxic compounds being released to the environment. Biological approaches by utilizing microorganisms, although because in the rise of practicality and cost-effectiveness, are still flawed and require more analysis and development. One of such approach that is often being researched is the utilization of biofilm for treating industrial waste, and among those is about the use of fungal and bacterial consortium. This research was conducted to examine and acquire a more stable biofilm formed by fungal and bacterial consortium for decolorization process of textile wastewater. Isolates were selected by examining their decolorization capability, antagonistic activity, and mixed culture formation (consortium). The selection continued with biofilm forming in material plastic LLDPE. Superior consortium from bacteria and fungi in the form of biofilm in material plastic LLDPE then was applied for the decolorization of Vat Violet 1 dye from textile industrial wastewater. The selection resulted in two superior fungal isolates coded as strain JYGC1 and K2; and three bacterial isolates were coded as strain ATA6, PK29, and PK65. These isolates were then combined to form biofilm on the surface of material plastic LLDPE and examined for their applicability to decolorize wastewater containing Vat Violet 1 under variation of pH condition of 5, 7, and 8. Biofilm with regular thickness was formed by the fungal bacterial consortium and capable of increasing the rate of decolorization activity. The highest biomass yield before and after application to the wastewater was found to be at pH 7 at about 0.66 g and 0.45 g, respectively. The thickness and biomass corresponds with decolorization activity, which is also the highest on pH 7, with difference of 1.155 between before and after application; much higher than without biofilm application at 0.714.

1. INTRODUCTION

According to Selvam et al. [1], there were about 10,000 kind of dyes used in textile industries and 7 x 10^5 tons dyes produced every year. During the coloring process, 10-15% of textile dye will be wasted together with industrial textile wastewater. The effluent of dye in industrial textile wastewater is about 60-70 mg/l ([2]. Besides polluting the environment Mathur et al. [3] stated that the dye also can be harmful to biodiversity and health, such as irritation to skin, eyes, and cause cancer and mutations.

The molecule of dye contains a saturated organic substance combined with chromophore that responsible for its color and auxochrome that intensifies the color of a substance or fibre. Unsaturated organic compound encountered in formation of the dye is generally derived from aromatic compounds and their derivatives (benzene, toluene, xylene, naphthalene, anthracene), phenol and its

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derivatives (phenols, ortho/meta/para cresol), and hydrocarbon that is containing nitrogen (pyridine, kinolin, korbazolum) [4].

Chromophore from vat dyes is a carbonyl group. It has an anthraquinone structure with large molecular mass, and the absorption of the fiber is high enough [5]. Vat dyes are also classified as a derivative oxazole included in the anthraquinone dye that is difficult to degrade because of the fused aromatic structures. These dyes are not soluble in water, so that it needs to be reduced using reducing agents such as sodium dithionite. In addition, vat dyes are also carcinogenic and mutagenic in humans [6] [7].

The benefit of bacterial and fungal ability to degrade textile wastewater in remediation allows these two species to grown in the form of a consortium. Consortium of bacteria and fungi is expected to improve the degradation of vat dyes because the complex interactions compounds between two isolates in intracellular or extracellular, such as enzymes in the metabolic process can be more numerous and complex. The use of mixed cultures are more effective in the process of biodegradation of pollutant compounds. In addition, the effectiveness of biodegradation process is also influenced by environmental factors such as pH, temperature, availability of nitrogen and phosphorus. These factors affect the degradation rate [8].

In this study, the formation of a consortium of bacteria and fungi carried out in the form of biofilm. Biofilm can be formed by a single species of bacteria and can also be composed of many species of bacteria, fungi, algae, and protozoa. Biofilms attached to the surface of abiotic or biotic substrate through extracellular polymeric substances (EPS).

Biofilms can be alternatives that are considered safer for bioremediation compared with planktonic microorganisms. It is because the cells in the biofilm are protected in the matrix so that they have a good level of adaptation and survival (especially during periods of stress). The interactions between microorganisms mutually beneficial physically and physiologically in biofilms make possible for the degradation process of xenobiotic compound happens quickly, so in the last few years, biofilm has been used in industrial plants to assist in immobilization and degradation of pollutants [9].

Therefore, consortium of fungi and bacteria in the form of biofilm is possible to increase the degradation of Vat Violet 1 dyes because of the interaction and the production of complex compounds in the metabolic processes. The biofilms formed is expected to be able to decolorize Vat Violet 1 dyes in textile industrial wastewater with higher efficiency levels.

2. METHODS

Research was conducted at Laboratory of Agricultural Microbiology, Faculty of Agriculture, Gadjah Mada University, Yogyakarta. Dyes wastewater was obtained from Towel Factory Lumintu 1001, Ngendo, Polanharjo, Klaten, Central Java. Fungal isolates used were coded as TPA4 (Isolated from landfill in Palembang), KRMS5 (Isolated from local sawmills in Palembang), JYGC1 (Isolated from wastewater PT GKBI Sleman, Yogyakarta), K1 (Isolated from purification process of JYGC1), K2 (Isolated from purification process of JYGC1), K3 (Isolated from purification processes of KRMS5). Bacterial isolates used were coded as ATA6 (*Bacillus* sp.) [10], PJ39 (Isolated from activated sludge PT Pajitex, Pekalongan), PK29 (Isolated from the silt of the river in Pekalongan), and PK65 (Isolated from the silt of the river in Pekalongan).

Plastic material (caps of mineral water bottle) made from LLDPE (Linear Low Density Polyethylene) was used as the material sticking biofilm. It would also require a wire gauze, wire tie, HCL 1 N, 1 N NaOH, and Lactophenol blue (as fungi staining).

The main materials used in this study, include shaker, spectrophotometer UVmini-1240 Shimadzu, pH paper (universal test paper), microscope Nikon SE, Haemacytometer, Petroff-Hauser Bacteria Counter, test tubes, petri dish, centrifuges, Whatman filter paper no .1 Ø 125 mm, bunsen burner, microtubes, cuvette, micropipette, microtips, vortex, ose needle, ent needle, drigalski, glass beaker, Erlenmeyer (125 ml, 250 ml, 500 ml), measuring

pipette (5 ml and 10 ml), kimax tubes, glass bottles (300 ml) and a digital camera.

Antagonistic activity - Tests performed by Kirby-Bauer method using paper disc [11]. Each fungal isolates were grown first on a PDA media with a spread plate method and incubated for 2 days. During the growth of fungal isolates, each isolate bacteria was grown on Nutrient Broth (NB) media for 48 hours. Then, it has shaken out with a speed of 125 rpm. Furthermore, the sterile paper disc was prepared to be immersed in the bacterial suspension with NB media. Each sterile paper disc soaked about ± 1 minute and then drained to eliminate excess fluid. Paper disc was placed in petri dish with PDA media and fungal isolate and incubated for 48 hours. The growth of fungal and bacterial isolates is said to inhibit each other if there were formed antagonistic zone around the colonies of bacteria or paper disc. Antagonistic value can be measured by dividing the diameter of antagonistic zone with diameter of bacterial colonies

Table 1. Antagonistic activity value between fungal and bacterial isolates

Bacteria	Inhibition Zone (mm)					
	Fungi (*spread*)					
	KRMS5	TPA4	K1	K2	K3	JYGC1
PJ39	1.75*	1.10*	1.53*	0	0	0
PK65	1.89	1.64	1.48	0	0	0
PK29	1.53*	1.50*	1.47*	0	0	0
ATA6	1.37	1.14	1.13	0	1.36*	0

*after day-8 antagonistic activity was stopped

Mixed cultures test - This test is based on methods O'Tolle and Kolter [12], using the medium Basal Glucose Salt (GBS) as a minimal media and media for biofilm formation coupled with tannic acid as an inducer ligninolytic enzyme synthesis. Fungal isolate was put in Erlenmeyer containing 100 mL GBS media as a starter and incubated for 5 days. Furthermore, the fungal spores were calculated using Haemacytometer (10^5 spores/ml). Fungal spores inoculated into other GBS media (150 mL) and incubated for 5 days to form clumps of hyphae. During the growth period of fungi, bacteria were grown and incubated as the exponential phase period of bacteria (48 hours). The number of bacterial cells with the cell density was calculated using Petroff-Hauser Bacteria Counter (10^5 cells/ml). Once fungal hyphae formed, bacterial isolates were inoculated and incubated at room temperature for 7 consecutive days with 80 rpm of shaking speed. The formation of mixed culture was observed by taking 1 ose inoculant of each culture using microscope with magnification 400x to 1000x and stained by lactophenol blue.

Biofilm formation on LLDPE – The formation of biofilm used sterile glass bottles attached with bottle cap made from LLDPE. GBS media and tannic acid 200 mL inserted into the bottle and then inoculated with fungal and bacterial isolates. Bacterial isolates were inoculated into the bottle after the formation of clumps of fungal hyphae and after the bacteria were incubated for 48 hours at an exponential period. Incubation or biofilm formation was performed for 7 until 14 days at room temperature with speed of shaking about 80 rpm. Biofilm formation was observed macroscopically by measuring biomass of biofilm and microscopically.

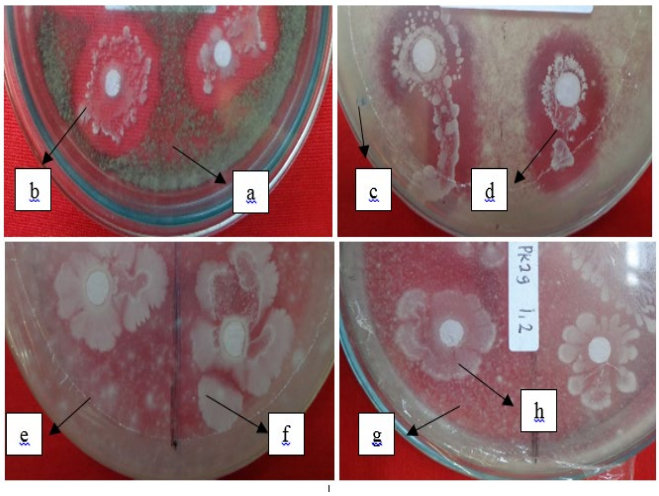

Fig. 1. Growth Inhibition of fungal isolate K1 (a) by bacterial isolate PK65 (b) (left) and fungal isolate TPA4 (c) by bacterial isolate PK65 (d) (right). Growth normally of fungal isolate JYGC1 (e) with bacterial isolate PK29 (f) (left); fungal isolate K2 (g) and bacterial isolate PK29 (h) (right)

Decolorization of Vat Violet 1 dye by biofilm with pH variation - Biofilms formed on the surface of LLDPE weighed prior to application to the Vat Violet 1

($C_{34}H_{14}Cl_2O_2$) dye wastewater. Furthermore, LLDPE material transferred into the bottles that had contained vat violet 1 dye wastewater with three variations of pH (5, 7, and 8). The cultures were incubated for 14 days at room temperature with speed of shaking about 80 rpm. Observations were done every 2 days by measuring the Optical Density (OD) of dye with a wavelength of 312 nm. The wavelength determined by recording the absorbance over some range and the highest absorbance was recorded. Before measuring OD values, vat dyes contain biofilm centrifuged at a speed rate about 12,000 rpm for 5 minutes. Microscopic observation was done by observing the structure of biofilm.

Data Analysis - Data were statistically analyzed using ANOVA test on R software and Microsoft Excel. If there was a significant difference, data was tested further by Duncan's Multiple Range Test (DMRT).

3. RESULT AND DISCUSSION

Antagonistic activity value (Table 1) used for selecting the combination isolates (fungi and bacteria that can live together) in the same habitat which in turn could potentially form a mixed culture, or in particular to form biofilms.

Based on antagonistic activity test, selected bacteria and fungi isolate that does not mutually inhibit growth each other was PK29, PK65, PJ39 and ATA6, and K2, K3 and JYGC1. However, further testing, isolate PJ39 were not selected because it low in produce exopolysaccharide (EPS) [13]. Low produce of EPS indicates that the biofilm formation was not formed. K3 also was not selected because it inhibited the growth of ATA6 isolate. Fungal isolates (K2 and JYGC1) and bacterial (PK29, PK65, and ATA6) were tested in the formation of mixed cultures in liquid media.

In the mixed cultures test (Fig. 2) the single culture (fungal isolates only) was found differ with mixed cultures. In single culture, there was no attachment of bacteria on fungal hyphae ((A) and (E)), whereas, in mixed cultures, the bacteria seem to have grown into or attached to fungal hyphae ((B), (C), (D), (F) ; (G), (H)). Attachment of bacteria on fungal hyphae can not be confirmed as a biofilm, nevertheless, it may be the reason that the isolated fungi and bacteria isolates can grow together in the same habitat.

