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DETERMINING CARBON DIOXIDE (CO₂) EVOLVED DURING THE DECOMPOSITION OF ORGANIC MATTER IN THE SOIL BY FLOW INJECTION ANALYSIS (FIA)

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ABSTRACT

A laboratory method to determine the carbon dioxide (CO₂) evolved during the decomposition of organic matter in the soil by Flow Injection Analysis (FIA) was observed. The objective of this experiment was to evaluate the accuracy of FIA to analyze CO₂ evolved from organic matter. The CO₂ gas was trapped by NaOH solution in an original designed incubation flask and its concentration was measured by FIA at 590 nm. A calibration curve was established using standard solutions of 0, 40, 80, 120, 160, and 200 mg CO₂ L⁻¹ prepared from NaHCO₃. The quadratic form $y = -0.0007x^2 + 0.983x + 17.4$ was obtained where y is the absorbance and x is the concentration of CO₂ with the correlation coefficient (r) of 0.9999. There was a rapid increase in CO₂ (more than 3.9 mg L⁻¹ min⁻¹) after the sample solutions made contact with the atmosphere which compelled the isolation of the samples and the atmosphere. Using FIA, the determination time of one sample was 30 seconds, faster than the other instruments nowadays available. The volume of solution needed for measuring the CO₂ was only 100 µL possible to measure a small volume of solution contain of CO₂. For application of the above method, half gram of 3 kinds of chicken manure (CM-1, CM-2, and CM-3), which were subjected to the differences in their C/N ratio (11.5, 8.54, and 4.79, respectively), were applied to 50 g of soil. The rate of CO₂ evolved from the soil applied by chicken manure was greatest in the first three days, then decreased gradually until the end of the 30-day incubation period. The cumulative amount of CO₂ during 30-day incubation was 2510, 4010, 6080, and 1460 mg kg⁻¹ in the soil applied by CM-1, CM-2, CM-3, and control, respectively. The cumulative amount of CO₂ correlated with the C/N ratio of chicken manure where the cumulative amount of CO₂ decreased as the C/N ratio increased. It was concluded that although there are some constraints in relation to the increasing CO₂ concentration, FIA is considerable as a convenient instrument for determining CO₂ evolved from the soil. The production of CO₂ is considerable for estimating the nitrogen mineralization since the relation between the amount of CO₂ and nitrogen mineralization during incubation has been given off.

Key words: Carbon dioxide (CO₂), C/N ratio, decomposition of organic matter, Flow Injection Analysis (FIA), trapping reagent

INTRODUCTION

Knowledge on the mechanism of CO₂ evolved from the soil is very useful in relation to the microbial activity in the soil [1], decomposition of organic matter [2], and soil acidification [3]. Decomposition of organic matter in the soil by the action of microorganism such as bacteria, fungi, and actinomycetes produce gases, resistant residues, and numerous other essential plant nutrient element in smaller quantities. In this process, much of the carbon is evolved into the atmosphere as carbon dioxide (CO₂). Some papers have described the relationship between CO₂ enrichment and plant activities, such as the increase of photosynthetic rates of leaves [4], the increase of yield [5], and enhanced nitrogen fixation [6]. Additionally, the concern over the increasing CO₂ level in the atmosphere has stimulated efforts to quantify both the CO₂ evolved from the soil surface and its change in the soil [7].

The quantity of CO₂ evolved from the soil can be measured either in the field, where it is subjected to natural conditions, or in the laboratory where the environment can be more exactly controlled. The method

chosen depends on the objectives of the experiment. Field measurements of CO₂ have been widely used to assess the general activity of below grown biomass as it is influenced by climate, physical, and chemical properties, and agricultural practices [8]. In the laboratory, either basal or stimulated soil respiration can be measured more accurately. Basal respiration has been widely used to study the influences of moisture, temperature, aeration, and other definable physical conditions on the rate of organic matter mineralization. The production of CO₂ can be made through the correlation with other physiological properties such as enzyme and the activity of micro flora [8].

Some methods have been used to measure the CO₂ evolved from the soils. These methods include trapping the CO₂ and measuring its concentration in the trapping reagent. Generally, the trapping reagent uses an aqueous solution of alkali (usually KOH or NaOH) or soda lime [9]. The presence of NaOH in the sample solution increases CO₂ concentration after making contact with the atmosphere. However, less data is available in relation to the increasing CO₂ concentrations after they made contact with atmosphere.

