



*Research Paper*

**THE IMPACT OF CALORAGE CHANGES ON BIO-URINE QUALITY FROM AEROBIC AND ANAEROBIC FERMENTATION PROCESS IN A BIOREACTOR**

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**Abstract**

The content of cow urine has nutrients as much as N = 1.00%, P = 0.50%, K = 1.50%, water as much as 95% and this material has the potential to be fermented into bio-urine. The aim of this research is to analyze the dynamics of heat in the fermentation process of cow urine in a laboratory scale bioreactor. Experiment with a two-factor factorial design, the first factor was the fermentation system (aerobic and anaerobic), the second factor was the added nitrifying bacteria, namely 0%, 1%, 2%, 3% and 4% of the volume of fermented cow urine. The parameters observed on biomass were: biomass temperature, environmental temperature, air humidity, pH, EC and TDS, content of C and content of N. The temperature of the cow urine fermentation reaction in the bioreactor was 27.7 - 36.2°C. The total heat of the fermentation reaction of cow urine is 2.8 - 7.1kJoules/liter-urine. Heat insulator in the form of 2 cm thick cork is able to inhibit heat loss to the environment by 18.9%. Bio-urine which is fermented aerobically in a heat insulated bioreactor has a quality that meets POC standards from the Decree of the Minister of Agriculture of the Republic of Indonesia No: 261/KPTS/SR.310/M/4/2019. Bio-urine has C/N 11.8, TDS 13899 ppm, EC 54868 µS/cm.

Key words: urine, fermentation, heat, bio-urine.

**INTRODUCTION**

Cow urine organic fertilizer contains micro and macro nutrients, so it is appropriate to be used as liquid fertilizer. According to Mahajan [1], the content of cow urine contains 95% water, 2.5% urea and other substances. Other elements such as: minerals, salt, hormone, enzymes as much as 2.5%. Chemically cow urine contains sodium, nitrogen,

sulfur, Vitamins A, B, C, D, E, minerals, manganese, iron, silicon, chlorine, magnesium, citrate, succinate, calcium salts, phosphate, lactose, carbonic acid, enzymes, creatinine and hormone [2]. In addition, bio-urine contains growth stimulants that can be used as growth regulators and has a distinctive smell of livestock urine, this distinctive odor can prevent various plant pests from coming. Therefore, bio-urine has 3 main functions, that are: (1) as a liquid fertilizer for plants, (2) as a plant growth regulator and (3) as a vegetable pesticide [3].

In the aerobic or anaerobic fermentation process, cow urine will decomposed into energy, minerals, microorganism cells, and exhaust gases [4] and [5]. The fermentation process of cow urine enriched with biological fertilizers with microbes as much as 3 ml/liter-urine, and molasses 75 ml/liter-urine. Enrichment of substances in urine can increase N-total 860 percent, organic matter 282 percent, and microbial population by 1229 percent [6]. The composition of bio-urine enriched with biological fertilizers is: (1) 200 liters of cow urine, (2) 5 kg of physalis plants, (3) 5 kg of brown sugar, (4) 2 kg of salt without iodine, (5) cow manure 10 kg, (6) EM4 plant 1 liter, (7) EM4 Fishery 0.5 liter, and (8) breadfruit leaves 5 kg [8]. According to Rasyid [9], one adult cow can produce as much as 8 liters of urine/day. Some SIMANTRI groups in Bali have 10-25 cows, while horticultural or rice farmers also have 1 - 4 cows, which is a potential for processing urine by fermentation processes. The final fermentation product of cow urine is bio-urine which can be grouped into organic liquid fertilizer or POC.

The process of fermentation of cow urine into bio-urine is a complex bio-chemical reaction involving microbes. Microbes can work optimally under ideal biomass conditions and optimal processing conditions. The initial biomass must have a C/N value of 15-20, and a pH close to neutral or 6 to 7,5 [10]. For this reason, in the urine fermentation process at the beginning of the process, it is necessary to: (1) add molasses or a carbon source of  $\pm 3\%$  [11], (2) add 3% urea [12], (3) add oxygen with an aerator for aerobic fermentation [13] and (4) the addition of 3 - 5% nitrifying bacteria [14] and [15]. Urea and sugar are the initial nutrients for microbes to start the urine fermentation process.

Based on the temperature area, microbes can be divided into 3 groups, namely psychrophile microbes (microbes that can grow at temperatures ranging from 0-30°C); mesophile microbes (microbes that grow at temperatures ranging from 30-60°C) and thermophile microbes (microbes that grow at temperatures ranging from 40-80°C) [16].

Bacteria that break down cow urine into bio-urine are a type of mesophile microbe, because they can have optimum activity at temperatures ranging from 30-40°C. Based on the pH area of their life, microbes are divided into 3 groups, namely acidophilic microbes (microbes that can grow at a pH range (2.0-5.0); mesophile microbes (microbes that can grow at a pH range of 5.5-8.0) and Alkaliphile microbes (microbes that can grow at a pH range of 8.4-9.5). The microbes that decompose cow urine are mesophile microbes, because they live at a pH range of 4.5-7.5 [16].

