Biofiltrasi Gas Amonia Menggunakan *Nitrosomonas Sp.* dan *Nitrobacter Sp.* untuk Industri Karet

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**ABSTRAK**


Ammonia Gas Biofiltration by Nitrosomas Sp. and Nitrobacter Sp. for Rubber Industry

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ABSTRACT

The objective of this study was to investigate the effectiveness of bacteria consortium of Nitrosomonas sp. and Nitrobacter sp. for ammonia removal in air. The laboratory scale biofilter, inoculated with Nitrosomonas sp. and Nitrobacter sp. Bacteria, was used for degradation of ammonia in air. Activated carbon and cocopeat were used as the filter media. This study analyzed the effect of bacteria colony number on ammonia reduction. The ammonia concentration of gas outlet biofilter columns were analyzed by indophenol methods based on SNI 19-7119.1-2005. The result indicated that there is a positive correlation of bacteria colony number and ammonia reduction. The 20 mL of bacteria inoculum contains $1.78 \times 10^8$ colony/mL is effective for reducing ammonia from 3.66 ppm to 0.26 ppm in 0.087 m$^3$ air.

Keywords: Ammonia, cocopeat, Nitrosomonas sp., Nitrobacter sp.
INTRODUCTION

Ammonia (NH₃) odor arises from the production process and waste water in rubber industry, it may cause eye irritation and respiratory tract and in high concentration can cause death (Gandu et al., 2015). Ammonia has high deposition velocity which makes it easy to spread (Nanda et al., 2012). It spreads in short distance through dispersion and dilution (Joshi et al., 2000). In Indonesia, there is a regulation from the ministry of environment related to odor intensity which mention that ammonia concentration in air should be less than 2 ppm (KLH 1996). The correlation between odor intensity and ammonia concentration is linear (Jinanan & Leungprasert, 2015).

The natural rubber intermediate products produced by Indonesia are rubber sheets, block rubber, crepe rubber, crumb rubber, and concentrated rubber latex. The rubber sheet is classified as ribbed smoked sheets (RSS) and air dried sheets (ADS). The air pollution in rubber industry is from various gases, vapors, fumes, and aerosols due to the leaching out of chemicals and high temperature vulcanization (Jagadale et al., 2015). The air pollution of rubber sheet production is caused by smoke of fuel wood burning which contains hazardous components, while the air pollution of rubber latex industry is caused by waste water and ammonia odor from latex preservation (Tekasakul & Tekasakul, 2006). The latex is usually treated with 0.2% or 0.7% ammonia solution during the production which causes a strong ammonia odor (Tekasakul & Tekasakul, 2006). The ammonia concentration in rubber storage warehouse can be so high to 11 ppm (Yani et al., 2012).

The common methods for ammonia removal are based on physical and chemical processes, however there is much attention on biofiltration which includes biological process recent years (Gandu et al., 2015; Gopal et al., 2014). The main equipment in biofiltration is biofilter, it is a column filled with the porous and humid packing materials and microorganisms that is able to convert targeted pollutants into less hazard or less toxic materials (Chetpattananondh et al., 2005).

Gas phase biofilter can be applied for high and low air flow rates with pollutant concentration less than 1000 ppm (Zhu et al., 2016). However, there is a previous study which mention that there is a reduction on ammonia removal when the gas contains high ammonia concentration (more than 2000 mg NH₃/m³) (Pagans et al., 2005). It is in accordance with previous study that mention biofiltration good for low concentration gas pollutant (Kumarettal., 2011).

There are two simultaneous processes in biofiltration, the chemicals process (absorption/adsorption) and biological process (biological oxidation/biodegradation) in pollutant removal scheme (Chen & Hoff, 2009). The pollutants diffuse from the gas into the thin layer.
biofilm of microorganisms (Kavyashree, et al., 2015). The microorganisms use the targeted pollutants as a carbon source, or an energy source, or both. The microorganisms convert the targeted pollutants to odorless, less toxic, and less hazard compounds such as CO₂, water vapor, and organic biomass (Chan, 2006). Each microorganism has certain characteristics on pollutant conversion.