The CO₂ that has been trapped by the trapping reagent, is quantitatively measured by gravimetric analysis [10], titrimetric analysis [10;11], conducti-metric analysis [12], and gas chromatography [13]. However, among of these instruments, Flow Injection Analysis (FIA) has not been used yet to determine the CO₂ evolved from the soil.

The objective of this study was to evaluate the possibility of FIA for determining CO₂ evolved during the decomposition of organic matters in the soil under laboratory condition.

MATERIALS AND METHODS

Calibration curve of CO₂

FIA used in this experiment was FIAstar 5010, which consists of a flow injection analyzer with spectrophotometer, a controller, a sampler, and a recorder. The reagents used were free-CO₂ water as carrier (C), 0.05 M H₂SO₄ as reagent one (R1), and an indicator solution (R2). The indicator solution was prepared by dissolving 1 g of indicator mixture (tecolor, No. 5000-0295) in 5 ml of 0.1 M NaOH, then diluted with CO₂-free water to 200 ml. Ten ml of that solution were taken and 0.5 ml of 0.1 M NaH₂PO₄·H₂O was added before diluting to 500 ml CO₂-free water. In the FIA instrument, the sample solution is injected into C stream and mixed with R1. The joint stream passes along a PTFE membrane in a gas diffusion cell and the CO₂ forms diffuse through the membrane into R2. The resulting color change of R2 is measured by spectrophotometer at 590 nm (Figure 1).

Correlation between increasing CO₂ concentration and time during the solution making contact with the atmosphere before injecting by FIA

The FIA used in this experiment was combined with an automatic sampler to allow 40 samples to be sequentially analyzed. However, using NaOH as CO₂-trapping reagent, its exposure in the atmosphere containing CO₂ caused the increasing CO₂ concentration. To calculate the increasing CO₂ concentration in the NaOH solutions, two series of solutions contained of 0, 40, 80, 120, 160, and 200 mg L⁻¹ CO₂ were prepared from NaHCO₃ which were diluted in 0.04 and 0.2 M NaOH. The CO₂ concentrations were then analyzed by FIA 0, 5, and 10 min after exposing in the atmosphere.

Evaluating the capability of incubation flask to capture CO₂

An original incubation flask was designed to capture the CO₂ evolved from the soil under laboratory condition. The apparatus needed consists of a modified 200-ml incubation flask and a trapping system containing 4 ml NaOH (Figure 2). Aeration during incubation is by diffusion through a layer of granulated soda lime and 1 ml of 5 M sulfuric acid stored in glass column. The soda lime protects the atmospheric CO₂ from entering into the incubation flask while the sulfuric acid protects the CO₂ out of the flask. To evaluate the capability of incubation flask to capture CO₂, 1, 2,

5, 10, and 15 mg CO₂ prepared from NaHCO₃ were injected into the incubation flask. A 10 ml concentrated H₂SO₄ was injected into the flask to convert NaHCO₃ into CO₂ gas. The volatile CO₂ gas from the surface of the solution was captured by 4 ml of 1 and 5 N NaOH. The CO₂ concentration was detected periodically every 6 hours during the 2-day incubation period. The NaOH in the glass column of the incubation flask which had captured CO₂ was released to a 100-ml measuring flask containing about 80 ml CO₂-free water through a silicone pipe. After filling-up to 100 ml, the concentration of CO₂ in the solution was determined by FIA.

CO₂ evolved during the decomposition of organic matter in the soil. Organic matter used in this experiment were three kinds of chicken manure (CM-1, CM-2, and CM-3) obtained from the chicken manure companies. The chicken manure were milled to become powder and passed through a 1-mm sieve. The total carbon and nitrogen were 266 and 23.1, 293 and 34.3, and 313 and 65.3 g kg⁻¹ for CM-1, CM-2, and CM-3, respectively. Half gram of each of the chicken manure was mixed thoroughly with 50 g of air-dried soil and filled into the incubation flask. The soil used was Volcanic-ash soil with the following chemical properties: pH (H₂O) 6.1, EC 0.03 dS m⁻¹, total carbon 18.4 g kg⁻¹, total nitrogen 1.50 g kg⁻¹, CEC 6.23 cmol (+) kg⁻¹, and base saturation 113%. Distilled water equivalent to 60% of its water holding capacity was added to the soil. The incubation flask was then placed in a room temperature of about 25°C. The CO₂ evolved from the soil was periodically determined every 6 hour in the first day, every 12 hour in the second and third days, everyday in the 4th to 7th days, and once every 3 day until the 30-day incubation period.