In processes other than the pH value of the biomass, the process temperature must always be controlled, the bioreactor, which is a fermentation tool, must have a construction that makes it easy to control the reaction temperature. Reaction Therefore, the construction of the bioreactor must be able to regulate the amount of heat produced by the reaction which is intended: (1) to increase the biomass temperature ( $Q_T$ ), (2) to evaporate water from biomass ( $Q_U$ ), and (3) to dispose of it into the environment ( $Q_L$ ). The construction of the bioreactor is that the temperature of the fermentation reaction is still not optimal for microbial breeding, because the temperature of the fermentation process is still close to the ambient temperature. Fermentation temperature of cow urine occurs between 28 - 35°C [11], [12], [13], and [15].

Therefore, an analysis of the dynamics of heat or heat in the fermentation process of cow urine for bioreactor design recommendations needs to be done. The minimum capacity of the bioreactor and the presence of insulation on the bioreactor walls need to be studied to optimize the fermentation process, because the heat of the reaction, ( $Q_R$ ), heat to increase temperature ( $Q_T$ ), heat to evaporate water ( $Q_U$ ) and heat lost to the environment are a function of the volume of urine in bioreactor and the heat conductivity of the bioreactor walls. The analysis was carried out on: heat to increase the temperature of the biomass, heat to evaporate water, and heat loss to the environment.

## II. MATERIALS AND METHODS

### 2.1 Time and Place of Research

The research was conducted at Br. Mayungan Anyar, Antapan Village, Baturiti District, Tabanan Regency at 2017 - 2019. The research location has an altitude of  $\pm$  900 m above sea level, with an average daily temperature of 28°C, humidity of 70 - 90%.

## 2.2 Materials and tools

The main ingredient for the study was cow urine with a pH value of 5.4 - 6.5, EC = 54200 - 55320 mh, TDS = 9400 - 10211 ppm, and C/N = 15-18 [17]. Supporting materials are palm sugar, urea fertilizer and nitrosomonas microbes. The tool used is a bioreactor in the form of a plastic bucket with a volume of 60 liters equipped with a temperature measuring device (digital thermometer) and humidity (hygrometer) and a gas exhaust hose from the fermentation reaction.

## 2.3 Experimental design

Experiment with a two-factor design, the first factor is the fermentation system (aerobic and anaerobic system), the second factor is the bioreactor wall construction (the bioreactor is given a heat insulator and the bioreactor is not given a heat insulator). Nitrifying bacteria population added to cow urine is 5% of the volume of fermented cow urine. The volume of fermented cow urine is 500 liters, palm sugar added is 3% or the equivalent of 15 kg [11], [13] and [15]. Each experimental unit was repeated 3 times, so that there were 12 experimental units. In the aerobic fermentation process of cow urine, the bioreactor is given an additional aerator which functions to add oxygen to the cow urine, the aerator has a power of 30 watts with an air flow of 0.038 liters/second [15]. The experimental construction is shown in Figure 1.

