The International Journal Of Engineering And Science (IJES)
||Volume||2 ||Issue|| 8 ||Pages|| 86-94||2013||
ISSN(e): 2319 – 1813 ISSN(p): 2319 – 1805

Assessment of Nitrogen in Municipal Household Bio-Waste Using Kjeldahl-Nesslerization

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----------ABSTRACT----------

Quantification of Total Kjeldahl Nitrogen determination is done with various methodes such as titimetry, photometry, spectrophotometry and it depend the form of solid or liquid sample. The nitrogen determination of EPA 350.2 model according to colour complex substance of NH_2Hg_2IO is used for the sample that liquid form with Kjeldahl method-Nesslerization. Aim of research is first, transformation of EPA 350.2 quantitative model for solid sample with evaluation through verification of the unit. Second, percentage determination of bio waste nitrogen is compare with the precision of measurement with prior research. Using action research method in laboratory was done with two variables: first, modification of Kjeldahl digestion of Janssen and Koopman and second, Nesslerization-spectrophometry technique. EPA 350.2 quantitative model transformation to determination % N has three parameters i.e. N concentration in mgL^{-1} unit, total volume of N-organic digestion result in litre (L) unit, and sample weight in mg unit. The third of those parameters gave procedure more efficient. Differences of accurate value of research measurement and reference were – 0.08 until – 0.02. Using comparator with photometry and titimetry method were – 0.13 until + 0.14. Accurate value of measurement in this research and comparator was not different.

KEYWORDS: *Bio-wastes, Kjeldahl-Nesslerization method.*

Date of Submission: 1 August2013, Date of Acceptance: 20 August2013,

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I. INTRODUCTION

The knowledge of total nitrogen quantity that closed on the intrinsic of value in the environment is very important such as the happen of eutrofication. Developing the organic or super intensive agricultural, the agricultural that based organic fertilizer like the compost from organic waste material [1,2] as well as inorganic have support on soil fertility that the plant is growth. The characteristic of nitrogen in waters is N-organic, N-NO_3, N-NO_2, and N-NH_4. The decomposed organic material expressed by %N to determination of C/N ratio. The waste from food in household has protein molecule as sources of N-organic and transformed only become NH_3 substance. Total nitrogen in result of compound digestion is determined follow unit of measurement that is desirable.

Kjeldahl method is used in various analyses recently. Redondas [3] used Kjeldahl procedures to determined quantity of total nitrogen, the composting activity of market organic waste material [4], result of decomposition as the compost [1,5] for plant of *Zea mays* growth or agricultural activity. Determination of nitrogen and carbon can show the value of C/N ratio that is desirable respectively in percentage. Decomposition of organic material to other substances by microbes in natural organic solid waste (bio waste) depends of C/N ratio value. Kjeldahl method consist three of part i.e. digestion, neutralization, and distillation. The first, digestion has function for forming $(NH_4)_2SO_4$ and every differences of procedures compared with the original named modification method. The second, neutralization has function of forming acid condition to be alkali and that is purposed toward NH_3 substance. The third, distillation has function to escape the NH_3

substance and it is reacted with borax acid. The quantification of nitrogen is done with various approaches method such titimetry, photometry, and spectrophotometry.

Transformation N-organic substance of plant and/or bio waste [6] material to NH_3 is non N-NO_3 and N-NO_2 categories; however nitrogen in waters has two that category. Photometry and spectrophotometry method, mean a technique, is alternative from titrimetry method that use reagent of borax acid. That alternative method follows Lambert-Beer law in interaction of color complex substance and the spectrum of visible ray. The Nesslerization technique form the colour complex substance of NH_2Hg_2IO and maximum absorbance happen on 425 nm. Similar technique is inophenol that complex color substance on 630 nm spectrum.The Nesslerization technique of NH_2Hg_2IO with reagent of HgI_2, KI, and NaOH is used by Environmental Protection Agency (EPA) 350.2 and still has its function for long one year [7, 8]. The Total Kjeldahl Nitrogen (TKN) of EPA 350.2 in concentration unit of mgL^{-1} and it is done from liquid form of N-organic sample with spectrophotometer instrument. The formula of TKN is

$$\text{TKN, mgL}^{-1} = [(A \times 1000) / D]\,[B/C] \quad \ldots\ldots (1)$$

Where: A = mgL^{-1} unit as value of N (determination N using standard curve)
B = Total volume (mL unit) of distillate include H_3BO_3
C = mL unit of aliquot volume from result of digestion and distillation then adding Nessler reagent.
D = mL unit of liquid sample from environment