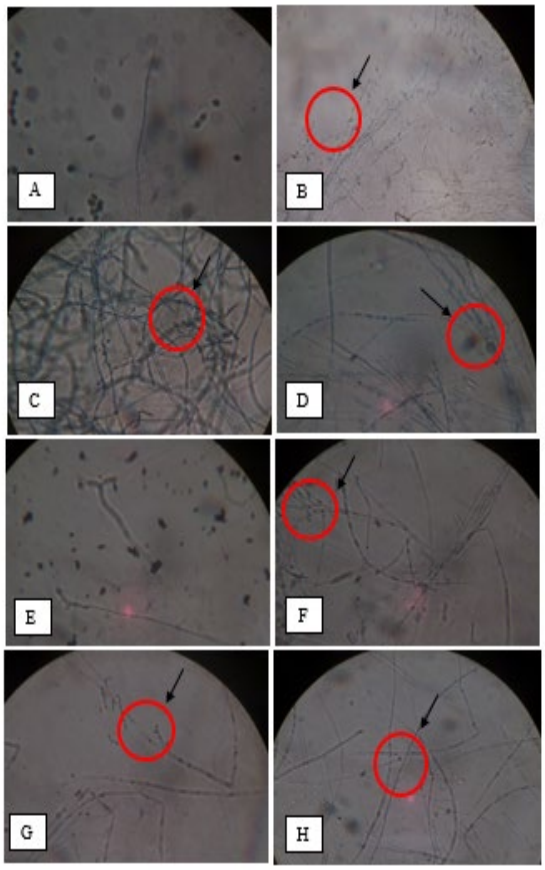

Fig. 2. Microscopically observation mixed culture (zoomed out 400x). (A) Fungal isolate K2; (B) Fungal isolate K2 and bacterial isolate PK29; (C) Fungal isolate K2 and bacterial isolate ATA6; (D) Fungal isolate K2 and bacterial isolate PK65; (E) Fungal isolate JYGC1; (F) Fungal isolate JYGC1 and bacterial isolate ATA6; (G) Fungal isolate JYGC1 and bacterial isolate PK29; (H) Fungal isolate JYGC1 and bacterial isolate PK65. Arrow showed the attachment of bacteri on fungi hypae

Table 2. Biofilm mass in the surface of LLDPE

pH	Biofilm mass (g)		
	a	b	c
5	0.56	0.36	(-) 0.20
7	0.66	0.45	(-) 0.22
8	0.63	0.35	(-) 0.27

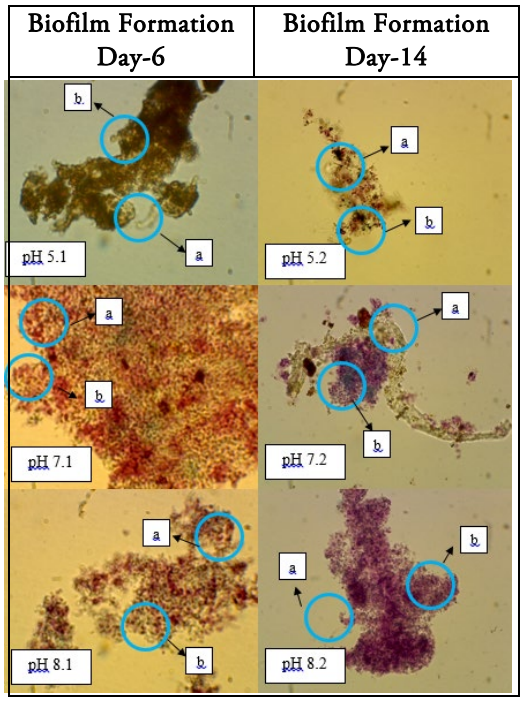

Fig 3. The biofilm formation of bacterial and fungal consortium in the surface of plastic material LLDPE (by microscope, zoomed out 1000x). Fungi (a); bacteria (b)

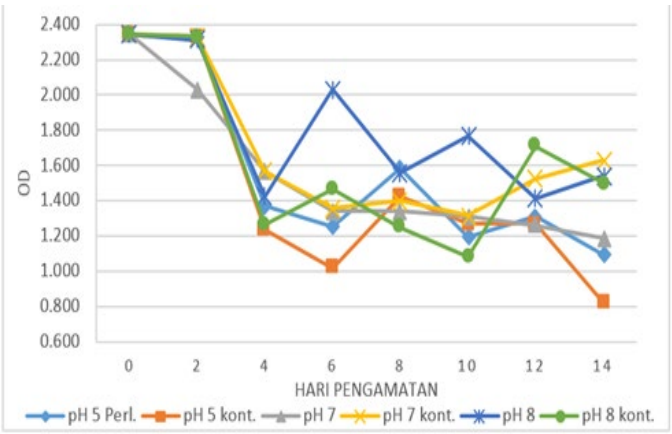

Fig 4. Decolorization of Vat Violet 1 dye wastewater by biofilm of bacterial and fungal consortium in varied pH (pH 5, pH 7, pH 8).

Biofilm formation was occurred after mixing cultures test using bottle cap (LLDPE material) that has a rough surface. On the inside, there is grooves lid that allows the biofilm to attach in it. Rough surfaces may be a good place for the attachment of biofilm-forming microorganisms [14]. Based on the results (Table 2), the mass of the bottle cap material increased after inoculation fungal and bacterial isolates but decreased after the application into the waste mass.

Biofilm mass after cultures inoculation, but before application to dye wastewater (a); biofilm mass after 14 days application to dye wastewater (b); differences in biofilm mass before and after application to dye wastewater (c).

Biofilm mass reduction was also supported by microscopic examination (Fig. 3) which indicates that biofilms were observed in the microscope is reduced from days or the previous observation. It shows that the mass of biofilms that attached on the surface of plastic material is comparable with density of biofilm that was observed in the microscope.

The application of biofilm in the material LLDPE to decolorize Vat Violet 1 dye on the graph (Fig. 4) shows that the OD values of dye at all pH conditions fluctuated, but overall OD values decreased from OD initial starting on the second day of measurement. Impairment OD value of vat dye showed a decrease in the intensity of the dye which also shows the decolorization of dyes by biofilm.

The highest rate of decolorization of vat dye by biofilm at pH 7 with difference at 1.155 between before and after application; much higher than that of without biofilm application at 0.714. It shows that treatment with biofilm can be an alternative treatment to decolorize Vat Violet 1 dye wastewater. Nevertheless, the ANOVA test results still showed no significant difference between the treatment and control group of decolorization at pH 7 because the calculated F value was still smaller than the F table.

4. CONCLUSION

Biofilm with regular thickness was formed by the fungal bacterial consortium and capable of increasing the rate of decolorization activity. The highest biomass yield before and after application to the wastewater was found to be at pH 7 at about 0.66 g and 0.45 g, respectively. The thickness and biomass corresponds with decolorization activity, which is also the highest on pH 7, with difference

of 1.155 between before and after application; much higher than without biofilm application at 0.714.

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Nitrogen Removal in the Anammox Biofilm Reactor using Palm Fiber as Carrier in Tropical Temperature Operation

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ABSTRACT

Anaerobic ammonium oxidation (anammox) is the process of converting ammonium directly into nitrogen gas with nitrite as an electron acceptor under anaerobic conditions. This process is more effective than conventional nitrification-denitrification, but this process is very dependent on several parameters, one of which is temperature. The optimum temperature range for the growth of anammox bacteria is 30-40^{0}C. The purpose of this research was to determine the efficiency of nitrogen removal by anammox process using palm fibers in the Up-Flow Anaerobic Sludge Blanket (UASB) reactor in the tropical temperature. The experiment was conducted at a laboratory scale with a variation of Hydraulic Retention Time (HRT) 24 h and 12 h using artificial wastewater. The reactor was inoculated with anammox granule genus Candidatus Brocadia. The concentration of ammonium, nitrite, and nitrate in the influent and effluent was measured using a UV-Vis spectrophotometer based on standard method. Based on the experiment, the ratio ΔNH_4^+-N:ΔNO_2^--N and ΔNO_3^--N:ΔNH_4^+-N similar with stoichiometry of anammox. The maximum Nitrogen removal performance (NRT) achieved 0.11 kg-N/m^3.d at Nitrogen Loading Rate (NLR) 0.14 kg-N/m^3.d and 0.20 kg-N/m^3.d at NLR 0.29 kg-N/m^3.d. The removal efficiency for Ammonium Conversion Efficiency (ACE) and Nitrogen Removal Efficiency (NRE) in HRT 24 h were 79% and 76%, respectively. While decreased in HRT 12 h were 72% and 69%, respectively. Anammox process can be applied in the tropical temperature at a laboratory scale using a UASB reactor with palm fibre as the carrier. The utilization of palm fiber as an anammox biofilm carrier increased the nitrogen removal performance of the reactor

1. INTRODUCTION

The presence of high nitrogen concentration in effluent wastewater can have a negative impact on receiving water bodies such as reduced dissolved oxygen, promoted eutrophication, and increase the level of toxicity of a water body. Therefore, it needs to be treated before being discharged into water bodies to meet the regulation of quality standards. One of the preferred treatment is a biological process by utilizing microorganisms to degrade organic compounds and to reduce the nitrogen content in wastewater (Gerardi 2002).

Conventional process for removing nitrogen from wastewater was nitrification-denitrification. These process required high costs for aeration and external organic carbon sources for the denitrification process (Szatkowska and Paulsrud 2014). Nowadays, application nitrification-denitrification replaced with anaerobic ammonium

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oxidation (anammox) process. Anammox is a biological process in which nitrite is used as an electron acceptor in the conversion of ammonium to nitrogen gas (Mulder et al. 1995). Anammox process compared to other conventional nitrogen removal processes is more effective because anammox can reduce aeration by 64%, organic carbon 100%, and sludge production 80-90% (van Loosdrecht 2008).

Research related to the anammox process has been carried out with various operational variations such as the type of reactor used (Fluidized Bed Reactor, Membrane Bioreactor (MBR), Sequencing Batch Reactor (SBR), Up-Flow Anaerobic Sludge Blanket (UASB), Anaerobic Baffled Biofilm Reactor (ABBR)), Gas Lift Reactor and other modifications; mode of operation (batch and continue), bacterial species (generally *Candidatus Brocadia* and *Candidatus Kuenenia*), variations of substrate, temperature, pH, salinity, using supporting media (non-woven, membrane, sponge, bamboo charcoal, Polyvinyl Alcohol sodium Alginate Gel Beads, Acrylic Resin Material, Polyethylene Sponge Strips, Spherical Plastic, and string wound filter) (Zulkarnaini et al. 2018). At present more than 114 anammox reactor operated in the world, most of them in Europe, China, and North of America (Ali and Okabe 2015) as sub-tropical countries.

Indonesia, as a tropical country, has a potential opportunity for application of anammox process because of the temperature is suitable for the growth of anammox bacteria (Marc Strous, Kuenen, and Jetten 1999b). The purpose of this research was the analyze the nitrogen removal performance of anammox biofilm reactor in the tropical temperature using palm fiber as a carrier in a UASB reactor. Palm fiber was used as supporting media for anammox biofilm. These fibers have the characteristics: not easily decomposed, durable, and waterproof (Widyawati 2011). As a carrier, palm fibers also reduced the biomass wash-out from the reactor. The use of palm fiber media in the reactor as intended to influence reactor performance. Minimizing the amount of anammox bacteria wash-out and increasing the retention rate is a strategic key in starting up an anammox reactor and stabilizing operating conditions (C. Chen et al. 2012). Biofilm reactors are the best way to process anammox in reducing the biomass wash-out from the reactor (M. Strous, Heijnen, Kuenen, and Jetten 1998).

2. METHODS

A 300 mL UASB reactor was operated for anammox biofilm reactor. For the start-up, granular anammox bacteria genus *Candidus Brocadia* from Kanazawa University, Japan, inoculated into the reactor, Figure 2(a). Palm fiber as the carrier was filled into the reactor with 50% in volume, Figure 2(b). The substrate with the composition described in Table 1 was pumped into the reactor from the bottom, using a peristaltic pump. The substrate tank was connected to a gas bag containing nitrogen gas to maintain anaerobic conditions and regulate the balance of atmospheric pressure in the tank. The effluent will flow to the top of the reactor. This experiment was carried out with variations in HRT 24 h and 12 h. The research installation scheme can be seen in Figure 1.

Table 1. Composition of artificial wastewater (Graaf et al. 1996)

Substrates	Concentration (mg/L)
$(NH_4)_2SO_4$	330 (70 mg-N/L)
$NaNO_2$	345 (70 mg-N/L)
$MgSO_4 \cdot 7H_2O$	300
$CaCl_2 .7H_2O$	180
KH_2PO_4	27.2
$KHCO_3$	500
Trace element I and II	1 mL/L

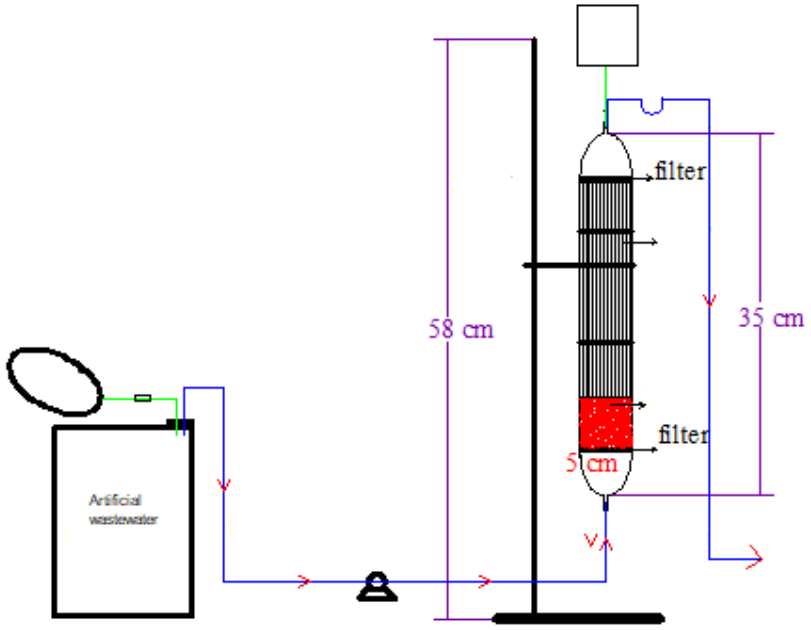

Figure 1. Reactor installation scheme.

Figure 2. Anammox granular as seeding sludge (a) and palm fiber as the carrier (b) for start-up reactor.

Samples collected twice a week and measured directly using UV-Vis Spectrophotometer based on standard method (American Public Health Association, American Water Works Association 1999). Performance of anammox biofilm reactor in the tropical temperature was expressed with nitrogen removal rate (NRR, kg-N/m^3.d), ammonium conversion efficiency (ACE, %), and nitrogen removal efficiency (NRE, %), calculated based on the following equations :

$$NRR=\frac{[NH_4^+\text{-}N]_{in}+[NO_2^-\text{-}N]_{in}-[NH_4^+\text{-}N]_{eff}-[NO_2^-\text{-}N]_{eff}-[NO_3^-\text{-}N]_{eff}}{HRT} \quad (1)$$

$$ACE=\frac{[NH_4^+\text{-}N]_{in}-[NH_4^+\text{-}N]_{eff}}{[NH_4^+\text{-}N]_{in}} x\ 100\% \quad (2)$$

$$NRE=\frac{[NH_4^+\text{-}N]_{in}+[NO_2^-\text{-}N]_{in}-[NH_4^+\text{-}N]_{eff}-[NO_2^-\text{-}N]_{eff}-[NO_3^-\text{-}N])_{eff}}{[NH_4^+\text{-}N]_{in}+[NO_2^-\text{-}N]_{in}} x\ 100\% \quad (3)$$

The inhibition parameters, the concentration of free ammonia (C_{FA}, mg/L), and free nitrous acid (C_{FNA}, mg/L) were calculated based on the following equations:

$$C_{FA}=\frac{17}{14}\frac{C_{t,NH_3}x10^{pH}}{e^{6344/(273+T)}+10^{pH}} \quad (4)$$

$$C_{FNA}=\frac{47}{14}\frac{C_{t,NO_2}}{e^{-2300/(273+T)}x10^{pH}+1} \quad (5)$$

3. RESULT AND DISCUSSION

Stoichiometry anammox

Anammox process can be observed with the conversion of ammonium, nitrite, and produced nitrate in the reactor, Figure 3 - 4. Identifying the anammox processes in the reactor can use the stoichiometric reaction relationship proposed by (M. Strous, Heijnen, Kuenen, Jetten, et al. 1998) as follows:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O0{,}5N_{0,15} + 2.03H_2O \quad (6)$$

The results showed an average ratio of ΔNO_2^--N/ΔNH_4^+-N was 1.24 and for the ratio of ΔNO_3^--N/ΔNH_4^+-N was 0.24. The rates were lower than stoichiometric that indicated the possibility of a denitrification process takes place in the reactor. Where nitrate is reduced by denitrifier bacteria to N_2 gas using organic compounds derived from biomass decay (dead anammox bacteria) (Kindaichi et al. 2007), this difference in stoichiometric ratio can be influenced by the physiological of anammox bacteria, experimental conditions (nitrogen loading, temperature, and pH) and microbial populations (Puyol et al. 2013).