The advantages of biofiltration are the system needs a relatively low capital and operating cost, it requires small amounts of energy during operations, and it produces a relatively low toxicity waste stream (Kumaret et al., 2013; Nanda et al., 2012). This biological treatment can be conducted at moderate temperature (10°-40°C) and atmospheric pressure (Barbunsinski et al., 2017). The major limitations of biofiltration are the necessities of large space and frequent media replacement due to the deterioration of media (Chan, 2006). Biofilter can be used for two to seven years (Yani et al., 2013), it depends on the materials and pollutants.

A biofilter system consists of packed material, filter media, and microorganisms. The media and microorganisms should be selected according to the targeted contaminant to be removed

**Packed material**

There are organic and inorganic biofilter packed material. The example of inorganic packed material commonly used is polyvinyl sulfonate. It has lower absorptivity than organic material (Soccol, et al., 2003). The organic material is not durable because of the reaction of the organic content as a supplement of alternative food source for the microbes (Soreanu et al., 2013).

**Microorganism**

The choice of species and preparation methods of a proper inoculum microorganisms is important in biofiltration (Chan, 2006). The targeted pollutants must be able to be converted to different compounds by selected microorganisms and non-toxic for the microorganisms. The high soluble organic compounds are easier to be converted than inorganic compounds (Chan, 2006). Ammonia is an easy converted organic compound.

There are several choice of microorganisms used in biofiltration includes bacteria, fungus, and actinomycetes. Bacteria has ability to convert air contaminant faster than fungus and actinomycetes (Estrada et al., 2013). Bacteria activity is affected by moisture content, pH, nutrient, and temperature (Pagans et al., 2005). Each species requires certain working condition.
This research was applied nitrification and denitrification process to convert ammonium pollutant. There are two steps of nitrification of ammonium into nitrate, this step should be under aerobic condition. The nitrification is faster in mixed culture than in pure culture bacteria (Kumar, et al., 2013). The first nitrification step is oxidation of ammonia to nitrite by ammonia oxidizing bacteria,

$$2\text{NH}_4^+ + 3\text{O}_2 \rightarrow 2\text{NO}_2^- + 2\text{H}_2\text{O} + 4\text{H}^+$$

The second step is oxidation of nitrite to nitrate by nitrite oxidation bacteria,

$$2\text{NO}_2^- + \text{O}_2 \rightarrow 2\text{NO}_3^-$$  (Bardskar, Monitoring of denitrifying activities in moving bed biofilm reactors with an ex-situ manometric batch test (Master Thesis) 2016).

Nitrification involves bacteria species from the *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, and *Nitrosolobus* genus (Pramanik et al., 2012). This process occurs in the biofilm of microorganisms (Leson and Winer, Biofiltration: an innovative air pollution control technology for VOC emissions 2012). The additional organic and inorganic compound can be used as carbon or energy source, it has good effect on nitrification process (Rodriguez-Sanchez, et al., 2014). After nitrification, there is denitrification process.

$$\text{NO}_3^- + \text{C}_x\text{H}_y\text{O}_z \rightarrow \text{N}_2 + \text{CO}_2 + 7\text{H}_2\text{O} + \text{OH}^-$$ (Bardskar, Monitoring of denitrifying activities in moving bed biofilm reactors with an ex-situ manometric batch test (Master Thesis) 2016).

The denitrification process can be in aerobic or anaerobic condition (Bardskar, Monitoring of denitrifying activities in moving bed biofilm reactors with an ex-situ manometric batch test (Master Thesis) 2016). The denitrification process involves *Nitrobacter* bacteria. The stoichiometric relationship depends on the carbon sources (C\text{X}H\text{Y}O\text{Z}). The end products of this process are CO\text{2}, water, and microbial biomass (Leson and Winer, Biofiltration: an innovative air pollution control technology for VOC emissions 2012).