Application of Kjeldahl method, Nesslerization-spectrophotometer for solid sample, and quantification of that TKN model need a study. Quantification approach is done with verification of unit that model of formula (1) and has consequence of working procedure in laboratory. Nesslerization technique procedure and using spectrophotometer is assumption of Lambert-Beer law scope. Therefore in this study has method domain and mode of Kjeldahl, Nesslerization, and spectrophotometry and those are termed with Kjeldahl-Nesslerization method. How the model of EPA 350.2 is used on nitrogen quantity determination of municipal household bio waste sample that solid form? The aims of research are the first, transformation of EPA 350.2 quantity model for solid form of sample with evaluation through measurement unit verification. The second, determining percentage of nitrogen bio waste and compare of measurement precision with previous study. The benefit of that model transformation and determining nitrogen percentage is applying to quantification of solid bio waste C/N ratio.

II. MATERIAL AND METHOD

2.1. Materials

This research used materials for chemistry analysis of nitrogen content of *nasi* (cooked rice bio-waste) in the laboratory. The materials were pure water with CLSI (0.1 MΩ-cm) standard that it was resulted by Micromeg type S and measuring of conductivity with instrument of DDB-6200 model. Standardization that instrument was done with NaCl 0.05M on 27 ^{o}C. The catalyst follows Merck i.e. K_2SO_4 p.a (Riedel-de-Haen), Selenium p.a (Merck) dan $CuSO_4.5H_2O$ p.a (Merck). Acid for digestion was used H_2SO_4 98% p.a (Merck) and Gerhardt type KI 24 apparatus for heater. Nessler reagent was made from HgI_2 p.a (Merck), KI p.a. (Merck), and NaOH p.a (Merck). The electronic balance (Denver instrument) has 0.01 mg precision. The Spectronic 20 (Milton Roy) 110 V on 425 nm spectrum was standardized with $CoNO_3$ on 505, 510 dan 515 nm spectrum.

2.1. Method

Study was done for three month i.e. September 2012 – December 2012 and used action research method in laboratory. Study consist two variables or bivariate [9]; first, modification of Kjeldahl digestion of Janssen and Koopman and second, Nesslerization-spectrophometry technique. The first variable was Kjeldahl modification that result NH_3 substance. The second variable was Nesslerization that result a color complex substance of NH_2Hg_2IO and it depend from the first variable. Hypothesis of this research is; Accuracy of Kjeldahl-Nesslerization-spectrophotometry modification and the Kjeldahl-Nesslerization-photometri modification with the nitrogen percentage indicator of bio-waste are not different. Data analysis showed on Fig. 1. Analysis was done toward differences of precision measurement compared references with Follin and Danish that owned differences of accurate measurement from method of Kjeldahl-Nesslerization-photometry and Kjeldahl-Titimetry. Quantification value of N concentration calculated with verification model of formula (1) above toward solid sample. Value of analysis precision found from comparing respectively between

modification result of Kjeldahl-Nesslerization-spectrophotometry and references. The reference values are analysis result of nitrogen [10] and the information of database in the internet.

Approaching to that analysis was done toward sample and it was chosen with the criterion; bio-waste has one kind content of material. That was *nasi* (cooked rice bio-waste) from *Kelurahan* Kasin with a code 6011014. Samples of organic waste (bio-waste) were taken from kitchen and yard of household in *Kelurahan* Kasin, Kauman, and Oro-oro Dowo of Malang City (TABLE 1).