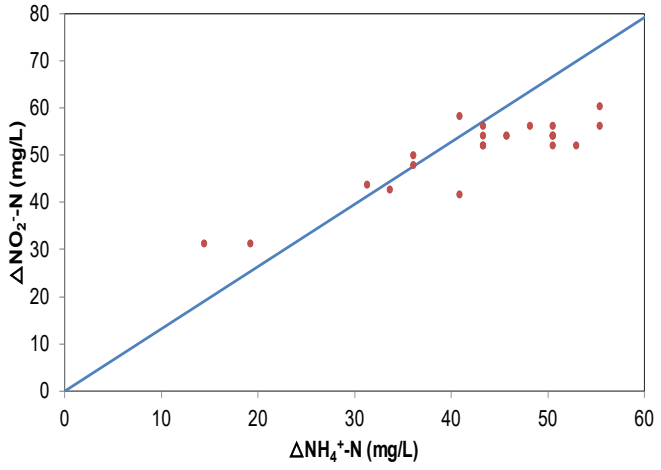

Figure 3. The experimental ratio of ΔNO_2^--N/ΔNH_4^+-N, the blue line is the ratio based on stoichiometry anammox process of 1.32

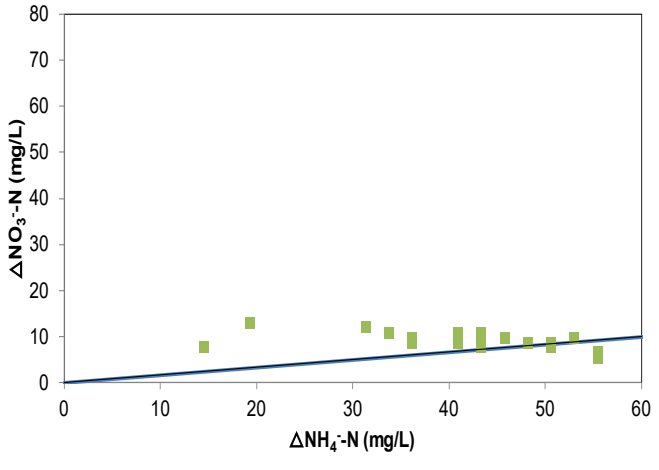

Figure 4. Ratio ΔNO_3^--N/ΔNH_4^+-N, the dark blue line is the ratio based on stoichiometry anammox process of 26

Profile of nitrogen in the operation

Ammonium

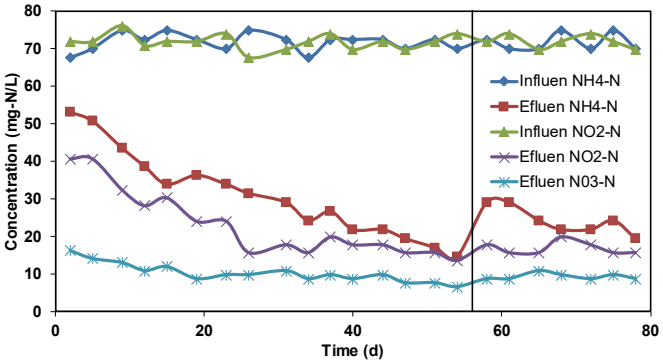

Figure 5. Profile of nitrogen in the operation

Profile of nitrogen concentrations in the influent and effluent during the experiment was illustrated in Figure 5. In the graph, it can be seen that in the period I the effluent concentrations of ammonium have gradually decreased from 53.25 mg-N/L to 14.70 mg-N/L. While in period II, there was an increased ammonium concentration from 14.70 mg-N/L to 29.16 mg-N/L due to changes in HRT from 24 h to 12 h. Then, decreased gradually and achieved 19.52 mg-N/L at the end of the operating reactor. Ammonium is a substrate for anammox bacteria. Decreased of ammonium concentration in experiments in both periods I and II indicated the occurrence of anammox process in the reactor.

Nitrite

Nitrite concentration has an essential role in the anammox process. Besides as a substrate for anammox bacteria, higher or lower nitrite concentrations can have a negative effect of inhibiting the growth of anammox bacteria, reversible or irreversible inhibition (Marc Strous, Kuenen, & Jetten, 1999a). The nitrite concentration in the artificial wastewater is 70 mg-N/L, which was below the threshold of inhibition of the anammox process by nitrite concentration of 0.1 g-N/L (Marc Strous, Kuenen, and Jetten 1999a). The concentration of nitrite in the influent and effluent illustrated in Figure 5, it can be seen that effluent concentrations of nitrite in the period I decreased gradually from 40.75 mg-N/L to 13.72 mg-N/L. The concentration of nitrite effluent in period II increased at the beginning of period II due to decreased HRT from 24 h to 12 h, from 13.72 mg-N/L to 17.88 mg-N/L and then decreased to 15.80 mg-N/L. The remaining nitrite in the effluent can be interpreted that the consumption of nitrite was lower than the stoichiometry. Successful initiation of the Anammox process is indicated by the removal of stable ammonium and nitrite (W. Chen et al. 2017).

Nitrate

A small amount of nitrate produced by anammox process in anaerobic conditions. Produced nitrate was illustrated in Figure 5. On the graph, it can be seen that the produced nitrate in the first period tended to decrease from 16.38 to 6.62 mg-N / L. In period II, nitrate concentration was 8.75 mg-N/L. The presence of nitrate concentrations in the effluent indicates the operation of the anammox process.

Performance of anammox biofilm reactor

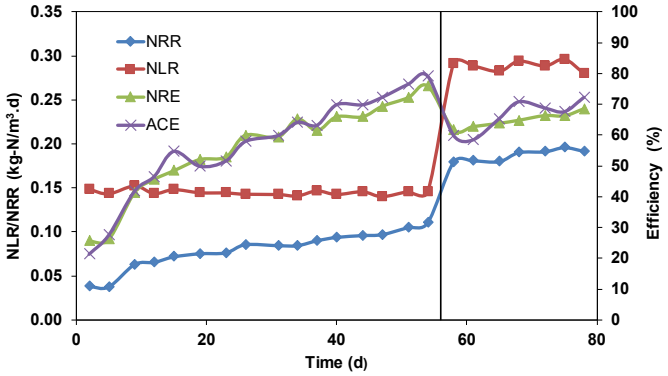

Figure 6. Performance of the anammox biofilm reactor.

Temperature, pH, free ammonia (FA) and free nitrous acid (FNA)

The reactor was operated at ambient temperatures with the range 25-28^{0}C. Anammox reactors are generally performed in the high temperature (≥30^{0}C) and treat high ammonia containing wastewater. The optimal temperature range for the growth of anammox bacteria is between 20-43^{0}C. The enzyme metabolic activity of anammox bacteria is reduced because of the temperature is lower than the optimal value of 37^{0}C (Marc Strous, Kuenen, and Jetten 1999a). A lower temperature operation in the reactor caused a decrease in NRR and the efficiency of ammonium and nitrite removal in the reactor (Ma et al. 2013). In this study, the pH of effluent ranged from 7.3 to 7.8 were still in the

range for the growth of anammox bacteria indicated that the experiments were carried out under optimum conditions. The results of this pH affect the concentration value of FA and FNA. Where if the pH is low, it will reduce FA but increased FNA concentration (Jin et al. 2012).

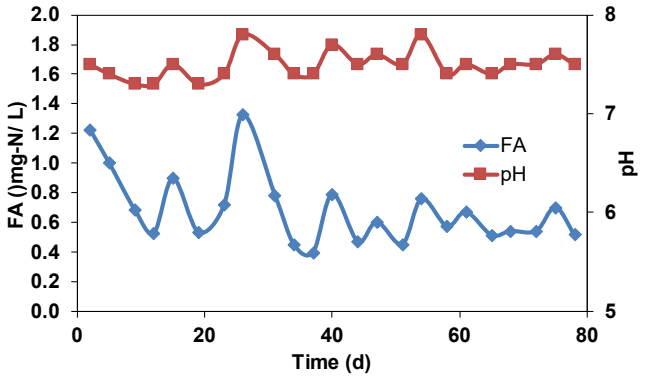

Figure 7. Free ammonia during reactor operation.

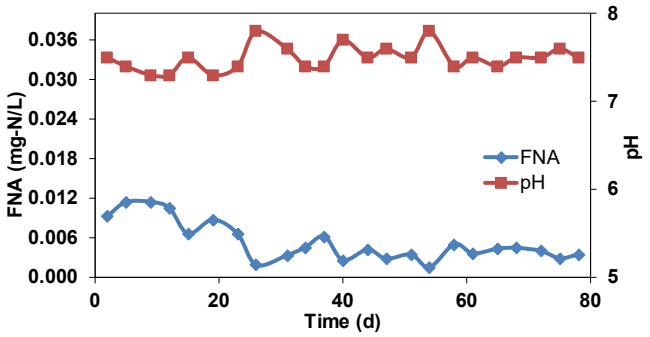

Figure 8. Free nitrous acid during reactor operation.

The concentration of FA and FNA is generally considered as essential parameter in stabilizing the reactor used in the anammox process. As indicated in the previous report, FA and FNA concentrations depend on substrate concentration, operating temperature, and pH in the reactor (Anthonisen et al. 1976). The Inhibition of anammox process was caused by FA is reversible. It has been reported that the recovery time was about one month, and FA did not substantially change the physical properties of the anammox. In a batch experiment, FA concentrations 38 mg/L can inhibit 50% the anammox process and 80% inhibition at 100 mg/L (Fernández et al. 2012). While in the continuous operation, higher concentration both ammonia and nitrite in the influent up to 1.500 and 500 mg/L did not inhibit anammox process. At FA concentration increased up to 150 mg/L, inhibition anammox was not observed. When FA reached 190 mg/L, anammox activity dropped to 10% (Aktan, Yapsakli, and Mertoglu 2012). FA concentration in the study was 0.783, while the FNA concentration was 0.005, below the threshold, Figure 7 – 8. The anammox process in the biofilm reactor with palm fiber was free from inhibitory factors during the study.

Anammox biofilm on the palm fiber

The utilization of palm fiber as an anammox biofilm carrier increased the nitrogen removal performance of the reactor. The large surface of palm fiber increased contact between anammox bacteria and artificial waste, Figure 9. Installation of a carrier along the UASB reactor also optimized by the distribution of anammox bacteria compared to anammox granule, where the removal of nitrogen mainly occurred at the bottom of the reactor due to the tendency of the settlement of granule. However, the small diameter of the fiber caused the anammox biofilm biomass failed to form a "bio-cake" as a step in the process of granulation anammox biofilm which will later be settled with increasing thickness of the biofilm and then detached from the carrier, because the anammox bacteria are likely to form granules (Vlaeminck et al. 2009).

Figure 9. Anammox bacteria growth as biofilm in the palm fiber.

The palm oil fiber, which is difficult to degrade, prevented inhibition of anammox process by degraded organic carbon. Chamchoi (2008) reported that concentrations of chemical oxygen demand (COD) over 300 mg/L decreased performance of the anammox process, while Yang et al. 2019 reported smaller levels of 200 mg/L. The use of palm fiber can be an economical alternative natural carrier compared to commercial carriers such as non-woven carriers, membranes, filters, carbon fiber, and sponges. This material is commonly used as a filter for traditional water filtration. Therefore, the application of the anammox process using palm fiber for removal of nitrogen in Indonesia could be an applicable technology to prevent effect of nitrogen pollution.

4. CONCLUSION

Anammox process stable operated in the tropical temperature with a stoichiometric ratio close to anammox stoichiometry. Performance of nitrogen removal during the study on HRT 24 h and NLR 0.14 kg-N/m^3.d obtained the optimum NRR 0.113 kg-N/m^3.d, while on HRT 12 hours and NLR 0.29 kg-N/m^3.d NRR increased to 0.196 kg-N/m^3.d. The efficiency of ACE and NRE in HRT 24 hours reached 79% and 76%, respectively and became 72% and 69%, respective, in HRT 12 h.

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Low Energy Bacteria Preservation of Extremely Halophilic Archaea Haloferax Lucentense and Haloferax Chudinovii Immobilized using Natural Zeolite

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ABSTRACT

The methods of microbial cells preservation were already known by liquid drying, freeze-drying, and freezing. Those methods could preserve bacteria cells in a long period of time but its survivability was relatively low and used relatively high energy during preservation. Immobilization was known as entrapping, attaching or encapsulating bacterial cells in a suitable matrix. This research was conducted to know the suitability of zeolite as immobilization carrier and also as preservation matrix of two halophilic archaea *Haloferax chudinovii* and *Haloferax lucentense*. Variable of this research was the type of the carrier which was raw zeolite, 110°C and 300°C heat-activated zeolite carrier, parameters measured in this study was physical and chemical of zeolite such as chemical content, Si/Al ratio, surface area and pore volume, and biochemical assay, bacterial cells numbers after immobilization and bacterial cells after preservation as bacterial response to the immobilization and preservation. Heat activation was significantly affecting the zeolite chemical composition, carrier surface area, and pore volume. Compared to other pretreated zeolite, highest quality zeolite was obtained in 110°C pretreated zeolite which has which has 65,625 m^2/gr for surface area 0,071 cc/gr pore volume and 5,13 Si/Al ratio. . The bacterial cells obtained after immobilization process was 1,8x10^7 cfu/g, 3,0 x 10^7 cfu/g, and 2,1x10^7 for raw zeolite, 110°C pretreated zeolite and 300°C zeolite respectively. After 4 months preservation, the slight reduction of the bacterial cells was observed. Immobilization halophilic archaeae using zeolite as carrier was proven as low cost and effective preservation method due to relatively simple process and unspecific preservation temperature requirements.