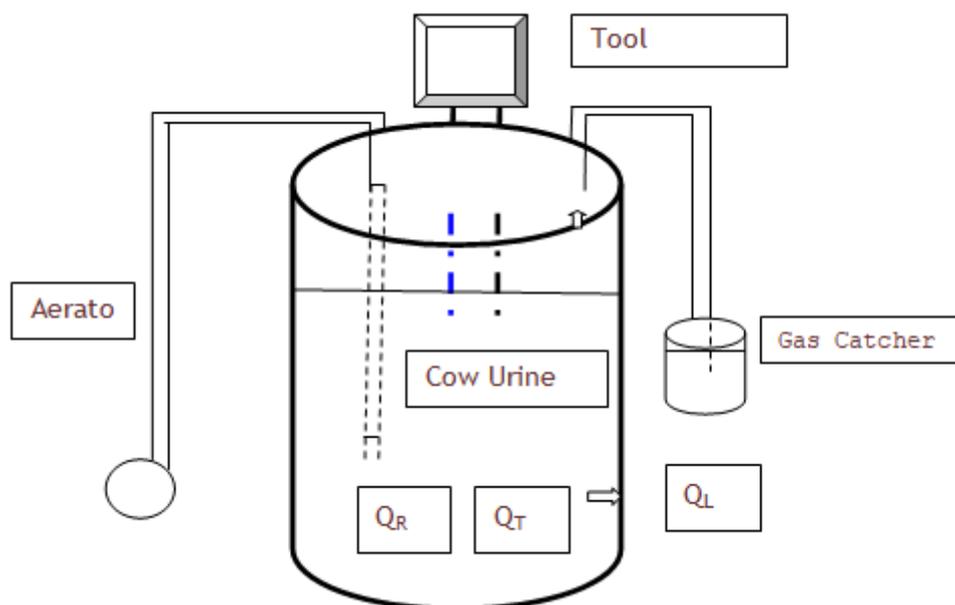


Figure 1 Schematic of cow urine fermentation bioreactor

## **2.4 Fermentation Experiment**

### **2.4.1 Aerobic Fermentation**

- (1) A bioreactor in the form of a tank profile with a capacity of 630 liters is prepared. For the insulating bioreactor, the walls are covered with 2 cm thick cork. In addition, for all bioreactors, add an aerator with 30 Watt power.
- (2) 500 liters of cow urine is put into a 630 liter volume tank (bioreactor insulating / non-insulating)
- (3) Urine is added with 2% sugar, 3% urea and 5% nitrifying bacteria [11] and [15].
- (4) During the fermentation process the aerator is kept on.

### **2.4.2 Anaerobic Fermentation**

- (1) A bioreactor in the form of a tank profile with a capacity of 630 liters is prepared. For the insulating bioreactor, the walls are covered with 2 cm thick cork.
- (2) 500 liters of cow urine is put into a 630 liter volume tank (bioreactor insulating / non-insulating)
- (3) Urine added 2% sugar, 3% urea and 5% nitrifying bacteria.
- (4) During the fermentation process the aerator is kept on.

## **2.5 Parameters Observed**

The parameters observed were: urine temperature and environment, air humidity in the bioreactor, pH value, EC and TDS biomass, C and N content of urine. Urine and environmental temperature, as well as air humidity in the bioreactor were measured every day with 3 replications each parameters. Meanwhile, the EC, TDS, carbon content and nitrogen content were measured every 7 days. The fermentation process was carried out for 21 days [18].

## **2.6 Data analysis**

### **2.6.1. Changes in urine temperature in the bioreactor in the fermentation reaction.**

Daily data on the average urine temperature in the bioreactor were measured using a thermocouple and digital thermometer at 3 measurement points, the relationship between the average daily temperature data and the fermentation time was graphed.

### **2.6.2 Heat to increase urine temperature in bioreactors.**

Heat to increase biomass temperature ( $Q_T$ ) is a function of the volume of cow urine in the bioreactor ( $V$ ), urine mass density ( $\rho$ ), biomass specific heat ( $C_p$ ) and changes in biomass temperature ( $\Delta T$ ), where  $\Delta T$  is the difference in mean urine temperature. on

day h ( $T_h$ ) minus the average urine temperature on day h-1 ( $T_{h-1}$ ). The values of  $Q_T$  and  $\Delta T$  are formulated:

$$Q_T = \rho \cdot V \cdot C_p \cdot \Delta T$$

$$\Delta T = (T_h - T_{h-1})$$

The specific heat value of urine is close to the specific heat value of water, the value of  $C_p = 4,18$  Joule/g-°C and the value of  $\rho$  is 1,01 g/cc.

### 2.6.3. Heat Dissipated Into the Environment

Heat loss to the environment ( $Q_L$ ) occurs as a result of the difference in the mean temperature of urine in the bioreactor ( $T_b$ ) with the average ambient temperature ( $T_1$ ). Changes in temperature of the urine in the bioreactor result in the transfer of heat from the bioreactor to the environment in an unsteady process. Heat will pass through the bioreactor wall which has a thickness of  $\Delta x_1$ , a solid insulator with a thickness of  $\Delta x_2$  and a surface area of A. The value of the combined heat conductivity is a combination of the convection heat transfer coefficient in the bioreactor wall ( $h_{di}$ ), the thermal conductivity of the bioreactor wall ( $k_b$ ), the heat conductivity of the insulator ( $k_i$ ) and the convection heat transfer coefficient outside the bioreactor wall ( $h_{dl}$ ).

$$Q_L = U \cdot A \cdot (\Delta T)$$

The U value for a bioreactor with heat insulated walls according to Holman [19] is:

$$1/U = 1/h_{di} + (k_d/\Delta x_1) + (k_i/\Delta x_2) + 1/h_{dl}$$

the U values for a bioreactor with heat insulated walls are:

$$1/U = 1/h_{di} + (k_d/\Delta x_1) + 1/h_{dl}$$

The heat conductivity value of the plastic is 0,25 Joule/m-°C and the conductivity of the cork type insulator is 0,095 Joule/m-°C. Meanwhile, the value of the convection heat transfer coefficient on smooth and vertical walls used is 10 Joule/m<sup>2</sup>-°C. The thermal conductivity values of the bioreactor walls without insulators and insulators are 1,14 Joule/m-°C and 0,43 Joule/m-°C, respectively [19].