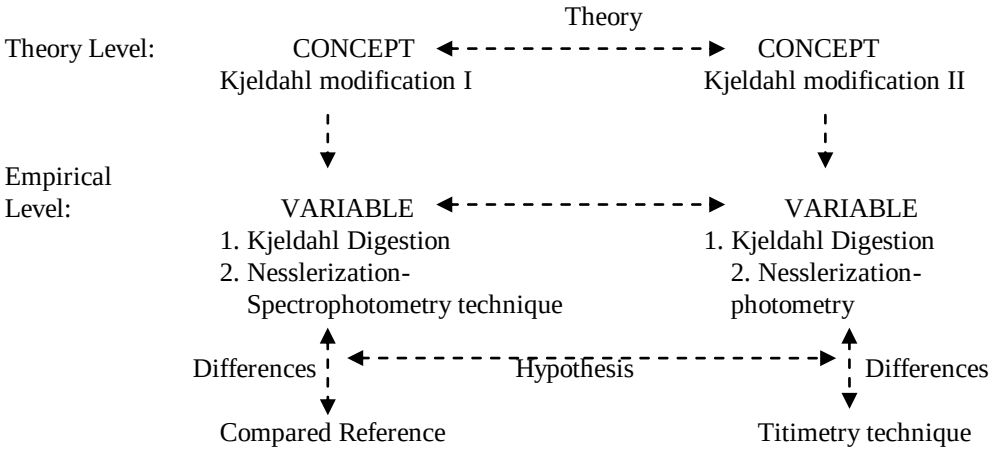


Figure 1. Diagram of Research that Two Variables and Hypothesis Test.
Adaptation of Data Analysis [9].

Table 1. Villages, Community group (*RW*) Location of Research Area, Household Population and Samples of household

No.	Village (*Kelurahan*)	Community group	Household		Samples Number
			Number	%	
1	Kasin	1,2,3 and 7	547	25.47	8
2	Kauman	1,5,8,9 and 10	469	21.83	7
3	Oro-Oro Dowo	1,2,3,4,5,6 and 9	1.132	52.70	17
Total			2.148	100	32

Organic material digestion used the procedures from Janssen and Koopman [6], however, the catalyst of Titanium metal was changed by Cu and Se [11, 12]. Digestion was done with 10 mL of Sulfuric Acid 98% + 3.5-4 g metal catalyst and K_2SO_4 follow the Merck, Kjeldahl Tablet, on less than 400 ^{o}C toward 0.2 – 0.5 g of sample. Netralization was done with adding 20 mL of NaOH 10 M on cool condition. Nesslerization and quantification of N follow the procedure of EPA 350.2 method [7].

Substance of $(NH_4)_2SO_4$ was heated on 110 ^{o}C for a long 1 hour before it was weighted [13]. Coloring substance on Nesslerization was done in the reaction tubes of 10 mL and six drops of Nessler reagent for standard curve. The reaction developed for a long 10 minutes.Analysis of uncertainty values of N measurement used regression equation between standard concentration of $(NH_4)_2SO_4$ 0.02 – 2.14 ppm and absorption of 425 nm spectrum. The statistic confidence was taken on 95% follow t distribution. Absorption value of λ=425 nm spectrum toward aliquot on more than value 0.5 for uncertainty 9.68%. Calculation of uncertainty used computer program.

III. RESULT AND DISCUSSION

3.1. Procedure of Nitrogen Analysis

Kjeldahl-Nessler Method is main stage series; digestion, neutralization, and Nesslerization. Respective stage in this research has procedures as is shown TABLE 2. Result of digestion stage is $(NH_4)_2SO_4$ and forming NH_3 on pH above 9.5 [7]. Reactor conditions on adding NaOH have attention to reaction that doesn't escape the vapor with providing cover for Kjeldahl flask. If doesn't like that than NH_3 substance will decreasing quantity. That stage is neutralization. Janssen and Koopman method add 20 mL NaOH 10 M that it is guaranteed to result NH_3. Those appropriate with [13] the color of Nessler reagent depend on alkalinity degree and the term of this is direct Nesslerization. Concentration of NaOH 10 M in the volume 20 mL has

shown pH 14. Reaction of escaping the ammonia follow [8] can be determined as indirect i.e. titration after NH_3 was reacted with borate ion $(NH_4)B(OH)_4$ as follows:

$$(NH_4)_2SO_4 + 2\ NaOH \longrightarrow Na_2SO_4 + 2\ H_2O + NH_3\uparrow$$

Table 2. Stages of Kjeldahl-Nesslerization Method for Analysis N Application toward Household Natural Organic Waste (Bio-Waste).