1. INTRODUCTION

Halophilic bacteria or halobacteria are known as single cell microorganism inhabit saline environment. Halobacteria are considered as halophilic archae living in hypersaline environment and having pigment ranging from yellow to red. Mostly halophilic archaea are found in crystallization ponds due to high salinity requirement (250-300 g/l) of NaCl (A Oren, 2010). Halophilic archaea have ability to coupe with high osmolarity pressure due to their ability to compensate intercellular osmotic pressure using salt in mechanism and compatible solute (Das sarma, 2001). Halophilic archaea were also known to their ability to survive inside the halite crystal in long period of time (Norton et al., 1993).

Bacteria cells preservation has been known for a long time. Lyophilization or freeze-drying method was the oldest methods used to preserved bacteria cell in which

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bacteria cell was frozen and then dried using freeze drying apparatus (Heckly, 1961). Freeze drying apparatus consisting of vacuum machine, freezing apparatus and drying apparatus, this method has relatively low survival percentage due to extreme temperature applied during its processes and also the requirement of suitable protective media are strictly needed. To obtain maximum preservation time the freeze dried sample must be stored in 1-4°C storage or even lower temperature (Kupletskaya and Netrusov, 2011). The limitation of freeze-drying method were very selectively depended on the microorganism characteristic. Preservation of bacterial cell commonly done by refrigeration (storage in < 4°C), ultralow freezer (-86°C), cryofreezing (-130 to -145°C) this preservation cost high energy to operate.

Zeolite is naturally found in nature and commercially used by many human activities. Zeolite has white-greenish soft minerals, it consists commonly of a tetrahedral crystalline microstructure built from alumina (AlO_4) and silica (SiO_2) (Jha, B; Singh, 2016). Zeolite composed in three categories which are extraframework cations, framework and sorbed phase due to its high adsorption properties. Upon incorporation of Al with SiO2 the surface of zeolite becomes negatively charged, in this condition extraframework of organic and inorganic cations is needed to made the surface positively charged (Payra and Dutta, 2003). Zeolite is also known to have molecular sieve, ion exchange and catalityc features.

Immobilization is known as a common technique to preserve and improve bacteria activity. Immobilization is microbial engineering by trapping, attaching and encapsulating the enzyme or bacterial cells into a matrix or carrier (Woodward, 1988). The suitable matrix characteristic for immobilizing bacterial cell must have high cell loading capacity, simple and nontoxic for targeted bacteria, mechanically stable, bacteria and matrices were easily separated (Abdelmajeed et al., 2012). Common carriers used for immobilizing bacterial cells are divided into inorganic materials such as zeolite, clay, porous glass, ceramics and organics materials /polymers (Suzana et al., 2015). The advantages of bacterial cell immobilization compared to enzyme immobilization is cheaper due to reduction of the separation process, the possibility of multiple reactions occurred due to various enzymes produced by bacteria cells, and the presence of bacterial biosynthesis that supports the existence of longer or complex chain of enzyme reactions (Jack and Zajic, 1977). Increased ability and biochemical activity of immobilized bacteria are well known, research done by Shindo et al., (2001) investigated continuously ethanol production using *Saccharomyces cerevisiae* immobilized in inorganic matrices (zeolite) carriers which have twice larger fermentation activity compared to un-immobilized *Saccharomyces cerevisiae*. In other hands (Omarova et al., 2012) shown that immobilized Rhodococcus spp using organic polymers were used in the degradation of crude and oil products. Many methods used to immobilizing bacteria cell and the most common bacterial cell immobilization was encapsulation using alginate polymer (Zommere and Nikolajeva, 2018). Otherwise, immobilization of bacterial cells could probably has a negative impact to bacterial biochemistry activities.

Immobilization processes are also used to preserve bacteria cells in order to keep the bacteria in perfect condition when it comes to use. Research done by Krumnow et al (2009) obtained that E.Coli and Bacillus subtilis were well preserved for 64 days in accacia gum and pulluan matrices with temperature and humidity variation, thus result are also affected by the bacteria characteristic such as avoid dehydration capability and ability to produce spore. There are many variation of immobilization carriers used for preserving bacteria cells such as sol gel matrix (Nassif et al., 2003) and PVA (Poly Vinyil Alcohol) (Efremenko and Tatarinova, 2007). The matrix selection used in immobilization process are adjusted with the bacteria characteristic and the purpose of immobilization. Bacteria cell immobilization are also has function for maintain bacteria ability to produce specific proteins. Desimone et al (2005) found that E. Coli producer of recombinant proteins were immobilized in silicone oxide matrix and it preserved perfectly in 4°C and 20°C storage for 60 days.

Haloferax chudinovii and *Haloferax lucentense* are an example of halophilic archaea which found in hypersaline environment from Sampang region in Madura Indonesia. Potential use of these haloferax species are still limited due to lack of study about this species. The aim of this research was to obtain information about possibility of low energy preservation of two halo arcahaeae species *Haloferax chudinovii* and *Haloferax lucentense* using immobilization method.

2. METHODS

2.1 Carrier Pretreatment, Morphological visualization and quantificationHalophilic bacteria preparation

a. Carrier Pretreatment

The Natural zeolite used as a carrier in this research was obtained from a local chemical store in Semarang. Pre-treatment of the carrier used in this research was done by activating the zeolite using heat treatment to remove organic impurities in the zeolite pore. Heat activation was done by heating the zeolite using furnace at 300°C for 4 hours (Djaeni et al., 2010) and heating using the oven at 105°C for 24 hours (West and Strohfus, 1997), treated zeolite was then cooled in a desiccator.

b. Morphological Visualization and Quantification

Quantification of immobilization carriers was done using Braunner-Emmet-Teller (BET) method and Barret-Joyner-Halenda (BJH) method (Quantachrome instrument), this quantification was to determine zeolite surface area and pore size for each pretreatment variations. Morphological visualization of immobilization carriers and the immobilized sample was done using SEM-EDX (Jeol JSM 6510 Analytical Scanning Electron microscope and JED 2300 EDS for EDX analysis) analysis was carried out to obtain the difference between two heat activation to zeolite physical and chemical properties.

2.2 Culture preparation and immbilization procedure

a. Culture Preparation

Extremely halophilic archaea *Haloferax chudinovii* and *Haloferax lucentense* strain used in this research was laboratory isolate. Pre-culture was conducted by inoculating each 2 loops pure culture of *Haloferax chudinovii* and Haloferax lucentens into different sterile conical flask glass filled with 50ml of modification of extremely halophilic liquid medium consisting NaCl 24% (w/v), yeast extract 0,25 gr/l, tryptone water 0,5gr/l, $MgSO_4.7H_2O$ 20 gr/l, KCl 2 gr/l, and trisodium citrate 3 gr/l (Nilawati et al., 2017), the cultures were incubated and shaked in shaker incubator at 39°C for 7 days. The culture Harvested after 7 days incubation and then mixed into 250ml sterile conical flask and stirred to make the mixture homogenous. The obtained mixture was stirred for 15 min and counted the cell using total plate count (TPC) method to determine the number of the viable cell, TPC was done by diluting 1 ml of harvested mixed culture and then diluted in series 11 fold in 9ml sterile distilled water, the number of bacterial were expressed as CFU g^{-1}. Obtained intial culture was 10^{11}

b. Immobilization Procedure

The immobilization was carried out by inoculating 50ml pure culture of *Haloferax lucentense* and *Haloferax chudinovii* each in sterile erlenmeyer flasks and then each 10gr natural and pretreated zeolite as immobilization carrier was added to the culture. Immobilization procedure was done under shaking condition at 200rpm for 24 hours in order to obtain maximum absorption of the cultured cell on the zeolite carrier matrices. Immobilized cells were then harvested by separating supernatant and the culture filtrate and dried in room temperature for 24 hours.

2.3 Analysis

a. Biochemical Assay

Biochemical assay of *Haloferax lucentense*, *Haloferax chudinovii*, and immobilized *Haloferax lucentense* and *Haloferax chudinovii* was determined using Vitek 2 Technology (Biomereux) by culturing 10 w/v immobilized sample in liquid modified haloferax medium and incubated in shaker incubator in 150rpm for 7 days at 39°C. The growth cultures then harvested and stored in a sterile glass tube with screw cap and then transferred for biochemical assay.

b. Viability cell during preservation

The number of viable cell were determined as viable counts (cfu) per gram immobilized sample. After dried in room temperature for 24 hours immobilized *Haloferax lucentense* and *Haloferax chudinovii* sample were transferred into a sterile plastic clip and then stored in room temperature for 4 months (Alvarez et al., 2007). Viability cell was done after immobilization process and after 4 months preservation using TPC method with triple replicates for each variation, 1 gram of each immobilized sample were serially diluted 10^{-9} fold dilution and each dilution was mixed using vortex to make homogeneous suspension, then 1ml aliquot of 10^{-4}-10^{-9} diluttion were plated in solid modified haloferax medium which was then incubated for 21 days at 39°C.

3. RESULT AND DISCUSSION

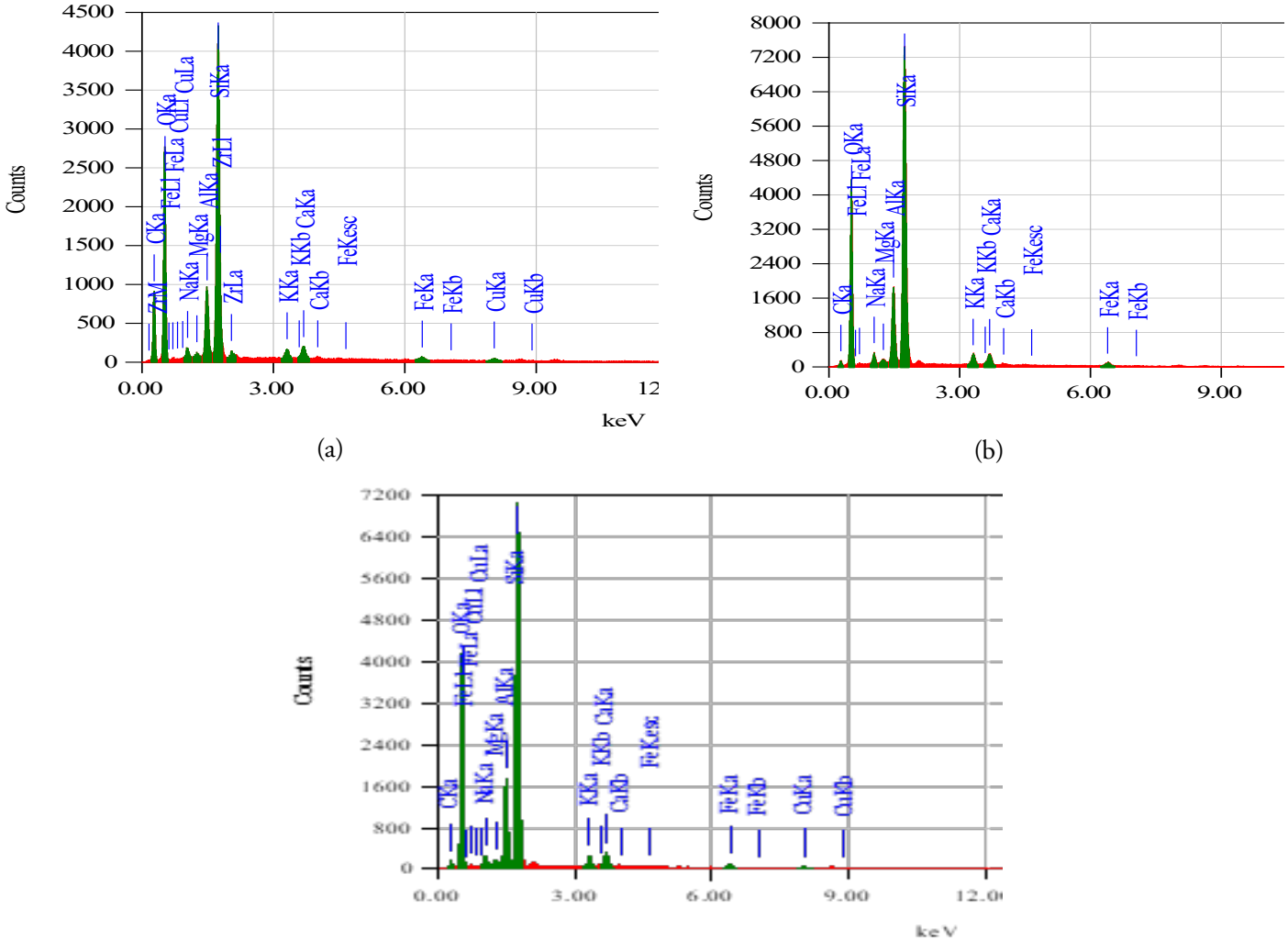
3.1 Physical and chemical characteristic of carriers

Zeolite is a porous material which consists of Al2SO3 and SiO2 bonded together in such way formed tetrahedral structure (Mery et al., 2012). CaCO3, organic and water vapor are the most common impurities found in zeolites that could cover the porous that led to decreasing the zeolites ability to adsorb targeted substances (Fuoco, 2012). Heat treatment and chemical additionare the most common pretreatment done to activate and or enhance zeolite ability to adsorb liquid and gasses. Chemically activated zeolite has several disadvantages when used as bacteria cell immobilization carrier which was longer activation process, the possibility of hazardous chemical left in the carrier matrices that has a negative effect on immobilized bacteria.