### 2.6.4. Heat For Water Evaporation

The heat to evaporate water from biomass ( $Q_U$ ) which is fermented into the environment is the difference in the water vapor content in the air in the fermentation process ( $\Delta H$ ) times the latent heat value at the biomass temperature ( $h_{fg}$ ). The value of  $\Delta H$  is the difference from the moisture content in the bioreactor day h ( $H_h$ ), minus the water vapor content in the bioreactor day h-1 ( $H_{h-1}$ ). Psychrometric charts were used to find  $H_h$

and  $H_{h-1}$  values with the help of air temperature data at the top of the bioreactor and air humidity. Likewise, to find the  $h_{fg}$  value. The heat is defined:

$$Q_U = h_{fg} \cdot \Delta H$$

The heat from the urine fermentation reaction ( $Q_R$ ) will then be used to raise the temperature of the biomass, evaporate water from the biomass to the environment and disappear through the bioreactor walls into the environment. Heat of fermentation correction is a combination of (1) heat to increase temperature, (2) heat of water evaporation and heat that is lost to the environment if there is an increase in temperature of the biomass.

$$Q_R = Q_T + Q_L + Q_U$$

However, if there is a decrease in the temperature of the fermented biomass, the heat of the reaction is only a combination of heat to evaporate water and heat that is lost to the environment.

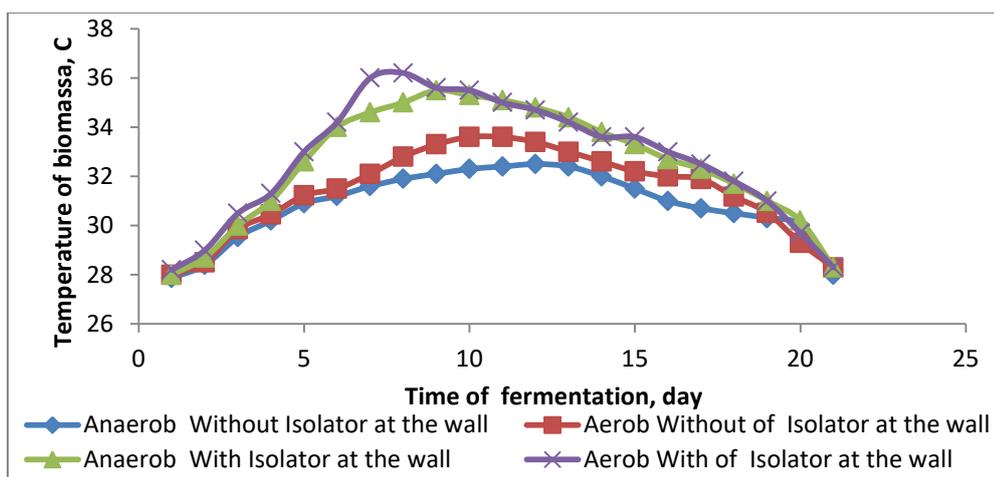
$$Q_R = Q_L + Q_U$$

### III. RESULTS

#### 3.1. Reaction Dynamics of Cow Urine Fermentation in Bioreactor

##### 3.1.1 The temperature of the fermentation

The temperature of the fermentation reaction of cow urine in the bioreactor in aerobic and anaerobic processes is illustrated in Figure 2. The urine temperature of each treatment increased from day 0 to day 12, after which the temperature reached a peak of 32,5 – 36,2°C the temperature then decreased to 28,0 – 28,3°C [20]. Based on the temperature of cow urine in the bioreactor of the four treatments, the reaction temperature was initially in the fermentation process by psychrophile bacteria (temperature 27,7 - 30°C), then the processes on fermentation by mesophile bacteria (temperature 30 – 36,2°C) and after that the fermentation process returns to the process by bacteria psychrophile. . Based on the fermentation temperature pattern, in general the chemical reaction process of cow urine fermentation is quite good, because the relationship between fermentation time and urine temperature is a quadratic equation with an  $r^2$  value of  $0,92 \pm 0,02$ . At a temperature of 30-37°C, enzymes, microbes are able to work optimally to carry out the urine fermentation process with small daily temperature fluctuations [20]. At temperatures below 30°C or above 37°C the biochemical reaction of fermentation is ineffective [21].