Stages	Procedure Modification	References
I. Digestion	- Adding 10 mL H_2SO_4 p.a. (*pro analysi*)	[6]
	- Using temperature < 400 ^oC with application Technique of Gerhardt KI 24 type of heater Gerhardt Manual on knob 50%	[6]
	- 3.5 – 4g metal catalyst of $CuSO_4.5H_2O$ p.a. and Se p.a. Adding K_2SO_4 p.a as salt mixture follow Merck	[11, 12]; Merck Catalog
II. Neutralization	- Adding 20 mL NaOH p.a 10 mol/L (M)	[6]
	- pH > 9,5	[7]
III. Nesslerization	- Decreasing concentration of $(NH_4)_2SO_4$	[7, 11]
	from digestion for spectrophotometry method	[7]
	- Adding the reagent of Nessler with composition 100 g HgI_2, 70 g KI + little pure water in flask. Adding to 500 mL NaOH (160g). Compound was solved until 1 L.	[7]

Concentration of NH_3 that resulted from digestion and neutralization influence the spectrophotometric technique (TABLE 2). However, Lambert-Beer law require aqueous concentration (*part per million* = ppm), in order to color that forming from Nessler reagent can be read the absorption of 425 nm spectrum. Complex substance of NH_2Hg_2IO is the end stage of transformation N-organic solid sample in the dry condition. Transformation of weight unit (mg) changed to concentration unit (mg/L or ppm) has shown the quantitative model follow the EPA 350.2.

3.2. Verification of Quantitative Model

Consideration of quantitative model is forming follow the unit of parameter model. Expression of measurement unit from (1) can be written:

$$\text{TKN, mg/L} = \frac{[\text{mg/L}] \times 1000.\ [\text{mL}]/[\text{mL}]}{[\text{mL}]} \quad \text{.......} \quad (2)$$

The model has balance value 1000 for mgL^{-1} unit of TKN from mL unit of sample. That equation form is appropriate with mathematical equation and can be done with conversion factor. The example of case above the balance value is 1000 and found as follows:

mg/L = ? mg/mL

= mg/L x 1

$$= \cancel{\text{mg/L}} \times \frac{\text{mg/1000mL}}{\cancel{\text{mg/L}}}$$

The desirable quantitative model to comparing weight with weight (w/w) need mg or g unit for nitrogen. Expression comparing in percentage (%) between nitrogen and sample can be written:

TKN, % = (mg N / mg sample) x 100

Value of absorption of 425 nm spectrum is transformed to concentration expression N mgL^{-1}. This form of unit per liter result value of weight (mg) as follow:

$$\text{TKN, mg} = [\text{mg}/\cancel{\text{L}}].\ [\cancel{\text{L}}]$$

Therefore the percentage can be determined:

TKN, % = {[mg] N / [mg] sample} x 100

Grounded on that unit concept of model proposed the equation that analogue with (1) as follow:

$$\text{TKN, \%} = \frac{A \times B}{\text{Sample weight}} \times 100 \quad \ldots\ldots (3)$$

Where; A = mg/L nitrogen that was found from standard curve
B = Total volume (L) of aqueous Kjeldahl digestion result

Unit expression of formula (3) such as the expression (2) can be written:

$$\text{TKN, \%} = \frac{[mg/L].[L]}{[mg]} \times 100 \quad \ldots\ldots (4)$$

Finding a problem is; does the volume that was used for absorption the spectrum influence in that quantification? Formula (3) and unit expression (4) is verification I show not to be a problem and action that is needed in procedure to know total volume of digestion result (B). It becomes a parameter in nitrogen quantification without volume calculation for reading absorption.