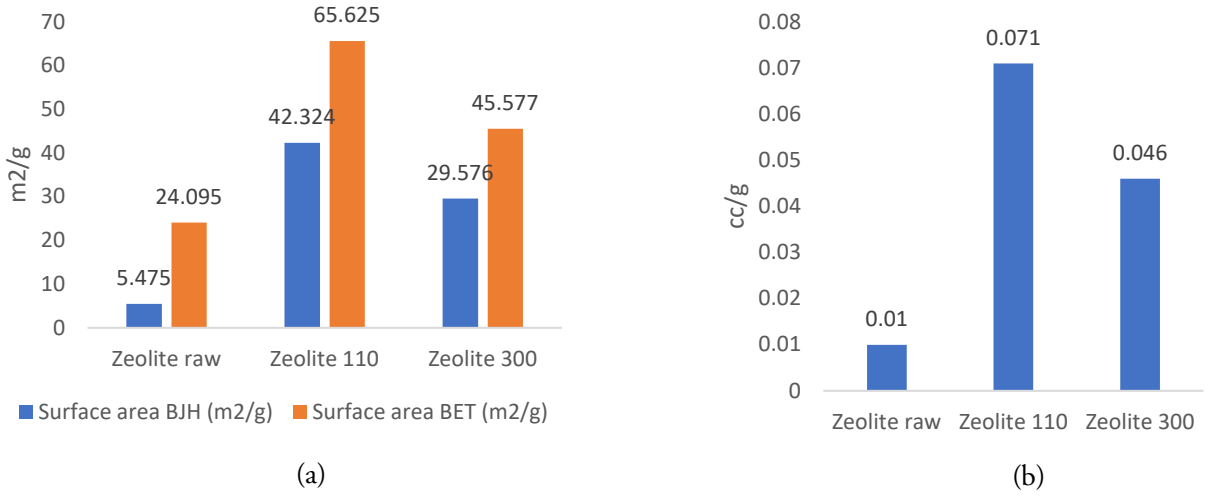
Table 1 shows the quantification of zeolite composition in all pretreatment variable used in this research. Carbon content considered as impurities in zeolite and it has significantly decreased due to heat treatment applied, it decreased from 54,41% to 13,07% and 15,45% in 110°C pretreatment and 300°C pretreatment respectively. In other hands physical heat treatment increased the percentage of SiO_2 and Al_2O_3, initial percentage of raw zeolite was 32,48% and 5,88% while the 110°C and 300°C pretreated zeolite has increased twice which was 64,89/12,47% and 62,79%/11,82% respectively. The increasing of $SiO_2/Al2O_3$ percentage occurred because of elimination or reduction of organic impurities and evaporated water vapor which trapped in zeolite pore by heat treatment. Heat treatment applied in this experiment was not impacting other impurities such as CaO and K_2O minerals this phenomenon occurred because the heat applied was not high enough to decompose the substances due to zeolite calcination temperature was ranging between 1200-1300°C (Shindo et al., 2001). The importance of heat tratment was to enhance the Ca+, K+, Na+ ions in zeolite, thus needed for making the zeolite surface become more positively charged to accomodate adhesion or adsorption of negatively charged bacteria cell wall. Calcination was not needed in this experiment because of the physical and chemical properties needs for immobilizing bacteria cell was not specifically required, the importance of heat treatment was reduction of organic and vapor impurities that could caused blocking or inhibition of bacterial adsorption or adhesion.

Table 1. Chemical Composition of Carriers

Element	Mass %		
	Raw Zeolite	110°C Pretreated zeolite	300°C Pretreated zeolite
C	54,41	13,07	15,45
Na2O	0,96	2,02	1,64
MgO	0,29	0,57	0,75
Al2O3	5,88	12,67	11,82
SiO2	32,48	64,89	62,79
K2O	0,89	2,13	1,84
CaO	1,38	2,57	2,44
FeO	0,9	2,08	2,13
CuO	1,53	-	1,13
ZrO2	1,29	-	-
Si/Al ratio	5,52	5,12	5,31



Figures 1. EDX visualization Raw Zeolite (a), Pretreated 110°C zeolite (b) and 300°C (c)



Figures 2. Physical properties of carrier, Carrier surface area (a) and Carrier pore volume (b)

Another impact affected by heat treatment was Si/Al ratio, untreated zeolite has 5,52 Si/Al ratio, 110°C and 300°C heat treated zeolite has Si/Al ratio 5,12 and 5,52 respectively, the lower ratio of Si/Al and higher SiO_2/Al_2O_3 content means higher quality of the zeolite (Mustain et al., 2014). Hydrophobicity and ion exchange capacity of zeolite could be known from its SiAl ratio, higher ratio means higher hydrophobicity and lower ion exchange capacity (Kubota et al., 2008). Si/Al ratio are also affect zeolite pore shape and its distribution. Zeolite modification could be done by physical or chemical treatment or both depend on needs, especially Modification were needed to obtain specific shape and size of zeolite pore. For example, zeolite modified to become nano-pore zeolite was used as immobilization carrier of α-amylase enzyme (Talebi et al., 2016). Another physical properties affected by heat activation was zeolite surface area and pore volume.

Figures 2 shows the BET and BJH surface area and pore volume analysis of untreated and treated zeolite carrier. Since the organic impurities were reduced and the water evaporated due to heat application the surface area of the carrier was increased it was confirmed by the BET surface area in mother carrier (raw zeolite) was 24,095 m^2/g and the highest surface area was 110°C pretreated zeolite which has 65,625 m^2/g and followed by 300°C pretreated zeolite which has surface area 45,577 m^2/g. Surface area analysis using the BJH method has the same trends with BET method. Highest BJH surface area was occurred in 110°C treated zeolite which has 42,324 m^2/g, followed by 300°C treated zeolite and zeolite raw which has 29,576 m^2/g and 5,475 m^2/g respectively. Increased pore volume has same trend with surface area, pretreated 110°C zeolite has 0,071 cc/g it was the highest volume compared to two others carriers.

3.2 Immobilzation

Immobilization bacterial cells onto zeolites matrix has been investigated by many reseracher, Weiß et al., (2013) investigated that anaerobic bacteria grows and colonized in activated zeolites, another work done by Hrenovic et al., (2011) has investigated phospate-accumulating bacterium A. Junii could immobilized in zeolitized tuff.

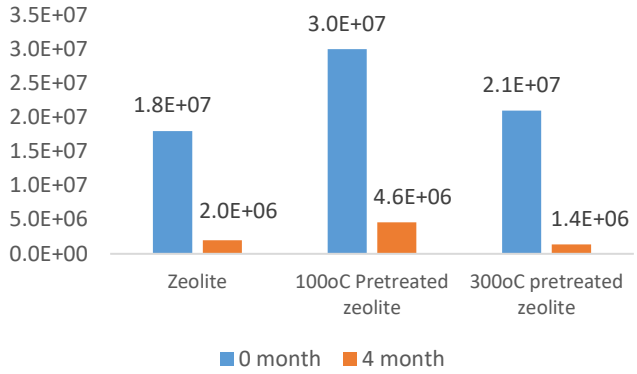


Figure 3. Number of immobilized bacteria cells (Cfu) in different zeolite carriers

After 24 H of bacterial immobilization process and then dried achieved natural zeolite has the lowest number of bacteria which was 1,8x107 cfu/g and followed by 300oC pretreated zeolite with 2,1x107 cfu/g. The highest immobilized bacteria cell was achieved using 110oC pretreated zeolite as immobilization carrier which has the number of bacteria 3,0 x 107 cfu/g. The result After 4 months preservation observed that reduction of bacterial cell in immoblized carrier were occurred in all carrier variations, raw zeolite carrier was reduced to 2x106 cfu/gr, 110oC pretreated carrier was 4,5x106 cfu/gr and 300oC pretreated zeolite was 1,4x106 cfu/gr. Reduction of viable cells number was normally happened during initial preservation. Previous research done by (Sakane et al., 1992) found that several halophilic archaebacteria preserved using liquid drying method could survive approximately to more than 3 years at 5oC storage but reduced survival rates was still occurred and cell viability was reduced until 2 weeks preservation but relatively stable after 2 weeks. Preservation using liquid drying method has several disadvantages such as obligation to use protective media as immobilization carrier, the number of viable cells after immobilization process was regarded low due to extreme heat exposure during liquid drying process, and the immobilized cell must be stored at 5oC to gain maximum preservation condition. Compared to another research, Alvarez et al (2007) obtained the number of viability cell of

E. Coli immobilized in silica oxide and its derivate which stored at 20oC has a reduction variying from 102 to 103 cfu/ml in 60 days preservation.

SEM visualization of un-immobilized and immobilized carriers used in this research were presented in Figure 4. Under microscopic visualization, halophilic archae used in this research has irregular shape and attached each others with slime-like substances (fig 4.g), those slime-like covering haloarchaea microorganism was exopolymer secreted by halophilic archaea microorganism as compatible-solute mechanism to adapt with hypersaline and other environmental stresses (Dassarma and Dassarma, 2016). Halophilic archaea also has "salt in" and "salt out" mechanism for osmoregulation or osmo-protection in order to balance the osmotic pressure with the extracellular environment (Vreeland, 2012). Salt-in or salt-out mechanism is a srtategy to accumulating inorganic ions escpecially K+ in their cytoplasm or secreting inorganic ions to their environment in order to maintain their ion gradient equilibrum (Kunte, 2009). Salt-in and salt-out mechanism works not only for osomregulation purpose but also for intracellular enzymatic activity (Empadinhas and Costa, 2008).

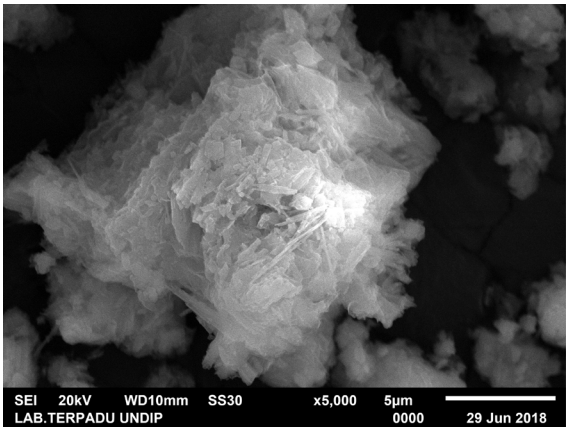
Figure 4a is SEM visualization of natural zeolite before immobilized with halophilic archaea, compared to other carriers, natural zeolite surface and pores covered by impurities. Pretreated 110^{o}C and 300^{o}C zeolite has more clearer surface and pore (figure 4 c and 4 e). Immobilized bacteria was colonizing mostly in the surface area of the carrier, some colony in pore was observed but not fully covering or plugging the pores. The presence of *Haloferax chudinovii* and *Haloferax lucentense* were marked with cubic shape or cubic like crystal which specifically produced by extreme halophilic archaea due to salt in and salt out mechanism (Castanier et al., 1992; Malik et al., 2019). In the hypersaline and dry environment the presence of halophilic archaea and formation of NaCl crystal or ooids were inefitable due to osmoregulation mechanism described below, this phenomenon happend due to drying in immobilization process. When the formation of NaCl crystal some fluid brine would form fluid inclusion, in the presece of halophilic archaea when the fluid inclusion was formed the halophilic archaea would use it as a shelter to survive in unfavorable condition (Lowenstein et al., 2011).

Figure 4 shown that immobilization was done mainly by adhesion mechanism, adsorption only happend due to the presence of pores in zeolite that utilize capillary pressure so it pull the culture (liquid phase) near onto zeolite surface. Most of gram-negative bacteria has negative charge while the zeolite it self has the same ionic charge, but negatively charged bacteria could also adhere on negatively charged materials (Klein and Ziehr, 1990), another possibility for bacteria to attached in zeolite surface was heat treatment that significantly turn the ionic charge of zeolite surface from negatively charged to positively charged . Adhesion or attachment of Halophilic archaea on the surface of zeolite carrier are also supported by the presence of S-layer proteins on haloarchaea cell wall that help the bacteria to attach on solid surface (Fendrihan et al., 2011). Another mechanism of halophilic archaea adhesion was exopolymers secreted by halophilic microorganism which surrounds the bacteria cells or colony that makes the bacteria attached to the zeolite surface.

Physiological and biochemical changes during immobilization may occurred due to unfavorable condition (Chauhan and Singh, 1999). The changes of Halophilic archaea biochemical pathways was investigated when trapped in halite fluid inclusion (Zerulla et al., 2014). Biochemical activity before and after immobilization procces was obtained by biochemical assay and shown in table 2.

Biochemical assay shown that immobilization of halophilic archaea *Haloferax chudinovii* and *Haloferax lucentense* was not changing halophilic arcahaea metabolism ability (Table 2). halophilic archaeae enzymatic activity such as Lactatae alcalinization, Lipase enzyme, Tyrosine arylmidase, and ellman test was present in all immobilized carriers, only succinate activity who not present. From the data above shown that immobilization carriers and immobilization process were not giving a negative impact to halophilic archaea biochemical activity. Otherwise after immobilization another enzymatic activity

who were not present in single culture enzymatic assay was present in immobilized culture assay such as Phospatase, Fermantation procces, adonitol, A-glukosidase enzyme activity. Thus data confirmed that immobilization process was enhancing bacteria ezymatic activity. Advantage of bacterial cells immobilization is the stability of protein and enzyme and this is not limiting their industrial utilization (Alfani et al., 1994).