RESULTS AND DISCUSSION

Calibration curve of CO₂

The absorbance of 0 to 200 mg L⁻¹ CO₂ of standard solutions ranged from 17.5 to 186 mA.U with the coefficient of variation (CV) ranging from 1.0 to 5.1% (Table 1). The absorbance of 0 mg L⁻¹ CO₂ of standard solution was higher than 0 mA.U as shown since the solution contained CO₂. The CO₂ may have been absorbed from the atmosphere by NaOH during the preparation of the solution. Efforts were made to minimize the contact between the solution and CO₂ in the atmosphere, such as using column containing soda lime, putting the solution in the sampler seconds before injecting by FIA, and using fresh reagents and standard solutions when measuring the CO₂. However, although only for a moment, there was still contact made between the solutions and the atmosphere. Therefore, the absorbance of standard solution of 0 mg L⁻¹ was higher than 0 mA.U. To reduce the amount of CO₂ in the samples, a blank treatment was included to subtract the CO₂ value from the samples.

The correlation between CO₂ concentrations and their absorbances was a quadratic of $y = -0.0007x^2 + 0.983x + 17.4$, where y is the absorbance measured by

FIA and x is the CO_2 concentration of the standard solutions (Figure 3) with the correlation coefficient (r) of 0.9999. The correlation coefficient of the calibration curve depended on any factors such as the pH of solution after mixing with R1 and the contamination of standard solution with CO_2 in the atmosphere. The pH of the sample stream after mixing with R1 was maintained below 3. When the pH of that stream was higher than 3 due to higher concentration of alkali in the trapping reagent, the concentration of R1 (H_2SO_4) was increased to neutralize the excess of alkali. Using fresh standard solution, a good calibration curve was obtained easily.

Correlation between increasing CO_2 concentration and time during the solution making contact with the atmosphere before injecting in the FIA

The increasing CO_2 concentration in the 0.04 M NaOH after the solution made contact with the atmosphere 5 and 10 min before injection in the FIA was 23.3 and 42.8 mg L^{-1} or 4.66 and 3.90 $\text{mg L}^{-1} \text{min}^{-1}$, respectively. In the same time interval, the increasing CO_2 concentration in the 0.2 M NaOH was 24.3 and 69.7 mg L^{-1} or 4.86 and 9.08 $\text{mg L}^{-1} \text{min}^{-1}$, respectively (Table 2). In low concentration of NaOH (0.04 M), the increasing CO_2 concentration in the second 5 min was lower than the first one, but in high concentration of NaOH (0.2 M), the value almost doubled. In low concentration of NaOH, the unreacted alkali is small causing the capability to capture CO_2 is weak and to be weaker in longer time. On the contrary, in high concentration of NaOH, the CO_2 concentration sharply increased in longer time indicating that the solution was very reactive. In choosing the NaOH concentration as the trapping reagent, the amount of CO_2 that will be trapped should be considered. When the amount of CO_2 production is predicted small, low concentration of trapping reagent is better. However, there is a time interval when sample solutions are laid in the sampler before injecting by FIA. By considering the sample-injection time and injector-washing time of 15 seconds, respectively, the reasonable time interval of sample-solution lay in the sampler before injecting in the FIA is a maximum of 15 seconds.

Capability of incubation flask to capture CO_2

In the first 6 hours, from 1 to 15 mg CO_2 injected into the incubation flask, 45.9 to 89.9% and 66.4 to 87.5% of CO_2 was captured by 4 ml of 1 and 5 M NaOH, respectively. There after, the CO_2 trapped by the alkali decreased sharply until the end of the 2-day incubation period (Table 3). At the end of the incubation period, 86.7 to 98.5% and 95.0 to 98.3% of CO_2 was trapped in the 1 and 5 M NaOH, respectively. The higher amount of CO_2 injected into the incubation flask tended to decrease the CO_2 trapped in the 1 M NaOH but not in the 5 M NaOH solution. The reduced amount of CO_2 trapped by the alkali in the higher amount of CO_2 was considered to be due to the decrease amount of unreacted alkali. In this experiment, the highest amount of CO_2 injected into the incubation flask was 15 mg and if all CO_2 was absorbed

by 4 ml of 1 and 5 M NaOH, the unreacted alkali was 91.47 and 98.3%, respectively.