Next verification II shows volume for absorption the spectrum become a parameter in quantification, other B above. Assume the volume for reading absorption is 8 mL and drop with Nessler reagent, unit expression as follows below. Parameter of volume for reading absorption is expressed in the quantification formula mean nitrogen weight necessary to be known. Therefore it is needed compared factor between total volume (B) and the volume for absorption the spectrum (C) as multiply volume toward total volume, result that:

$$\text{TKN, \%} = \frac{[mg/L]\ \frac{[1/1000\ L]}{[mL]} \times \frac{[mL]}{[mL]}}{[mg]} \times 100$$

$$= \frac{[mg]\ [1/1000\ mL]}{[mg]} . \frac{B}{C} \times 100$$

Verification II show unit of volume; mL and therefore must be done in the procedure to measure the volume that use for absorption the spectrum. Next consequent of B and C parameters in the formula are known the compared value. Verification II show not to be efficient compare with verification I as formula 3 and unit expression 4 above. Evidence of quantification result of verification I and II as this follows:

Assume absorption of λ 425 nm spectrum has value = 1.2 and follow standard curve is found nitrogen concentration value per liter = 5.101 mg/L. Total volume (B) is 1,505.55 mL, volume for absorption the spectrum (C); 8 mL and sample weight is 0.5078 g, then;

Verification I: TKN, % = [(5.101 x 1,505.55)/ 507.8] x 100
= 1,512
Verification II: TKN, % = {[5.101 x 0.008 x (1,505.55/8)]/ 507.8} x 100
= 1.512
Verification I = Verification II

3.3. Comparing Measurement Precision

Accuracies test of nitrogen percentage on confidence 95% have value interval 0.234 – 0.316%. Analysis data is shown T ABLE 3.

Upper limit value of nitrogen content is 0.316 % and reference of protein content 2.1%[i] or nitrogen value 0.353 % [10]. Both result difference – 0.037%. Generally the information in internet, protein of *nasi* about 2 – 2.38%[ii] in Indonesia or nitrogen content 0.336 – 0.4 % and result difference – 0.02 – 0.08 %. But the

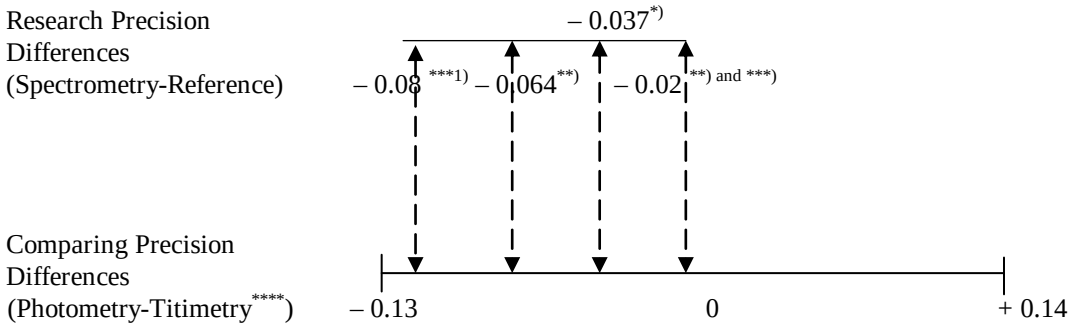
sample was analysed not long rice grain[iii]. The foreign information of protein content is 2.9%[iv] or nitrogen 0.487% in 128 g white *nasi*; grounded of USDA data about protein 2.01%[v]. That content of substance is influenced by the rice material like form and variety of *padi* among the sample that was used in analysis. Technique of cooked rice result the *nasi* influence of protein content too in every it's weight. Difference of precision toward value between Nesslerization and reference method of Kjeldahl using titimetry laid – 0.13 until + 0.14.

Table 3. Total Nitrogen Analysis Data of Municipal Household Bio-Waste: Cooked Rice.