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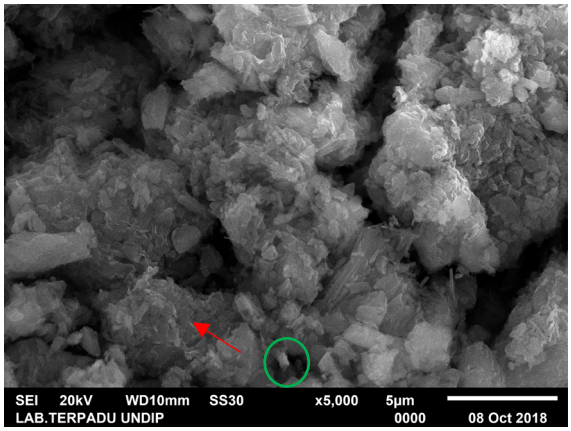

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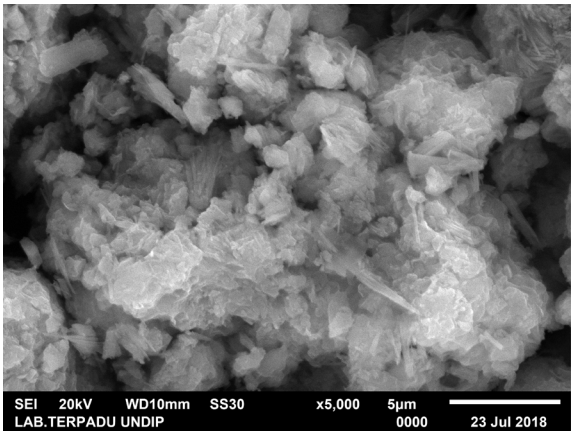

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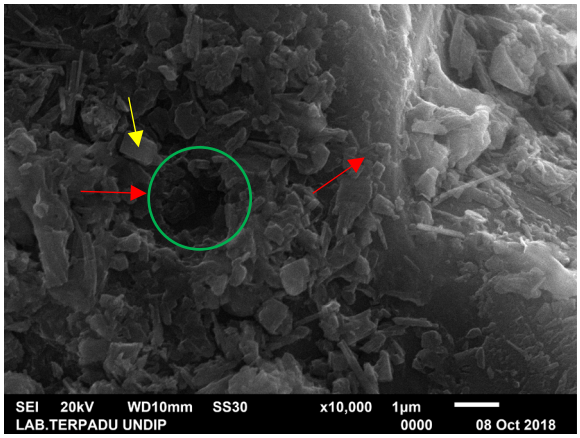

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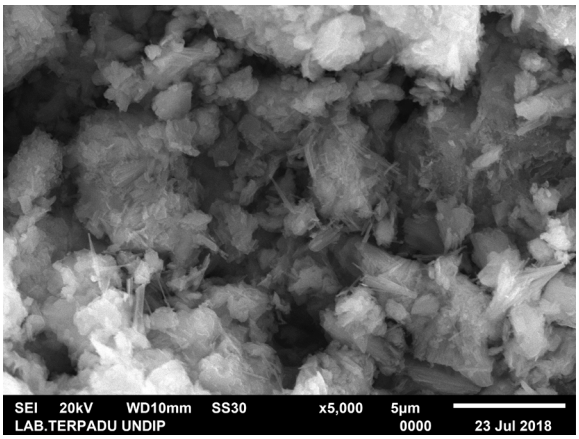

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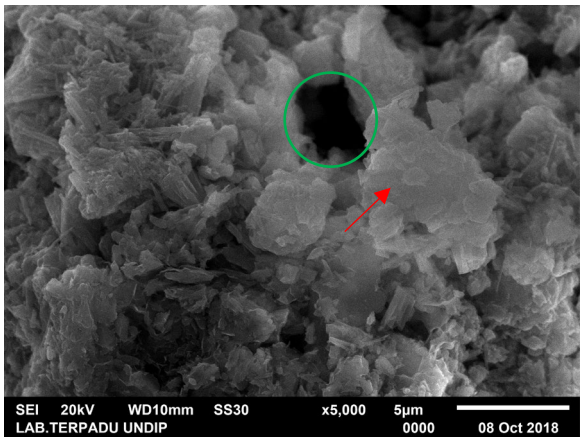

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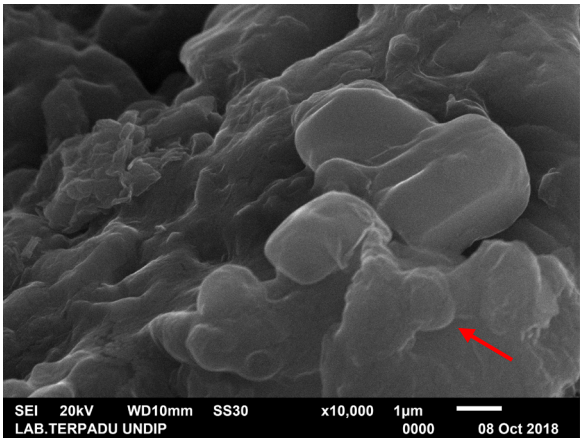

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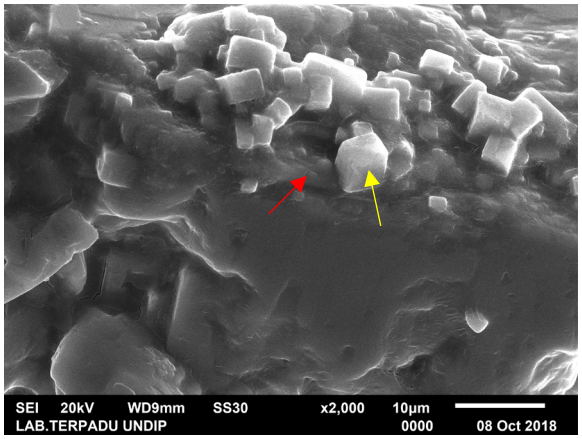

(h)

Figure 4. (a) Un immobilized Raw zeolite (b) immobilized Halophilic bacteria in raw zeolite matrices (c) Un immobilized 110°C pretreated zeolite (d) immobilized 110°C pretreated zeolite (e) Un immobilized 300oC pretreated zeolite (f) immobilized 300°C pretreated zeolite (g) SEM visualization of *Haloferax chudinovii* and *Haloferax lucentense* used in this work (h) NaCl crystal in Halophilic archaea colony; red arrow: Halophilic arcaea; Yellow arrow: NaCl crystal (cubic shape); green circle: pores

Table 2. Biochemical assay

No.	Test Parameter		Isolate / sample variation				
	Code	Measured parameter/activity	*Haloferax lucentense*	*Haloferax chudinovii*	Immobilized in zeolit carrier	Immobilized cell in 110°C pretreated Zeolit	Immobilized cell in 300°C pretreated Zeolit
1.	ILATk	L-Lactate alkalinization	+	+	(-)	+	+
2.	BGLU	B-Glukosidase	(-)	-	-	-	(-)
3.	Lip	Lipase	+	+	+	+	+
4.	ADO	Adonitol	-	-	+	-	+
5.	AGLU	A-Glukosidase	(-)	-	+	+	+
6.	SUCT	Succinate	-	+	-	-	-
7.	dMAN	D-Mannitol	-	-	(-)	-	-
8.	TyrA	Tyrosine Arylamidase	+	+	+	+	+
9.	ELLM	Ellman test	+	+	+	+	+
10.	CMT	Courmarate	-	-	+	-	-
11.	OFF	Fermentation/glucose	-	-	(-)	-	+
12.	PHOS	Phospatase	-	-	-	-	+

3.3 Survivalability of Halophilic archaea Haloferax chudinovii and Haloferax lucentense

The aim of Immobilization procedure is to preserve tergeted bacteria into selected carriers. Almost all bacteria known could be preserved when it become dormant. Dormant bacteria has very slow metabolic and other biochemistry activity which impact on decreased cell division rates. Survivalability of immobilized *Haloferax lucentense* and *Haloferax chudinovii* in zeolite carrier happend due to the ability of halophilic archaea to survive in unvaforable or starvation condition (Winters et al., 2015). Exopolymer secreted by halophilic archaea were also affect the survivalability of halophilic archaea during preservation due to formation of halophilic archaea biofilm and keeping the cell together in a three dimensional structure (Toyofuku et al., 2016). Biofilm formation are considered as strategy for bacterial survivalability, since biofilms protect microorganism from variable environmental conditions (Zur et al., 2016). The preservation condition in this research were placed under room temperature in closed pastic clip which immobilized halophilic archaea is on dry state. Halophilic archaea survival in dry environment was already known (Aharon Oren, 1994), also halophilic archaea enzymatic proteins were adapt to heat and dry environment and this phenomenon made halophilic archaea could survive in dry environment for long period of time (Tehei et al., 2002).

Due to the presence of exopolymer that bind and covering the bacteria together, thus promote reduction of dehydration in bacteria micro envirnoment which avoiding bacteria to environmental stress so the survivalability of *Haloferax lucentense* and *Haloferax chudinovii* were remain high. Another mechanism of survivalability of *Haloferax chudinovii* and *Haloferax lucentense* were the formation of crystal inclusion in conjunction with saline water that trapped the bacteria inside and survive in prolonged preservation. The survivalability of immobilized bacteria was depend on the method, carriers or immobilization matrices used and targeted bacteria. All of the three points mention before have mutual functions in order to keep bacteria survivalability during post immobilization.

4. CONCLUSION

The numbers of viable cell was relatively high during 4 months preservation in room temperature. Heat activation of the carrier was changing its chemical and physical properties but it seems to be uneffecting bacterial survivalbility or the numbers of attached bacteria. Immobilization and preservation prcedure done in this reserach was not affecting bacterial biochemistry ability. Immobilization method using zeolite as carrier was proven to be used as suitable low cost preservation method for halophilic archaea *Haloferax lucentense* and *Haloferax chudinovii* since the immobilization method was simple and preservation condition do not need special (temperature) condition.

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Waste to Product : Bisolum-Bricks, Incorporating of WWTP Sludge of Textile Industry into Bricks for Wall Pairs

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ABSTRACT

The disposal of WWTP sludge is one of problems in textiles industry, which requires serious attention to find a way out. Utilization of sludge from the textile industry wastewater treatment, according to the Republic of Indonesia Goverment Regulation No.110 year 2014, can be used as a mixture of brick raw materials, must consider the availability of technology, meet environmental quality standards and meet technical requirements for use. Environmental feasibility refers to Goverment Regulation No.110 year 2014, carried out with TCLP toxicity tests on raw materials for soil, WWTP sludge and brick products. Acute toxicity test LD 50, carried out on brick products in which using a mixture of WWTP Sludge. Technical feasibility is carried out by testing the quality of brick products in accordance with solid brick Nasional Indonesian Standard (SNI) for wall pairs. Research results prove the toxicity test on raw materials and brick products with a mixture of up to 60% of waste, still meets the requirements of TCLP and LD.50 according to IGR No.110 year 2014. Test the quality of bricks at the use of 40% and 60% mixture of sludge still meet Nasional Indonesian Standard (SNI 15-2094-2000) solid red brick for wall pairs.

1. INTRODUCTION

The disposal of sludge from the wastewater treatment unit is one of the problems in the Dyeing-finishing textile industry, which needs serious attention from all parties, due to the nature of its characteristics which are considered to contain hazardous substances such as acids or alkalis substances and some hazardous metal such as chromium, which have the potential to cause environmental pollution, if not properly managed.

Balan & Monteiro (2001), stated that the wastewater treatment process in the Wastewater Treatment Unit, produces a number of sludge from chemical treatment processes, and or sludge from biological treatment. The textile industry that treats wastewater at 50 m^3 per hour can produce 1 to 10 tons of sludge per day.

According to Indonesia Government Regulation No. 101year 2014, the management of Toxic and Hazardous Waste Material is intended so that the Toxic and Hazardous Waste Material produced by each production unit is as little as possible and even attempted to zero, by striving to reduce the source by processing materials, substituting materials, regulating operational activities, and using them clean technology. If toxic and hazardous

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material waste is still produced, the endeavored use of toxic and hazardous waste.

Utilization of WWTP sludge as a mixture of brick making has been done and has produced results that meet the technical feasibility and environmental feasibility, but this has not been able to encourage relevant parties to utilize this WWTP sludge, because understanding the concept of re-utilization of industrial WWTP sludge has only been raised since the issuance of the Indonesia Regulation Government No.110 of 2014. as an effort to solve problems in the textile industry, as well as efforts to reduce the impact environment due to exploitation of land use as raw material for bricks.

R. Baskar (2006) states that the use of sludge as a building and construction material not only converts waste into useful products but also minimizes the problem of sludge disposal WWTP and the reduction of exploitation of natural resources such as clay can also be prevented to avoid further environmental damage.

Jahagirdar (2013), stated another advantage of reusing sludge or sludge ash in burned clay bricks or tile is immobilization of heavy metals in the combustion process, organic matter is completely oxidized and removes all types of pathogens during the high temperature combustion process. Clay bricks with a combustion process, in general have been used as building materials with solid metric mainly because of its characteristics, such as good mechanical resistance, and satisfactory stability.

Utilization of various types of sludge waste for clay bricks mix shows various advantages in terms of physical and mechanical properties such as low density, lighter bricks, better compressive strength and even reduced energy consumption during combustion, although it also still shows some weaknesses. These studies have also shown that the use of sludge waste into a mixture of brick materials has a better impact on the environment.Chemical composition as well as heavy metals after combustion or solidification, on the final product according to standards Nasional Indonesian Standard (SNI 15-2094-2000)

.Utilization of sewage sludge can produce good quality bricks and provides environmentally friendly WWTP sludge disposal methods (Aeslina, 2014).

Toxic waste from the textile industry is solid waste in the form of sludge originating from the processing of liquid waste finishing and dyeing processes containing heavy metals (GR.No.101 / 2014).This study aims to find a solution of the problem of the textile industry's WWTP sludge.

2. METHODS

2.1 Total metal test on WWTP Sludge and Soil as raw material

Identification of potential metals contained in the WWTP sludge and soil raw material was carried out by total metal analysis with the reflux destruction method, according to the Standar Method for Examination Water and Wastewater APHA, 3111.B .(APHA,2017)

WWTP sludge used was taken from one of the textile industries with dyeing and finishing production processes. The process of treating the WWTP sludge has been carried out in factory with centrifuges dewatering, and continued by drying machine at temperatur 300°C.

The samples is was homogeneous, weighed and determined its water content. Samples were added with 10 mL HNO_3 1:1 refluxed at 95°C for 15 minutes. Cooling, add 10 mL H_2O_2 30%, reflux, heat at 95°C for 2 hours. Cool and add mienral mineral free water to exactly 100 mL. Filtered with Whatman 41, continued metal analysis with AAS. Calculate the metal content in the sampel.

2.2. TCLP test on WWTP Sludge and Soil

The extraction procedure in TCLP testing, refers to US.EPA, SW.846 Test Methode 1311, to determine the mobility of organic and inorganic analytes present in liquid, solid and multiphase wastes. Metal analysis using AAS, refers to the Standard Method for Examination of Water and Wastewater, APHA 3111.B

TCLP test was carried out by chrushing the material and shifting 9.5 mm in size. 5 gram of sample weigh dissolved in 96.5 mL of mineral free water. Take a pH measurement. If the pH is less then 5, use a sodium

acetate buffer with pH of 4.93, but if the pH of solution more than 5, use acetic acid with a pH of 2.88.The measured pH in the solution was 4.9 so a sodium acetat buffer solution was used.

Furthermore, exctraction was carried out using a rotary agitator with of 30 rpm for 18 hours. Next, the extracted soolution is filtered with a 0.6 - 0.8 µm size glass fiber filter. Removed the solids.

The solution obtained from this filtering, acidic with nitric acid, HNO_3, until the pH reaches 2. And then continued determination of metal by AAS.

2.3. Testing of Toxicity Characteristics of brick product

Test the characteristics of toxicity in brick products, carried out in 2 stages. The first step is TCLP testing using the procedure as in point 2 above. Then proceed with an acute oral toxicity test LD-50, using mice from Swiss strains aged 6-8 weeks, with an average weight of 27.6 grams. Acclimatization is carried out for 1 week, followed by testing for 14 days.