The trapping mechanism can be modified to increase the amount of CO_2 trapped by the alkali, such as the strength of the alkali and the surface area of the alkali in the trapping tube. Using a stronger alkali, the percentage of CO_2 trapped will increase, but at the same time, its contact with the atmosphere is also affected a higher increasing the CO_2 concentration. Therefore, a strong alkali as the trapping reagent is preferred since the increasing CO_2 concentration after contact with the atmosphere is still low. It is possible to maintain 90% absorption rate if the unreacted alkali remained at least 80% with stronger alkali concentration. If the total amount of CO_2 is less than 10 mg , the concentration of 1 M NaOH is suggested [14].

Using FIA, the determination time of one sample was 30 seconds, faster than the other instruments nowadays available. The sample solution needed for determination of CO_2 was only 100 μL possible to measure a small volume of solution contain of CO_2 . Although there were some constraints in relation to the increase of CO_2 when it makes contact with atmosphere, FIA was considerable as a convenient instrument for determining CO_2 evolved from the soil.

CO_2 evolved during the decomposition of chicken manure in the soil

The rates of CO_2 evolved during the decomposition of chicken manure in the soil were greatest during the first and second three days of incubation. Thereafter, the rates decreased sharply until 9 days, then decreased slowly until the end of the 30-day incubation period (Figure 4). In the first 3 days, the rates of CO_2 evolved were 174, 379, 574, and 217 $\text{mg kg}^{-1} \text{day}^{-1}$ and after 30 days, the rates remained at 28.4, 30.6, 40.2, and 1.7 $\text{mg kg}^{-1} \text{day}^{-1}$ for the soil applied by CM-1, CM-2, CM-3, and without chicken manure application (control), respectively. In the first 3 days, the rate of CO_2 evolved from the soil applied by CM-1 was lower than the control.

The cumulative of CO_2 during the decomposition of chicken manure in the soil increased sharply in the first and second 3 days. Thereafter, the cumulative of CO_2 increased gradually until the end of incubation period. The cumulative of CO_2 during 30-day incubation was 2510, 4010, 6080, and 1460 mg kg^{-1} in the soil applied by CM-1, CM-2, CM-3, and control, respectively (Figure 5). In the first 3 days, the cumulative of CO_2 evolved from the soil applied by CM-1 was higher than control. High C/N ratio of chicken manure tended to produce CO_2 less than that of the lower one. In the first 3 days, the rate and cumulative of CO_2 evolved from the high C/N ratio of CM-1 in the soil was slowly because it lacks sufficient nitrogen for the microorganisms to use for increasing their number. In such condition, microorganisms immobilized nitrogen already in the soil for building proteins in new populations.

The C/N ratio of organic matter is to be an important factor affecting its decomposition in the soil. The

decomposition rate of organic matter decreases as the C/N ratio increase. Chicken manure with low of C/N ratio was easier to decompose by microorganisms because this material rich in nitrogen which is important for energy source of microorganisms. As result, the decomposition of a low C/N ratio of organic matter was faster than the higher one and produce much of CO₂. Some treatments can be done to encourage the decomposition process of high C/N ratio of organic matter such as composting process or addition of ni-

trogen fertilizer into the organic matter. Principally, the efforts are to decrease the C/N ratio of organic matter.

Measuring the production of CO₂ can be use to estimate the activity of microorganisms in the soil. The activity of microorganisms is directly proportional to their number present in the soil and produce CO₂ when they decompose organic matter. High activity is also accompanied by higher production of CO₂.

Table 1. Absorbance of CO₂ standard solutions measured by FIA.

Concentration of CO ₂ standard solution (mg L ⁻¹)	Absorbance			Mean ± CV %
	1	2	3	
0	18.5	16.5	17.5	17.5 ± 5.1
40	56.5	54.5	55.5	55.5 ± 1.6
80	90.5	90.5	92.0	91.0 ± 1.0
120	129	125	126	127 ± 1.7
160	157	160	150	156 ± 3.4
200	187	183	188	186 ± 1.4

Table 2. CO₂ concentration trapped in 0.04 and 0.2 M NaOH solutions with time after making contact with the atmosphere before injecting in the FIA.