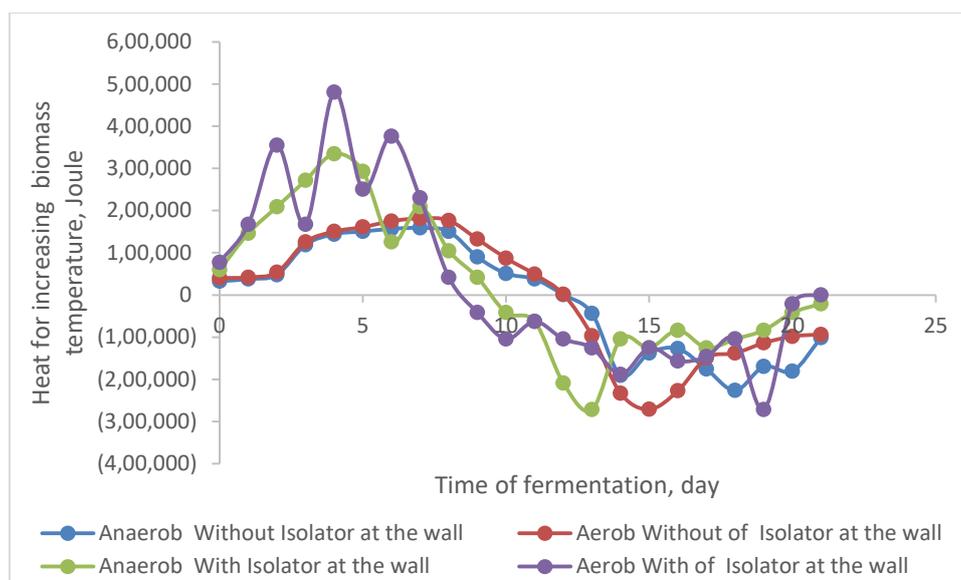


**Figure 2 Biomass temperature of cow urine in the fermentation process in a 60 liter volume bioreactor**

### 3.1.2. Heat to increase urine temperature in bioreactors

The heat to increase urine temperature in the bioreactor is related to the fermentation time as shown in Figure 3. The total heat to increase the temperature for aerobic treatment is 2,7 – 4,3 k.Joules/liter-urine, while the total heat to increase the temperature for aerobic treatment is 2,3 - 3,6k.Joules/liter-urine. The heat from the biochemical reaction of urine fermentation 71,6 – 85,14% is used to increase the temperature of fermented urine However, after the fermentation process reaches the peak of the heat of the biochemical reaction of urine, the amount of heat to raise the temperature becomes negative or there is a decrease in urine temperature in the bioreactor.

In the heat insulated bioreactor and urine fermentation carried out aerobic and anaerobic, the amount of heat to increase temperature is negative occurred on after days 9 and 12, respectively. For bioreactors without heat insulators and urine fermentation carried out aerobically and anaerobically, the amount of heat to increase temperatures is negative occurred after days 10 and 13.

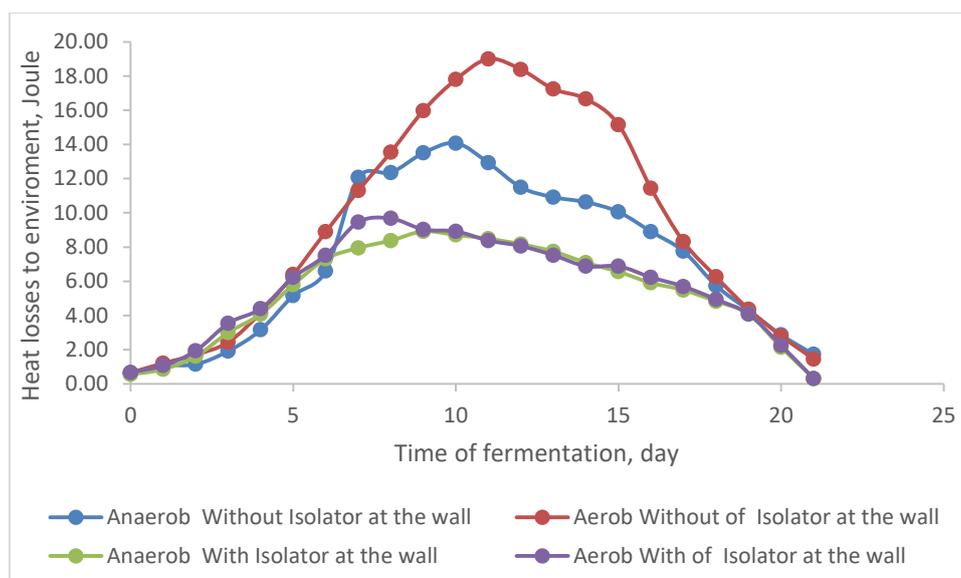


**Figure 3 Heat dynamics to increase the temperature of urine fermented in bioreactor**

### 3.1.3. Amount of heat dissipated to the environment during fermentation process

The effectiveness of the bioreactor with heat insulators is also illustrated in Figure 6, this figure explains the amount of heat lost through the bioreactor walls to the environment. In the aerobic fermentation process the insulator on the wall is able to inhibit heat by 284k.Joule or 45,1%. In the anaerobic fermentation process the heat loss to the environment is 135k.Joule or 27,6% which is retained in the bioreactor.

The relationship between fermentation time and the amount of heat to the environment is quadratic equation. The amount of heat lost to the environment is a function of the difference in urine temperature with ambient temperature ( $\Delta T$ ), wall thermal conductivity ( $k$ ), wall surface area ( $A$ ) and wall thickness ( $\Delta x$ ). Except for the difference in urine temperature with ambient temperature ( $\Delta T$ ), the other parameters are fixed in value, therefore because bio-urine temperature always changes with a quadratic pattern, the heat lost to the environment changes in a quadratic pattern.