The previous studies about ammonia gas biofiltration mentioned that the ammonia removal by nitrification could be achieved by adding the peat or media with nitrifying bacteria (Togashi et al., 1986). *Nitrosomonas europeais* effective for removing ammonia up to 100% with the maximum degradation rate 2.35 g N/day (Chungetal., 2007), the mix culture of *Pseudofulvimonas* and *Nitrosomonas* effective for removing ammonia up to 100% (Kimetal., 2015), and *Thiobacillus sp.* removes ammonia with critical rate 25 g/m\text{3}h (Kim, et al., 2003).

This research used Nitro-bac as nitrifying microorganism. Nitro-bac is a liquid blend of nitrifying bacteria of the strains *Nitrosomonas sp.* and *Nitrobacter sp.* contains 2x10\text{14} CFU/kg.
bacteria. The nitro-bac is specifically developed for aerobic wastewater treatment. In this research, this consortium bacteria was applied in the gas phase biofiltration with the main target for ammonia removal. The optimum growth and living condition for this bacteria is in pH 6.5 – 8.5 and temperature 8°C – 44°C. The main concern of this research is to investigate the optimum composition of media and bacteria for ammonia removal in gas phase biofiltration.

**Filter material and media**

The choice of filter material is based on price, lifetime, and structure (Pedersen, 2012). One criteria of structure is pore uniformity, it has effect on biofilter flow and efficiency (Soccol, et al., 2003). The filter medium should be good for microbial growth and easy for gas to flow through it (Utami et al., 2012). The material should be large in surface area, high in porosity, high in water retention capacity without becoming saturated and low in bulk density. The media should has a buffer capacity towards acidification and high contaminant volume to maintain pH in optimum working condition (Chan, 2006).

The media can be a natural organic or an inert synthetic media. The organic media, commonly used in biofiltration, are compost, peat, leaves, soil and wood chips (Sorial et al., 2001). Compost is commonly used because of its cheap price and it contains microbial communities for degrading various pollutants (Pagans et al., 2007). The inert materials in biofiltration are glass beads, perlite, and porous ceramics. The main different characteristic is the inert material is difficult to compact, while the organic is easy to compact (Tsang, Wang, et al., biodegradation of ammonia in biofiltration systems: changes of metabolic products and microbial communities 2017). The medium has effect on biofiltration performance because it provides the optimal environment condition for microbial growth or living.

There are advantage and the disadvantage of each support media. This research used the composite of cocopeat and activated carbon as media. The advantages of peat are cheap price, suitable for low contaminant concentration, and suitable for commercial technology. The disadvantages are: prone to channeling, limited ability to neutralize acid degradation, low degradation capacity, and limited supply of macronutrients. It is humid and frequent media replacement is needed. The advantages of activated carbon are high adsorption capacity, available for high contaminant, and high degradation capacity. The disadvantages are its higher cost compare to soil, peat, and compost and it is eventually plugging of bed requiring cleaning or media replacement (Yani et al., 2013).
Working condition

The pH, moisture, nutrients, and temperature have an effect on biofiltration. Each microorganism needs specific pH and temperature for the optimum growth or living. Most of the biofilter microorganisms performed well in the mesophilic temperature range (20 -45°C), with the optimum temperature range 35-37°C (Chan, 2006).

Moisture content is one important factor that determines biofilter performance. It has effect on ammonia mitigation and nitrous oxide generation (Yang, 2013). It influences the efficiency of the biofilter and pressure drop across the filter medium. The optimum operational moisture content in the biofilter is between 40% - 60% (Shahmansouri et al., 2005). Sheridan (2002) suggested that the filter bed moisture content should be greater than 63% to maintain overall efficiency, while other study mention that the optimum humidity is 60% -80% (Zagorski & Leksaitis, 2011).