CO ₂ concentration of standard solution (mg L ⁻¹)	CO ₂ concentration (mg L ⁻¹)			Increasing CO ₂ concentration (mg L ⁻¹)		
	Time interval (min)			Time interval (min)		
	0	5	10	0	5	10
Concentration of NaOH in the solutions: 0.04 M						
0	0.05	18.8	38.7	0.05	18.8	38.7
40	42.6	59.7	84.2	2.6	19.7	44.2
80	81.3	104	120	1.3	24.0	40.0
120	122	146	165	2.0	26.0	45.0
160	161	186	207	1.0	26.0	47.0
200	203	225	242	3.0	25.0	42.0
(Mean±standard deviation)				(1.7±1.1)	(23.3±3.2)	(42.8±3.2)
Concentration of NaOH in the solutions: 0.2 M						
0	1.50	21.9	45.0	1.521.9	45.8	40
80	86.9	114	163	6.934.0	83.0	120
160	162	183	243	2.0	23.0	83.0
200	204	224	279	4.0	24.0	79.0
(Mean±standard deviation)				(3.3±2.0)	(24.3±4.9)	(69.7±17.6)

Table 3. Percentage of CO₂ trapped in different concentrations of alkali.

Amount of CO ₂ injected into the flask (mg)	CO ₂ trapped (mg)					Total CO ₂ trapped	
	6	12	24	36	48	(mg)	(%)
1 M NaOH							
1	0.459 (45.9) ^a	0.201 (20.1)	0.139 (13.9)	0.075 (7.5)	0.076 (7.6)	0.950	95.0
2	1.193 (59.7)	0.329 (16.5)	0.114 (5.7)	0.037 (1.9)	0.114 (5.7)	1.79	89.4
5	4.49 (89.9)	0.038 (0.8)	0.089 (1.8)	0.154 (3.1)	0.152 (3.0)	4.93	98.5
10	7.63 (76.3)	0.636 (6.4)	0.190 (1.9)	0.204 (2.0)	0.152 (1.5)	8.81	88.1
15	11.6 (77.2)	0.302 (2.0)	0.343 (2.3)	0.229 (1.5)	0.543 (3.6)	13.0	86.7
					Mean	91.5	
5 M NaOH							
1	0.664 (66.4)	0.168 (16.8)	0.001 (0.1)	0.017 (1.7)	0.100 (10.0)	0.950	95.0
2	1.41 (70.7)	0.207 (10.4)	0.150 (7.5)	0.111 (5.6)	0.084 (4.2)	1.97	98.3
5	4.03 (80.6)	0.349 (7.0)	0.112 (2.2)	0.235 (4.7)	0.110 (2.2)	4.84	96.8
10	8.75 (87.5)	0.531 (5.3)	0.382 (3.8)	0.062 (0.6)	0.036 (0.4)	9.76	97.6
15	12.9 (85.7)	0.994 (6.6)	0.351 (2.3)	0.398 (2.7)	0.060 (0.4)	14.7	98.0
					Mean	97.1	

^a Figures in the parentheses indicate the percentage of CO₂

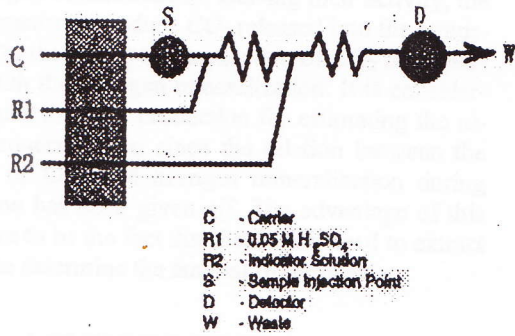


Figure 1. Schematic of solution flow in determination of CO₂ using Flow Injection Analysis (FIA)

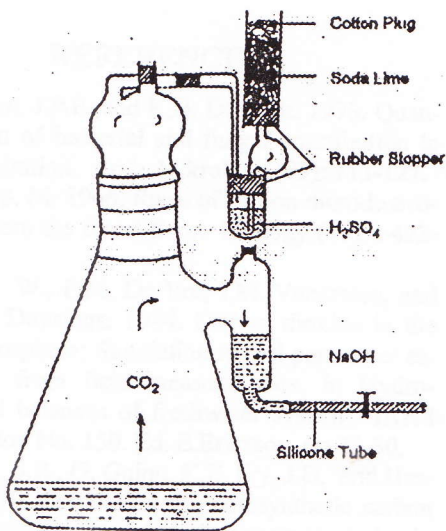


Figure 2. Incubation flask for measuring CO₂ production during manure decomposition