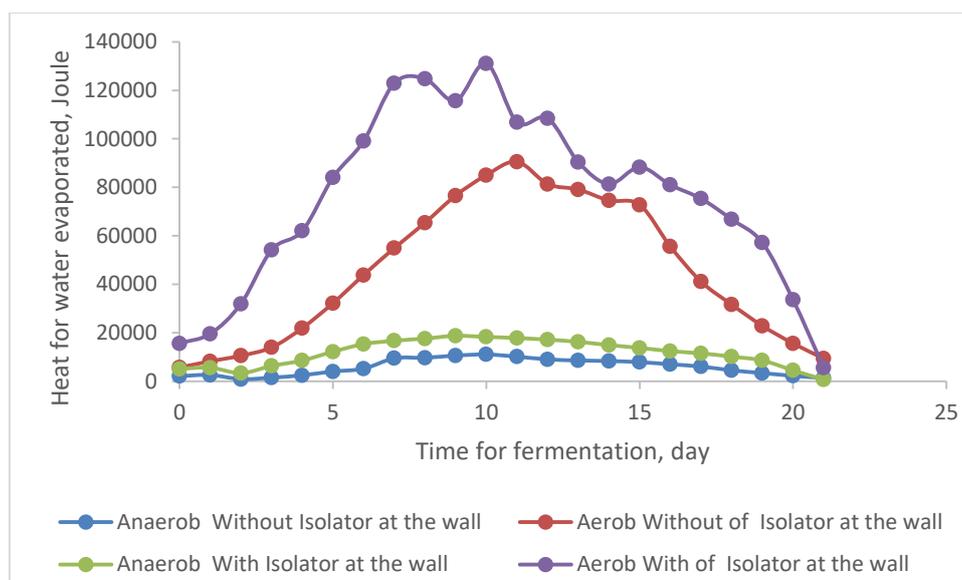


**Figure 4 Heat loss to the environment from bioreactor**

Based on Figure 4, from the heat loss image to the environment from the 630 liter volume bioreactor, it is suspected that the peak fermentation of urine into bio-urine occurs on days 10 - 11 for bioreactors without isolation and on day 9 for bioreactors with isolators. After the cow urine fermentation reaction reaches its peak, the fermentation reaction then slows down until the 21st day.

### 3.1.4. Heat to evaporate water

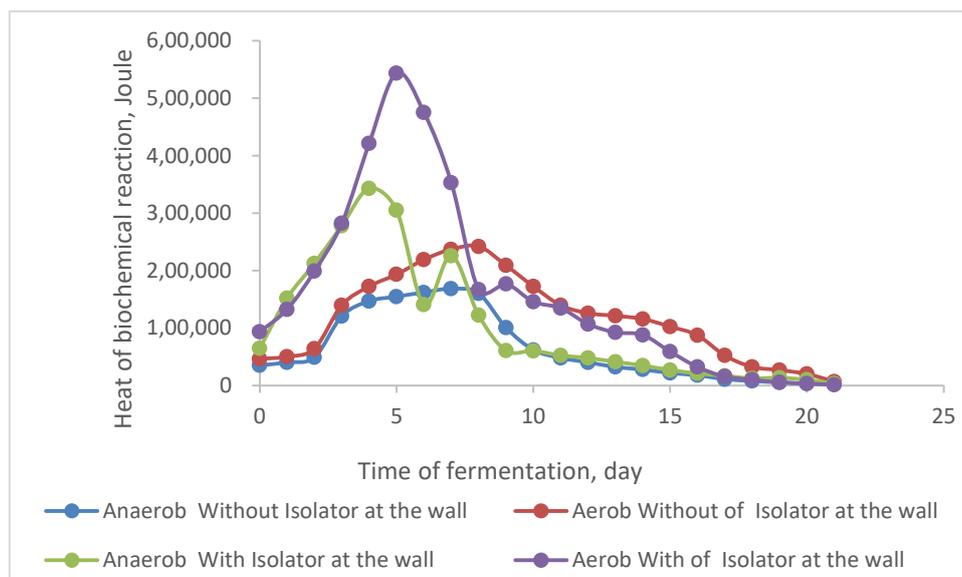
In addition to raising urine temperature and being lost to the environment, heat from the bio-chemical reaction of fermentation is used to evaporate the water in the urine into the environment. The amount of heat to evaporate water from the urine to the environment is 0,678 - 180k.Joule (as in Figure 5). The relationship between fermentation time and the amount of heat for water evaporation is a quadratic pattern, with evaporation peaks on days 9 - 11 for bioreactors without insulators and days 7 - 9 for bioreactors with heat insulators. Isolators are able to inhibit heat loss to the environment, so that the urine temperature in the insulator bioreactor is higher than the urine temperature in the bioreactor without an insulator. The increased temperature of the liquid causes the difference in water vapor pressure in the urine with the environment to increase as well, this has a direct impact on the amount of water that is evaporated.