MATERIALS AND METHODS

Preparation of biofilter materials

Biofilter column is a cylindrical polyvinyl chloride (PVC) pipe having a diameter 3 cm and a height 20cm. The columns are opened in the upper end which connected to ambient air environment within the chamber. The lower end of the columns are closed and equipped with the hose connected to the impinge air sampler for ammonia testing.

Biofilter media is the composite of 10 g powder activated carbon and 10 g cocopeat. Cocopeat in this experiment is cocopeat as growing media culture commonly sold in the market.

Preparation of nitrifying bacteria

Biofilter nitrifying bacteria in this experiment was the Nitrobac, it was the consortium of Nitrosomonas sp. and Nitrobacter sp. bacteria. 1 kg Nitrobac contains 2x10^{14} CFU bacteria. In this experiment, 0.5 gram nitrobac was added to 500 mL distilled water and then incubated in 35-37°C incubator for 24 hours.

Enrichment of nitrifying bacteria

The incubated nitrifying bacteria 0 -20 mL (Table 1) were added to 10 g cocopeat for 6 hours. After 6 hours, activated carbon were added to each sample and then homogenized. The composite media were transferred into biofilter column.
Biofilter experimental setup

The biofilter was connected to impinge air sampler. There were 5 sets impinge air samplers, each set equipped with an impinge for gas adsorption, an impinge for dehumidifier, air flow regulator, rotameter, and air suction pump. The regulatory knob was controlled for produced 1 L/minute air suction flow.

Gas Ammonia in this experiment was made from Ammonia glacial 85% produced by MERCK. Ammonia in chamber was generated from liquid ammonia solution.

<table>
<thead>
<tr>
<th>Column</th>
<th>Media</th>
<th>Volume bacteria (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>Cocopeat + activated carbon</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>Cocopeat + activated carbon</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>Cocopeat + activated carbon</td>
<td>15</td>
</tr>
<tr>
<td>E</td>
<td>Cocopeat + activated carbon</td>
<td>20</td>
</tr>
</tbody>
</table>

Experiment for ammonia biofiltration was carried out in a chamber. The experiments A-E were carried out simultaneously for 90 minutes. Experiment A represented the air contaminated ammonia without biofilter. After 90 minutes biofiltration process, the ammonia concentration in gas outlet was analyzed.

Determination of ammonia gas

Ammonia gas concentration was determined by Indophenol method based on SNI 19-7119.1-2005 (Badan Standardisasi Nasional, Standar Nasional Indonesia (SNI) 19-7119.1-2005. Udara ambien- Bagian 1: Cara Uji kadar amoniak (NH3) dengan metoda indofenol menggunakan spektrofotometer 2005). Standard curve for ammonia was determined using NH₄Cl. The chemicals for the analysis were sodium nitropruside (Na₂Fe(CN)₆NO.2H₂O 2%, sodium hydroxide 6.75M, sodium hypochlorite 3.7%, phenol (C₆H₅OH) 45% v/v, buffer (50 gr Na₃PO₄.12H₂O and 74 mL NaOH 6.75 M in 1000 mL distilled water), ammonia stock solution (3.18 gr NH₄Cl in 1000 mL with distilled water). The air sampler absorption solution was made by dilution of 3 mL sulfuric acid 97% in 1000 mL distilled water (Badan Standardisasi Nasional, Standar Nasional Indonesia (SNI) 19-7119.1-2005. Udara ambien- Bagian 1: Cara Uji kadar amoniak (NH3) dengan metoda indofenol menggunakan spektrofotometer 2005).
Figure 1. Scheme of the laboratory scale biofiltration system in the chamber

The sample was added with 2 mL buffer solution, 5 mL phenol solution, and 2.5 mL sodium hypochlorite solution. The sample were homogenized and allowed for 30 minutes color development. The color absorbance was measured using spectrophotometer at 630 nm.