Referring to the Regulation of the Head of the Republic of Indonesia Drug and Food Supervisor Agency No.7 year 2014, the testing procedures is shown in the following **figure 1.**

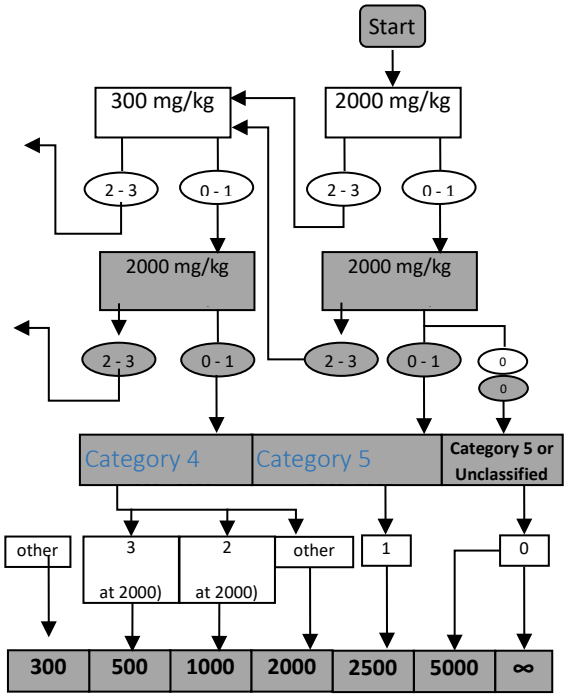

Figure 1. LD-50 Test Procedure

2.4. Brick Quality Testing

The bricks-making phase starts from mixing, curing, molding, drying and burning at the local brick-making site, following the usual manufacturing method.

Testing on bricks is done by physical obsevation and laboratory testing. Physical observations include size (dimension), cracks (pores), and color after burning. Dry srinkage is done after the bricks are dried for 5 days and dried in the sun for 3 days.

Testing the quality of bricks in the laboratory, refers to the Indonesian National Standard for solid bricks for wall pairs, SNI -15-2094-2000, which includes testing of compressive strength and water absorption.

3. RESULT AND DISCUSSION

The resarch process begins with the sampling of wastewater treatment sludge that has been dried in the textile industry, and clay sampling at the brick making location.

The first step is to conduct a total metal test to determine the potential of the metal contained in the raw material. Metal test result on material are shown in Table.1.

Table 1: Total Metal Test Results on Raw Material

No	Parameter	Result (mg/Kg)	
		Soil	Sludge
1	Barium, Ba	0.079	< 0.003(*)
2	Boron, B	80.01	38.04
3	Cadmium, Cd	< 0.005(*)	0.600
4	Chromium, Cr	7.598	2.120
5	Copper, Cu	9.597	63.75
6	Lead, Pb	< 0.030(*)	5.814
7	Mercury, Hg	0.070	0.088
8	Selenium, Se	0.034	0.127
9	Silver, Ag	<0.030(*)	1.900
10	Zinc, Zn	26.69	83.52

(*) Method detection limit

Test result in Table.1. shows that the metal contained in sludge and soil material, have the same tendency, in the sense that some metal such as Boron, Zink and Copper are quite dominant.

2.1. Metal Leaching Test Result on Raw Material

Based on TCLP Test Result in Table.2, it can be seen that some metals such as Boron, Chromium and Copper, show the potential for leaching, but when compared with the requirements of Quality Standards, it still meets the maximum allowable content

This is supported by the fact that the result of TCLP analysis conducted in the dyeing-finishing textile industry which were tested in laboratories since 2011 year until 2018 year, are presented ini Table. 3

Base on the test result, it can be seen that the sludge processed by the textile industry is still safe to be used as a mixture of bricks.

Table 2: TCLP Test Results for Bricks Raw Material

Parameter	Unit	Test Result			Quality Hazardous Waste Standard		
		Soil	WWTP Sludge	Mixture	TCLP-A	TCLP-B	before Landfill
Arsenic, As	mg/L	< 0.003(*)	< 0.003(*)	< 0.003(*)	3	0.5	0.5
Silver, Ag	mg/L	< 0.030(*)	< 0.030(*)	< 0.030(*)	40	5	5
Boron, B	mg/L	1.459	1.950	1.766	150	25	25
Cadmium, Cd	mg/L	< 0.004(*)	< 0.004(*)	< 0.004(*)	0.9	0.15	0.15
Chromium, Cr	mg/L	< 0.010(*)	0.093	0.014	15	2.5	2.5
Copper, Cu	mg/L	0.011	0.033	0.008	60	10	10
Mercury, Hg	mg/L	< 0.001(*)	< 0.001(*)	< 0.001(*)	0.3	0.05	0.05
Lead, Pb	mg/L	< 0.030(*)	< 0.030(*)	< 0.030(*)	3	0.5	0.5
Selenium, Se	mg/L	< 0.002(*)	< 0.002(*)	< 0.002(*)	3	0.5	0.5
Zinc, Zn	mg/L	0.144	0.337	0.035	300	50	50

(*) Method Detection Limit

Table 3: Metal Leaching Characteristics in WWTP Sludge of Dyeing-Finishing Textile Industry

PARAMETER	UNIT	Max Standar Quality	TCLP TEST RESULT							
		before Landfill	2011	2012	2013	2014	2015	2016	2017	2018
Arsenic, As	mg/L	0.5	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003
Silver, Ag	mg/L	5	< 0.030	< 0.030	< 0.030	< 0.030	< 0.030	< 0.030	< 0.030	< 0.030
Barium, Ba	mg/L	35	0.483	0.071	0.035	< 0.030	0.475	0.234	< 0.030	< 0.030
Nickel, Ni	mg/L	3.5	0.217	0.193	0.184	0.090	0.171	0.180	0.184	0.095
Cadmium,Cd	mg/L	0.15	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Chrom,Cr(VI)	mg/L	2.5	0.005	< 0.001	0.003	0.004	0.005	< 0.001	0.003	0.004
Copper, Cu	mg/L	10.0	0.007	< 0.005	< 0.005	0.005	0.005	< 0.005	< 0.005	< 0.005
Mercury, Hg	mg/L	0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Lead, Pb	mg/L	0.5	0.330	0.243	0.119	0.135	0.330	0.103	< 0.030	0.179
Zinc, Zn	mg/L	50	0.151	0.047	0.035	< 0.010	0.151	< 0.010	< 0.010	0.033

3.2. Toxicity Characteristics Test of brick product

Good combustion results in stable product. The existing metal content will burn to form oxides which are difficult to degrade naturally. Thus the properties as a Toxic and Hazardous material will decrease or disappear altogether. Another way to ensure that the brick products from textile waste are no longer toxic and hazardous, can be tested for acute toxicity.

Table 4 : TCLP Test Result

Parameter	Unit	Test Result	Quality Hazardous Waste Standard		
			TCLP-A	TCLP-B	before Landfill
Arsenic, As	mg/L	< 0.003	3	0.5	0.5
Silver, Ag	mg/L	< 0.030	40	5	5
Boron, B	mg/L	0.745	150	25	25
Cadmium, Cd	mg/L	< 0.004	0.9	0.15	0.15
Chromium, Cr	mg/L	< 0.010	15	2.5	2.5
Copper, Cu	mg/L	< 0.001	60	10	10
Mercury, Hg	mg/L	< 0.001	0.3	0.05	0.05
Lead, Pb	mg/L	< 0.030	3	0.5	0.5
Selenium, Se	mg/L	< 0.002	3	0.5	0.5
Zinc, Zn	mg/L	< 0.010	300	50	50

Table 5 : LD-50 Test Result

Sample Type	: Brick leachet extract
Test Animals	:
Type	: Swiss strain mice
Age	: 6 - 8 weeks
Sex	: Male & Female
Weight Average	: 27.65 grams
Examination	:
Acclimatization	: 1 weeks
Exam. Time	: 14 days
Dose Amount	: oral, 4 treatment & 1 control
Temperatur	: 23±3 °C
Humidity	: 75±10 %

LD-50 toxicity test result calculated based on the OECD Guideline 420 method are **> 5000 mg/kg body weigt**

According to table 4 Test Guideline Chapter IV Regulation of Head of the Republic Indonesia Drug and Food Supervisor Agency No.7 year 2014, concerning Guidelines for Non-Clinical Toxicity Test in Vivo, stated that oral LD50 in mice 5 - 15 gram (1000 - 15000 mg)/ body weight, classified as practically non toxic - relatively harmless.

3.3. Brick Quality Testing

The next step is to test the mixture as a raw material for bricks, to ensure technical feasibility as a raw material for bricks. Preliminary tests include plasticity test and dry shrinkage test on mixed materials and bricks before burning. The test result are presented in tables 6 and tables 7.

Table 6: Plasticity Test in Raw Material

Parameters	Units	Composition		
		V1	V2	V3
Liquid limit	%	49.00	53.00	52.00
Plastic Limit	%	25.26	31.42	35.65
Plasticity Index	%	23.74	21.58	16.35

Note :

V1 : Composition with 0 % sludge

V2 : Composition with 40 % sludge

V3 : Composition with 60 % sludge

Plastic is the character of a substance, allows the substance to change shape when externally acting on a force. The characteristic of plasticity in this case is very important to get an idea of the extent to which the material can be formed. This plasticity arises when clay is added with water while being resilient.

For bricks the good plasticity of the material ranges from 20-30 %. (R. Budi, 1995). Material with a plastic limit greater than 30 % will produce many cracks during the drying process.

Table 7: Properties Test of Brick Material before Burning

Parameters	Units	Composition		
		V1	V2	V3
Dry Shrinkage	%	7.55	6.76	6.46
Max. Forming Water	%	36.44	35.29	38.49
Min. Forming Water	%	28.56	26.72	33.70
Flexural Strength	kg/cm^2	46.27	43.30	33.36

Note :
V1 : Composition with 0 % sludge
V2 : Composition with 40 % sludge
V3 : Composition with 60 % sludge

The flexural strength is directly proportional to the plasticity index. The more mixed the waste, the smaller the flexural strength value and the smaller the plasticity index value. Based on empirical experiments that have been done by previous researchers, it shows that to get bricks that are goo's enough, the flexural strength must be above 28 kg /cm2 (R.Budi, 1995).

According to the flexural strength, the formulas V2, V3 qualify as raw material for bricks. The flexural strength value of raw materials reflects the compressive strength of brick products after burning(Author note: V1 was raw material, that no sludge to added)

Table 8: Physical Observations

Code	Parameters				
	Size (cm)	Form	Pores	Cracked	Color
V1	23.1x10.8x4.5	Elbow	-	-	reddish brown
V1	22.8x10.7x4.4	Elbow	+	+	reddish brown
V2	23.1x10.8x4.5	Elbow	-	+	reddish brown
V2	22.7x10.9x4.6	Elbow	-	-	reddish brown
V3	22.7x10.9x4.6	Elbow	+	-	reddish brown
V3	22.9x11.1x4.9	Elbow	+	++	reddish brown

Note :
V1 : Control bricks without sludge mixture
V2 : Bricks with a composition of 40% sludge
V3 : Bricks with 60% sludge
++ : enough / moderate
+ : little
- : none

The physical observations of size, pores, cracks, and color, do not show a certain tendency to mix variations. Seen in control bricks that do not use a mixture of mud, pores and cracks are also found.

Possible causes are the nature of the material, the way it is cured or the drying process, so that it can be seen that the addition of 40% and 60% sludge as a mixture, has no real effect on physical observation. The raw material for bricks taken from dry land gives a different physical appearance to bricks that use raw materials from paddy soil. Dry soil has a harder texture and has larger grains, so it requires sufficient ductility to get a softer and more uniform mixture. In addition, withering must be done the brick are made, so that the mixture obtained is more compact.

Figure 2: Physical images of Bricks

Table 9. Test Results of Bricks Products in Accordance with SNI

No	Code	Testing Parameters			
		Size (cm)	Form	Absorp-tion (%)	Compressive Strength (kg/cm^2)
1	V2	23,13x10,93	elbow	25,25	203,294
2	V2	22,96x10,83	elbow	19,89	212,133
3	V2	22,90x10,80	elbow	23,44	159,100
Average		**22,99 x 10,85**	elbow	**22,86**	**191,509**
Class Standard 100		**23±0,5 x 11±0,2**	**elbow**	**20**	**150 ± 22,5**
4	V3	23,20x11,1	elbow	26,69	119,325
5	V3	22,53x10,83	elbow	28,51	163,519
6	V3	22,70x10,63	elbow	29,07	150,261
Average		**22,81 x 10,85**	**elbow**	**28,09**	**144,368**

Observation data and test results of brick laboratories using WWTP sludge as a mixture of making

bricks raw material, show that the compressive strength and dimensions of brick products meet the quality requirements of National Indonesian Standard (SNI) Solid Brick for wall pairs with class 100 quality criteria.

4. CONCLUSION

Testing through TCLP (Toxicity Characteristic Leaching Procedure) test shows that the sludge used as a mixture of bricks meets the TCLP quality standard according to PP.110 of 2014. The ideal composition of industrial wastewater sludge is 40% and 60%. Testing of metal leaching with TCLP on brick products 40% and 60% of WWTP sludge, meets the TCLP Quality Standards according to PP 110 of 2014. LD-50 acute toxicity test shows that bricks with a mixture of 60%, obtained by the yield dose > 5000 mg / kg body weight, so it meets the allowable toxicity limit. Quality Test Results of Bricks using a mixture of WWTP sludge as much as 40% and 60% meet the requirements of National Indonesian Standard (SNI 15-2094-2000) solid red brick for wall pairs.

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Wet Scrubber Performance Optimization Application Assisted with Electrochemical-Based Ammonia Sensors

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ABSTRACT

Crumb rubber is one of Indonesia's agroindustry export commodities. This industry faces environmental problems due to their wastes, both liquid and air. The source of air pollution is commonly from drying process that emitted odor from its evaporation and heating phenomena. Industry uses wet scrubber technology as air pollution control from emitted odor from drying process. Preliminary identification in noncontrolled wet scrubber shown that wet scrubber efficiency around 47%. Low efficiency wet scrubbing process causes rain drop of water vapor around drying process. This research used electrochemical based sensor MICS 5524 as ammonia monitoring instrument, assisted with arduino as microcontroller to regulate water discharge through valve controlling scrubbing process. This electrochemical based sensor reads ammonia based on voltage reads by Arduino microcontroller. Ammonia reading then control scrubbing process by adjusting valve opening for spray water distribution. Wet scrubber efficiency increases to 66,96% due to water scrubbing control, also can save water utilization as high as 61,90%, followed by absence of rain drop contains ammonia around drying process area.