**Figure 5 Heat loss of water evaporation in cow urine fermentation**

### 3.1.5. Heat of cow urine fermentation

The heat of the fermentation reaction from the research results is depicted in Figure 6. The fermentation reaction of cow urine to bio-urine reaches a peak on days 4-5 for the insulator bioreactor process and on days 7 - 8 for the process in the bioreactor without an insulator. The heat retained by the insulator is used to increase the temperature of fermented urine so that it reaches a temperature of 38,5 – 40,1°C, at that temperature mesophilic bacteria develop more easily than if the fermented biomass temperature is below 35,8°C or in fermentation in a bioreactor. without an insulator.



**Figure 6 Heat of cow urine fermentation reaction in bioreactor**

### 3.2. Bio-urine Quality

The values of the parameters of C/N, EC, pH and TDS of bio-urine from the statistical test results of each fermentation treatment were different. The values for the parameters of C/N, EC, pH, TDS, macro and micro nutrient content of bio-urine for each treatment are shown in Table 1:

Table 1. Value of Bio-urine Quality Parameters after 12 days of fermentation in a 630 liter bioreactor

Quality Parameters Of Bio-urine	With Isolators on the Wall			
	Without Isolators on the Wall		Wall	
	Anaerobes	Aerobes	Anaerobes	Aerob
<b>Main Parameters</b>				
TDS, ppm	10289	10762	11843	13899
EC, $\mu\text{S}/\text{cm}$	54623	54765	54789	54868
pH	6,34	6,33	6,34	6,36
Microbe population, log cfu	7,40	7,54	8,60	8,80
C/N	21,00	19,60	12,00	11,80
C - organic, %	21,95	21,38	13,68	13,57
<b>Unsur Hara makro:</b>				
Nitrogen content, %	1,045	1,091	1,14	1,15
Content of $\text{P}_2\text{O}_5$ , %	5,7	5,6	5,8	5,7
Content of $\text{K}_2\text{O}$ , %	4,7	4,72	5,2	5,2
<b>Unsur Hara mikro :</b>				
Content of Fe total, ppm	732	736	834	888
Fe available, ppm	7.2	7.3	8	8.3
Content of Mn, ppm	654	678	689	672
Content of Cu, ppm	765	778	821	828
Content of Zn, ppm	1278	1331	1345	1362
Content of B, ppm	1231	1222	1265	1245
Content of Co, ppm	9,8	9,8	9,2	9,4
Content of Mo, ppm	4,6	4,6	4,8	4,7

## DISCUSSION

The speed of temperature increase in anaerobic fermentation in bioreactors without isolators and with isolators respectively is: 0,73°C/day and 1,73°C/day. In aerobic fermentation of urine in bioreactors, the rate of increasing urine temperature is 0,86°C/day and 1,75°C/day. The value of the speed of this temperature rise from each treatment from the results of statistical tests (ANOVA and LSD) is different, this reinforces the hypothesis that there is a difference in the rate of increase in urine temperature from aerobic and anaerobic fermentation processes and differences in temperature increase due to the use of heat insulators on the bioreactor. Oxygen, the process of stirring urine and heat insulators are needed in the fermentation process of cow urine into bio-urine.

Like the rate of temperature increase, the incidence of decreasing cow urine temperature in the anaerobic fermentation process with bioreactors without isolators and with insulators is -1,2°C/day and -1,6°C/day, respectively, whereas urine fermentation in bioreactor the aerobic rate of decreasing urine temperature was -1,5°C/day and -1,8°C/day [4]. The decrease in cow urine temperature reflects a decrease in the biochemical reaction rate of fermentation of cow urine into bio-urine as a result of decreased microbial activity and the microbial population that decomposes urine into simple minerals [18]. In addition, a decrease in the speed of the fermentation reaction occurs due to the low C/N value of the fermented urine or the microbes starting to run out of nutrients and die.

Isolators really support the increase in the temperature of fermented urine, because the insulating layer on the bioreactor walls is able to inhibit the amount of heat lost to the environment. In the aerobic fermentation reaction, the aerator in the bioreactor is able to supply oxygen and stir the urine continuously. The isolator is able to withstand the heat that will be lost to the environment between 13,2 – 24,8% and then the heat is used to increase the fermentation temperature, the increase in the temperature of urine fermented in the bioreactor due to the installation of heat insulators is 0,4 – 3,4°C.

The aerobic microbial fermentation of cow urine is more active than the anaerobic fermentation process by taking into account changes in the temperature of the fermentation reaction [18]. The increase in temperature of the fermentation reaction in the anaerobic process was 0,52 – 0,92°C/day, while in the aerobic process the increase in urine temperature in the bioreactor was 0,58 – 1,10°C/day. Adequacy of oxygen in

cow urine is in the bioreactor in the aerobic fermentation reaction process due to the presence of an aerator that is able to supply oxygen and carry out the stirring process resulting in a more complete fermentation chemical reaction to break down organic matter by microbes.

Changes in temperature and peak temperature of fermentation reactions describe the dynamics of biochemical reactions of fermentation of cow urine into bio-urine. The peak temperature of the fermentation reaction occurred on day 8 at a temperature of 36,2°C. Mesophilic group microbes are more active in this process than others because the reaction temperature occurs at a lower temperature (27,7 – 35,5°C).