Sample Weight*) (mg)	Absorption on λ_{425}	B (mL)	Regression Uncertainty with $t_{0.05\,(1)}$ (%)	TKN Value (%)
234.3	0.33	600	14.99	(0,275 ± 0,041)

*) Code 6011014

Comparing difference of precision is provided criterion; that the difference values of precision in this research there are same with compared value (Fig. 2). Both of resulted measurement value can be shown that respective accurate values have variation. Value in this research are – 0.08 until – 0.02; whereas compared value – 0.13 until + 0.14. The same values lay in the line with compared value. Therefore result of that comparing considers not different of its measurement precision.



*) Reference [10]
**) Reference of Information in *webpage of* foreign internet
***) Reference of Information in *webpage* of domestic internet
1) *Nasi* from medium rice grain
****) Reference [13]

Figure 2. Diagram of Precision Differences of Comparing Value of Nitrogen Content in White *Nasi* Material between Two Modifications of Kjeldahl-Nesslerization is. Adaptation from SNI 19-17025-2000 [14, 15].

3.4. Composition and Nitrogen Content in Bio-waste

Explanation above then EPA 350.2 model and modification Kjeldahl method can apply in determination of N percentage of municipal household solid waste. Applied Kjeldahl-Nesslerization method for nitrogen bio-waste analysis and value of the waste component is shown TABLE 3. Total % N value in column 2 dry basis grounded and quantity in wet basis can be calculated. Nitrogen percentage value of every sample is provided codes and cannot compare like in Fig. 2. However, every sample can be shown with its composition as in column 3 TABLE 3. The respective composition of bio-waste material is not same follow its weight.Origin bio-waste conditions from households have reasonable value of N percentage if to be seen the percentage nitrogen value of its component. This can explain as follows; The code 1103339 has nitrogen content (0.929 ± 0.069)% and reasonable if to be seen from % N value of its component. The sample code 1202002 has value (2.249 ± 0.168) % higher than its component. This may be caused egg white content in the higher number of weight. Those are different with the sample code 0802008 has value (1.041 ± 0.071) % lower than its egg white component 1.728% N. Those mean egg white in the bio-waste a little compare with sample code 1202002.

Table 3. Value of Nitrogen Percentage of Household Bio-waste with Wet Basis Correction and Its Respective Component Materials.

Codes	Material Samples *)	% of Total N and *Wet Basis* Correction	% N Component **)
6011014	*Nasi*	(0.275±0.041) 0.347	Cooked rice hull (*nasi*): 0.353 **1)
1103339	Young Jackfruit peel (*tewel*), Banana peel, Bamboo Vegetable, Broad bean, Garlic and its peel	(0.929±0,069) 0.114	Young Jackfruit: 0.32 Broad bean: 0.384 Garlic: 0.72 Banana and its peel: 0.72 – 0.96 ***)
0802008	Fruit and its peel Of cucumber and seed, Chicken Eggshell and egg White, Banana leaf (Steamed and coconut leaf rid)	(1.041±0.071) 0.135	Egg white: 1.728 Cucumber: 0.112 Soy bean sprout: 1.44 Banana leaf: 1.37 - 2.17 ***)
0903346	Zallaca (*Salak*) fruit and its peel, *Rambutan* fruit, Banana leaf (Steamed and coconut leaf rid) And it fruit peel, Chicken egg-Shell and its egg white	(0.544±0.043) 0.094	Egg white: 1.728 *Rambutan* Fruit: 0.144 Zallaca Fruit: 0.064 Banana leaf: 1.37 - 2.17; Banana peel 1.05 - 1.52 ***) *Ambon* Banana peel &*Raja*: 0.21 & 0.07****)
1202002	Onion peel and leaf Carrot, Broad bean Chicken Eggshell and Egg white	(2.249±0.168) 0.290	Egg white: 1.728 Broad bean: 0.384 Carrots: 0.192 Onion: 0.24
1003377	Banana leaf (not steamed), Leafy vegetable: leaf, stem And root; Carrot, *manesa* (Vegetable fruit), String bean, Broad bean	(2.215±0.165) 0.392	Broad bean: 0.384 Carrots: 0.192 Leafy vegetable: 0.41 String bean: 2.72 Banana leaf: 1.37 - 2.17 ***)
0503185	Tomato fruit, Potato peel, Condiment with big red chili (*sambal*), spinach stem, egg-Shell of chicken and its egg White, onion	(1.124±0.084) 0.131	Egg white: 1.728 Spinach: 0.56 Potato: 0.32 Red Chili: 0.16 Onion: 0.24
02033-4	Majority banana leaf (not Steamed), water lettuce Onion leaf, Slated boiled Fish (*pindang*) and its bones, Big Red chili 2.17 ***)	(1.512±0.091) 0.118	salted boiled fish: 4.8 Water Lettuce: 0.272 Onion leaf: 0.288 Red chili: 0.16 Banana leaf: 1.37 -