Determination of bacteria colony number

Bacteria colony number was determined based on total plate count method. 1 mL of prepared bacteria was added to 9 mL Buffered Peptone Water (BPW) media. The mixture diluted to 10⁹ times. Each diluted sample was pipetted 1 mL and transferred into 20 mL to plate count agar (PCA) media, and then incubated for 2 x 24 hours in 35-37°C incubator. The number of bacteria colony was calculated in counting chamber.

RESULT AND DISCUSSION

This research investigates the effect of microbial colony number on ammonia removal. The target of the biofiltration is the outlet contains low ammonia, less than 2 ppm. The ammonia in the chamber is conditioned between 3 – 4 ppm which represents process and storage room of rubber industry. The air flow is set in 1 L/ minute based on the National Standard method of ambient air sampling (SNI 19-7119.1-2005). The 1 L/minute represents the outdoor wind velocity which
mitigates air in outdoor environment or ambient air. The flow total volume of air through each biofilter for 90 minutes is 87 L or 0.087 m³.

The experiment compared gas outlet from five biofilter columns. Column A is column without media which represents air without biofiltration process. Column B, C, D, and E are columns for representing biofiltration with different bacteria volume (0 mL, 10 mL, 15 mL, and 20 mL). The finding indicates that the volume of bacteria has effect on ammonia absorption (Table 2). The ammonia concentration in gas outlet decreases or the removed ammonia increases by the additional volume of bacteria. The result indicates that the consortium bacteria containing *Nitrosomonas sp.* and *Nitrobacter sp.* is effective for reducing ammonia in air.

In this biofilter system, the effectiveness of ammonia removal is affected by number or volume bacteria added, while activated carbon and cocopeat has no significant effect on ammonia removal. There is no significant difference between ammonia content in experiment A and experiment B. The function of cocopeat in this system is not as ammonia remover, but as carbon source and nutrient for bacteria and as water absorbent for maintaining moisture content. The cocopeat and activated carbon provide large surface area and maintain media porosity.

<table>
<thead>
<tr>
<th>Column</th>
<th>Ammonia in gas outlet (ppm)</th>
<th>Ammonia reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3.64</td>
<td>0.62</td>
</tr>
<tr>
<td>C</td>
<td>1.26</td>
<td>65.71</td>
</tr>
<tr>
<td>D</td>
<td>0.82</td>
<td>77.47</td>
</tr>
<tr>
<td>E</td>
<td>0.26</td>
<td>93.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column</th>
<th>Bacteria number, CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>1.78 x 10⁷</td>
</tr>
<tr>
<td>C</td>
<td>1.8 x 10⁸</td>
</tr>
<tr>
<td>D</td>
<td>2.8 x 10⁸</td>
</tr>
<tr>
<td>E</td>
<td>3.72 x 10⁸</td>
</tr>
</tbody>
</table>

The chamber temperature in this experiment was 35°C, it represents the natural temperature of warehouse and processing room in rubber industry. This temperature is suitable for bacterial growth and living. The optimum temperature of *Nitrosomonas* is 35°C and the optimum temperature of *Nitrobacter* is 35°C-42°C (Titiresmi and Sopiah 2006). The bacteria can be applied in the biofilter, especially in the warm tropical country without temperature conditioning. The
number of bacteria after biofiltration process was determined (Table 3). The number indicated that the bacteria were living during the biofiltration process.

The biofiltration process in this experiment is effective for reducing ammonia content to less than 2 ppm. The 20 ml inoculum bacteria contains $2 \times 10^8$ CFU/ mL bacteria reduces ammonia content from 3.66 ppm to 0.26 ppm in 0.087 m$^3$ air.

CONCLUSION

Gas phase biofiltration by *Nitrosomonas sp.* and *Nitrobacter sp.* bacteria with cocopeat as media and nutrient source effective for ammonia removal.

REFERENCES