Based on Figure 3, the heat to increase the temperature for the fermentation process of cow urine in the insulator bioreactor is shorter (8 days) than in the bioreactor without an isolator (12 days). This shows that the effectiveness of the insulator in supporting the speed of the fermentation reaction, this condition is also supported by the amount of heat to increase the fermentation temperature in the bioreactor with a total insulator of  $2,0 \times 10^6 - 2,8 \times 10^6$  Joule, being in a bioreactor without an insulator the amount of heat to increase the temperature by  $0,9 \times 10^6 - 1,1 \times 10^6$  Joule. The increase in temperature of the fermentation reaction to a peak of 32,5 – 36,2°C is very important, this is to assess the effectiveness of microbial propagation as urine decomposers [18].

The role of the insulator in the fermentation process was assessed from the time of fermentation and the increase in fermentation temperature, its effectiveness increased by 25 - 33%. In addition, the total heat for increasing the temperature in the presence of an insulator is 310k.Joule in the anaerobic process and 1305k.Joule in the aerobic fermentation process, so that the aerobic fermentation process with an insulator bioreactor is more effective.

The amount of water evaporated in the fermentation process of cow urine is an indicator of the ongoing fermentation process. Therefore, based on Figure 5, the fermentation reaction lasts effectively until days 7 - 11 [20], after which the fermentation reaction slows down. Total water vapor in the fermentation process of cow urine for anaerobic treatment is: 1,1 – 2,7 g/liter of urine, while for aerobic fermentation is 8,7 – 16,7 g/liter of bio-urine. The aerator installed in the bioreactor increases the evaporation capacity of water in the bio-urine. Aerators increase the effectiveness of fermentation by 5 – 6,7 times, when viewed from the amount of water evaporated.

In a bioreactor without an aerator the air volume is only 130 liters, equivalent to 15,3 liters of oxygen, or at the beginning of the fermentation process the oxygen concentration in cow urine is 30 ml/l urine [22]. The fermentation reaction occurs anaerobic when oxygen is not present in the bioreactor. However, if the 3 watt power aerator is installed on the bioreactor, the availability of oxygen is 14,28 liters/hour or 28 ml/liters-urine, so that the fermentation reaction with oxygen sufficiency in the bioreactor takes place in an aerobic state.

Meanwhile, the insulator increased the fermentation temperature and subsequently increased the effectiveness of the fermentation by 1,5 - 2,1 times in terms of the evaporated water from the bioreactor. The main function of the insulator is to inhibit heat loss in the fermentation reaction so that the fermentation temperature will increase, while the main function of the aerator is to supply oxygen so that the fermentation reaction is more perfect and to stir the urine biomass to make it more homogeneous.

Based on the amount of reaction heat formed in the fermentation process of cow urine, the fermentation treatment in the bioreactor with heat insulators and an aerator is the best treatment. The fermentation reaction can be stopped on the 17 day, because the heat of the reaction has reached 15-16 k.Joule [23]. Fermentation treatment of cow urine in a bioreactor without a new isolator can be stopped on day 20.

The increase in the temperature of the fermentation biochemical reaction due to the addition of an insulator to the bioreactor by 0,4 - 3,4°C greatly impacts the increase in the population of microbes that are actively carrying out fermentation [16]. As a result of the addition of an isolator to the bioreactor, the number of active microbes also increased from  $2,6 \times 10^6$ cfu to  $5,9 \times 10^7$ cfu at the peak of the cow urine fermentation reaction and the fermentation time for cow urine decreased from 12 days to 9 days [24] and [ 25].

The benefit of heat insulators was also shown by the quality of bio-urine after fermentation for 12 days [25], and [26]. Bio-urine liquid fertilizer can save 40 - 60% fertilizer when applied to lettuce, tomato, potato, chili, and cabbage. In addition, the microbial population in the soil in the root zone increased  $5,6 \times 10^3$ cfu to  $7,9 \times 10^6$ cfu. The use of bio-urine liquid fertilizer is also able to strengthen the implementation of the LEISA system in horticultural cultivation [27].

## CONCLUSION

The aerobic fermentation process of cow urine in the heat insulator bioreactor is the most effective compared to the fermentation process in the bioreactor without heat insulator and anaerobic fermentation. Adequacy of oxygen, reaction temperature (27,7 – 36,2°C), urine circulation process and microbial activity (4,5 log.cfu – 8,8 log.cfu) are the main factors for the success of urine fermentation to become bio-urine in bioreactors. Bio-urine which is fermented aerobically in a heat insulated bioreactor has a quality that meets the POC standards from the Decree of the Minister of Agriculture of the Republic of Indonesia No: 261/KPTS/SR.310/M/4/2019. Bio-urine has C/N 11,8, TDS 13899 ppm, EC 54868 µS/cm, the content of macro and micro nutrients that meet the standards.

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