*) Sample weight >10% Waste weight every household
**) Protein factor 6.25 and 1) 5.95 [10]
***) From various sources [16]
****) [17]

Percentage of fast decomposition kinds of material and nitrogen content of organic waste samples are shown Fig. 3. Histogram show not always higher percentages of fast decomposition materials in bio-waste have nitrogen content those are higher too and show the opposite. Fast decomposition materials with lower % N

content mean the bio-waste has domination of carbohydrate. However, higher % N and lower fast decomposition materials was caused the bio-waste consist eggshell that with its egg white.

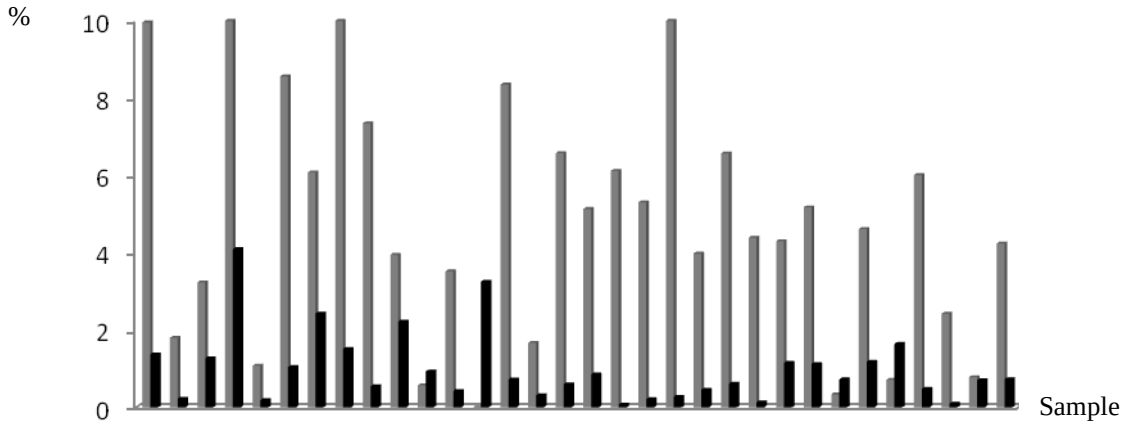


Figure 3. Histogram of Percentage Fast Decomposition (□) and N (■) in Bio-waste

IV. CONCLUSION

Transformation result of EPA 350.2 formula model and verification of its parameter unit is found Nesslerization quantification. Quantitative model showed more efficient procedure stage is

$$\text{TKN, \%} = \frac{\text{A x B}}{\text{Sample weight}} \text{ x } 100$$

Where; A = mg/L nitrogen that was found from standard curve
B = Total volume (L) of aqueous Kjeldahl digestion result

Quantitative model above has three parameters; first, nitrogen concentration in mg L^{-1} unit, total volume of N-organic digestion result in Liter (L) unit and weight of sample in mg unit. Kjeldahl-Nesslerization method and transformation of EPA 350.2 quantitative model for N percentage determination of bio-waste can be applied.

V. ACKNOWLEDGEMENTS

General Directorate of High Education of Education and Culture Ministry of Indonesian Republic and Coordinator of Private High Education at Zone VII East Java.

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