1. INTRODUCTION

Rubber as an agroindustry export commodity has great contribution for Indonesian trade income. Natural rubber also take important role for industry, especially manufacturing industry(Barlow, 1983). Mostly,natural rubber exports from Indonesia are semi-finished materials in crumb rubber with Standard Indonesian Rubber (SIR) quality (Anon, n.d.). SIR is the technical specification of rubber which is judged on the level of impurities, ash content and volatile substances. Energy requirement in line with amount of product, high quality SIR need the more energy (Utomo et al., 2010), and generally use the greater water in production process(Maulina et al., 2015).

Crumb rubber factory in general is a process of natural rubber from brown crepe or lump to crumb rubber with certain specifications in the form of *bandella* which will be a raw material for mixing rubber products such as tires. Crumb rubber processing in physicall process could be describe as treatment of raw material, starting from washing, chopping, grinding, drying, crumbing and drying (Setyamidjaja, 1993).

Crumb rubber processing provides potential pollution of the water and air for environment. They used water in washing process until brown crepe grinding, this process generates wastewater of washing from the impurities

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that stick to the raw material of rubber. Due to its high needs of water, this process generate high quantity of wastewater that must pass through a Waste Water Treatment Plant before being Include the numbers, literature or data from the rubber processing industry flowed into the river. The main pollutant parameters in crumb rubber waste water are BOD (Biological Oxigen Demand), COD (Chemical Oxigen Demand), and TSS (Total Suspended Solid). The highest levels of BOD and COD were 313 mg/l and 928.1 mg/l from an acid bath, TSS came from shreeder at 190 mg/l, while ammonia at 38.45 mg/l from the effluent scrubber (Andriani et al., 2019).

The main air pollutant produced by the crumb rubber industry is odor from the drying process. Ammonia (NH_3) sourced from the decomposition of proteins contained in raw materials from fungal/bacterial activity (Atagana* et al., 1999). Control of odor in crumb rubber industry has been carried out by a wet scrubber. Wet scrubber is an effective device to control gas and particle contamination from exhaust gas (US EPA, n.d.)(Byeon et al., 2012). The type of wet scrubber that is commonly used in industry is spray tower(Bhargava, 2017).

Wet scrubber as a controller of air pollution in the rubber crumb industry is some times still far from optimal in operation. Ammonia gas released by the wet scrubber does not meet quality standards of KepmenLH No. 50 of 1996 (> 2 ppm) (Andriani et al., 2019). The effect of this ammonia is the emergence of a pungent odor and the tendency of the wet scrubber's inefficiency.

The development of environmental technology, monitoring of emissions can be done using sensors(Dong Dong et al., n.d.)(Jeong et al., 2012). Low cost sensor types are widely used for monitoring ambient air(Popoola et al., 2018)(Munir et al., 2019), because is lower and simpler sensor. Now spreading rapidlly for non regulatory application.

This research as approach in applied research as a problem solving in industry, aiming to apply the ammonia sensor to assist the wet scrubber in the crumb rubber industry. Electrochemical-based ammonia sensor assisted not only for monitoring tool but also developed as a control instrument integrated with arduino based microcontroller. This control device is used to adjust the amount of water spray on the wet scrubber, and is expected to improve the performance of the wet scrubber by increasing the binding efficiency of ammonia.

2. METHODS

Wet scrubber used in experiment (Golsta model GS-AS2) has specification as follows : capacity 3 T/H, body material stainless steel, 2 scrubbing stage, chimney height 600 cm and 76 cm diameter with tellerettes packing equipped with water circulating pump 10 HP as shown in Figure 1.

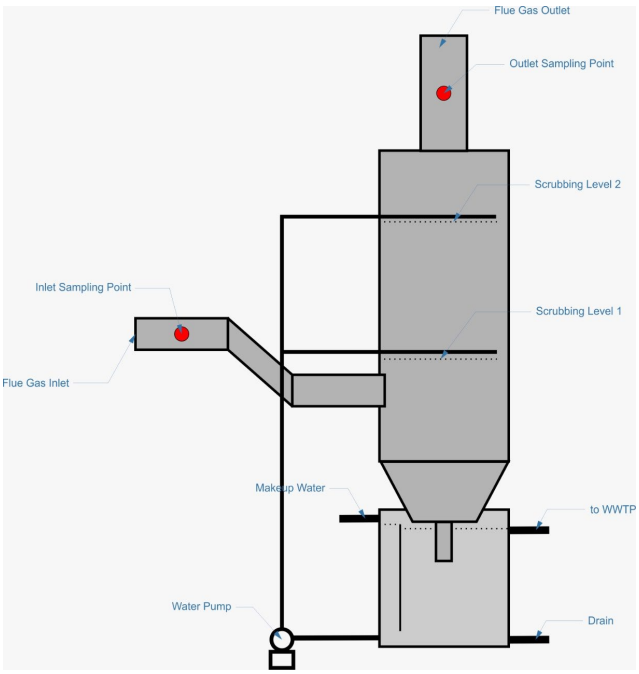


Figure 1. Wet scrubber existing

Micro-electro-mechanical System (MEMS) sensor for amonia (MICS 5524, China). Microcontroller (Mega 2560, Arduino UNO, China) and raspbery pi, motorized ball valve (CWX 15Q, China)

Procedure

This research was conducted in two stages, the first was observation and the second was application of a sensor-based control system

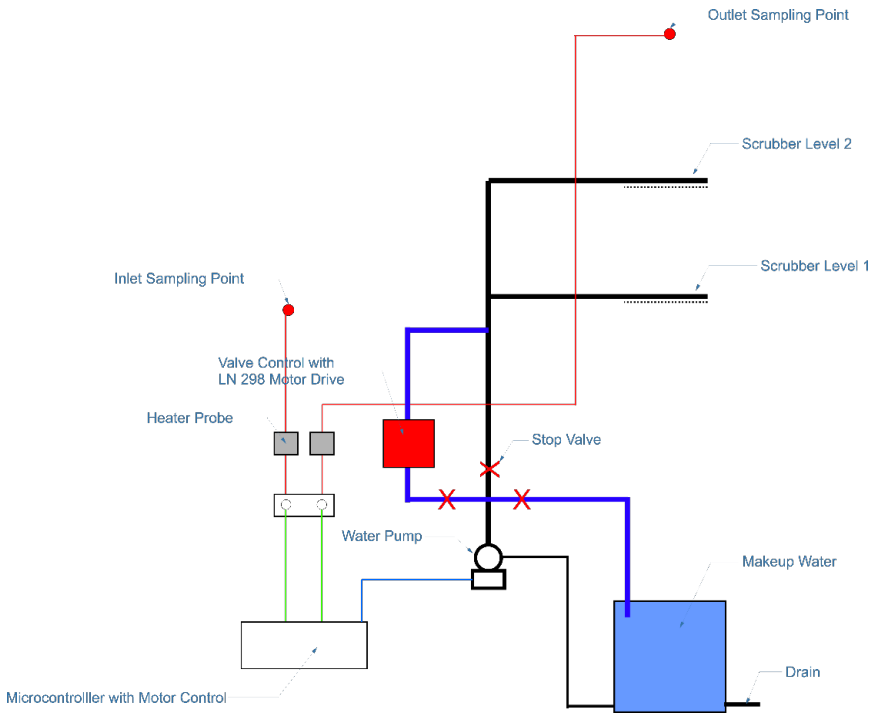


Figure 2. Experimental Setup

Observation of existing wet scrubber was carried out by visual observation and exhaust gas quality measurement during wet scrubber operation. Ammonia concentration of exhaust gas from dryer is measured as well as exhaust gas passed through the scrubber. This is done to determine the concentration of ammonia in and out of the scrubber as well as to determine the initial performance of the scrubber

Application step consists of designing a control system, installation in a wet scrubber, and evaluation. The design of the control system is carried out in the Center of Industrial Pollution Prevention Technology's laboratory. Sensor-based control system, consisting of a sensor, microcontroller, raspberry as LCD screen display, motorized ball valve.

The control system is installed on the existing wet scrubber by making some adjustments. Installation of control devices as shown in Figure 2.

After installed the control system, measurements were made of the concentration of ammonia released by the scrubber and the use of water used to absorb ammonia.

Ammonia outlet concentration data is used as a reference to drive the motorized solenoid valve. Controlled discharge is measured using valve open and close conditions

Quality of ammonia inlet and outlet scrubber were sampled by using air monitoring sampling standard method SNI 19-7119-1-2005 (Anon, n.d.) .

The efficiency calculation is done by analyzing the readings of the scrubber inlet and outlet using an ammonia sensor and data on water use savings are calculated from the control valve. And also we can calculate the using of spray water.

3. RESULT AND DISCUSSION

Visual observations of an existing wet scrubber in the industry showed that there was visible vogs and water droplets came out of chimney lead to local rain drop occurs around the plant. Spray water is manually pumped from the reservoir with a discharge of 0.675 liters/second.

Measurement results on ammonia inlet and outlet scrubber were shown in Figure 3.

Figure 3. Ammonia Emission Measurement Data Before Control

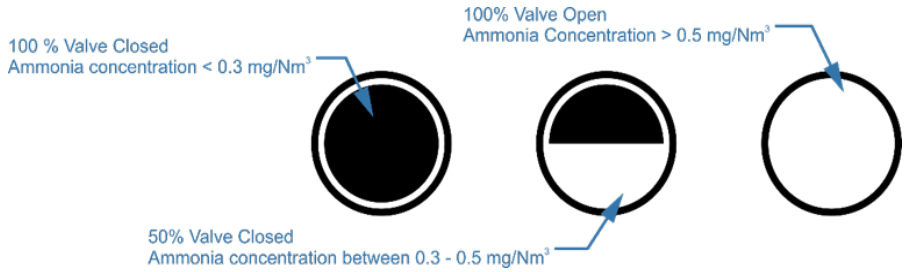
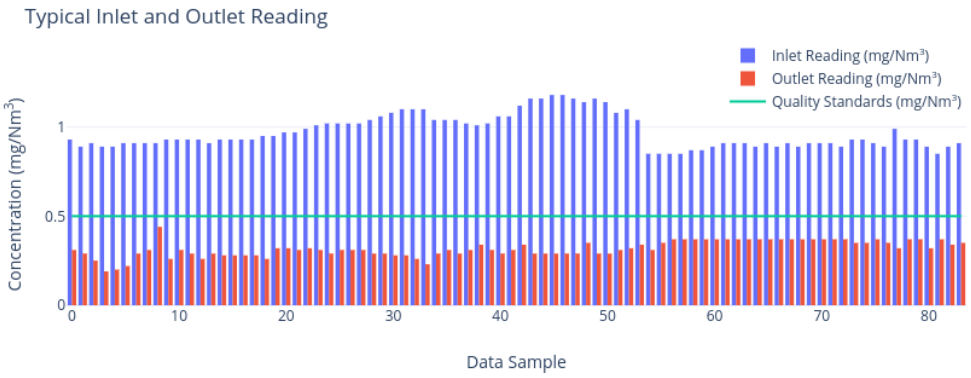


Figure 4. Trial Valve of Unit Control



(a)

(b)

Figure 5. Typical Inlet and Outlet Reading After Control (a), Use of Water After Control(b)

Average concentration of ammonia inlet were 1.175 ppm, while concentration outlet was 0.627 ppm. Fig. 3 showed that more than 50% measurements of ammonia emissions were excedded quality standard. An average efficiency of wet scrubber was around 47%. The existing wet scrubber average efficiency data shows the low efficiency of the wet scrubber, compared to the theoretical specification of the wetscrubber by 99%(Chien et al., 2015), besides that in the existing wet scrubber, the ammonia tendency to be discharged through the chimney is still above the quality standard.

Before designing the control system, ammonia sensors were verified using ammonia gas containers from liquid ammonia vapor, the verification results showed a linearity correlation> 0.9. The ammonia sensor verification results show the possibility of using electrochemical sensors as a monitoring and control tool (Fatkhurrahman and Sari, 2019).

The design of the control system uses the following mechanism: the sensor reads the concentration of ammonia gas in the scrubber. The sensor is connected to the micro controller which is drive the motorized solenoid valve to regulate the amount of spray water.

There were three setting control on opening or close valve, if the ammonia cons> 0.3 mg / nM^3, the valve will open 100%, if the cons was between 0.3 - 0.1 mg / Nm^3 the valve will open 50%, if the cons <0.1 mg / Nm^3 the valve will closed. Ammonia outlet concentration data is used as a reference to drive the motorized solenoid valve.

The design of the wet scrubber monitoring and control unit is based on an electrochemical sensor as shown in the figure 4.

Control system that has been made, installed in wet scrubber as Fig. 2. Trials of automatic valve control system was carried out to see the extent of improved NH_3 absorption efficiency and water use efficiency. Trials were carried out 3 hours, from 7 hours the regular production process. Controlled discharge is measured using valve open and close conditions, with typical data as shown in the figure below.

Using data from Figure 3, we calculate initial efficiency of this scrubber by comparing average outlet to average inlet concentration of ammonia, that is 47% efficiency. Application of water spray controlling using Arduino based microcontroller indicate different result specifically on outlet concentration of ammonia, as we see on Figure 5(a), no more ammonia reach national standard ammonia for emission. The average sorption efficiency of ammonia increased by 20% in the range of 66,96% by experimental data result as the rate of absorption is influenced by the contact area between the absorbing fluid and the gas (Maile et al., 2015), when water uncontrolled there is less time and area contact between ammonia as gas and water as absorbing liquid. We also can reduce the use of spray water until 62% during the period of experiment. This is in accordance with previous similar studies on the use of waterspray for particulate pollutants control which can save water use by an average of 59.8% (Fatkhurrahman et al., 2017).

4. CONCLUSION

Utilization of electrochemical-based ammonia sensors as ammonia emission monitor could be developed as assisted control device integrated with Arduino based microcontroller to control the use of water spray on wet scrubbers. This optimization process can increase the binding efficiency of ammonia by 20% from 47% to 66.96% and can save the use of spray water by 61.90%. Saving in water spray directly impact on reducing the burden of wastewater treatment.

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