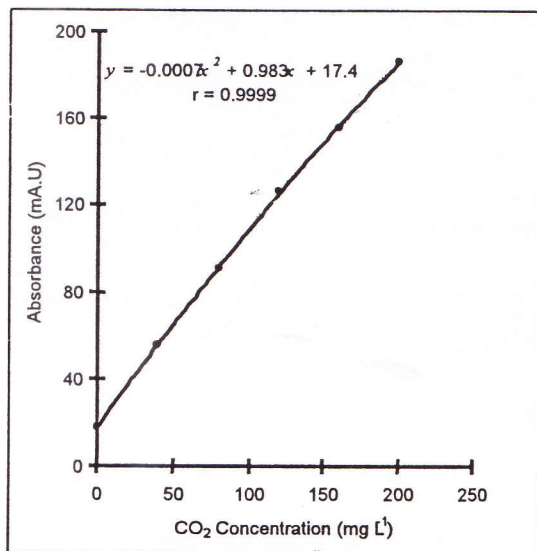


Figure 3. Correlation between CO₂ concentration and its absorbance measured by FIA

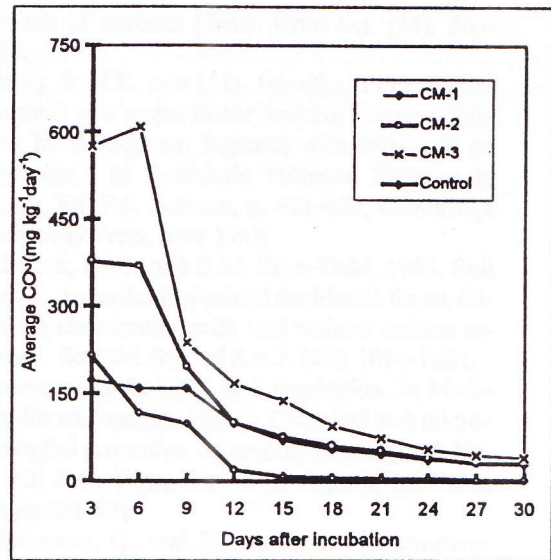


Figure 4. Rates of CO₂ evolved during the decomposition of chicken manure over 30-day incubation period

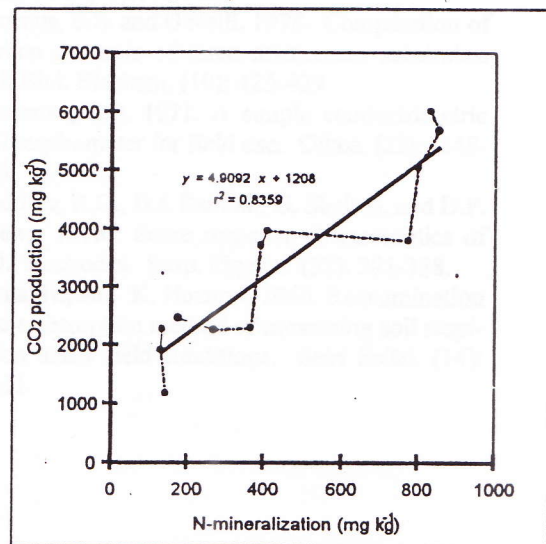


Figure 5. Cumulative CO₂ evolved during the decomposition of chicken manure over 30-day incubation period

Therefore, the production of CO₂ evolved from decomposition of organic matter reflected the activity of microorganisms in the soil.

The CO₂ evolved during the decomposition of organic matter in the soil can be used to estimate the nitrogen mineralization. Nitrogen mineralization is commonly estimated by incubation experiment. The incubated samples are then analyzed for the ammonium and nitrate-nitrogen. The incubation procedure has been considered to be the most satisfactory method for evaluating the availability of nitrogen to plants (Bremner, 1965).—Because the nitrogen released into the soil is originated from the body of microorganisms after die, the activity of microorganisms is responsible

for nitrogen mineralization. During their activity, the microorganisms produce CO₂ released into the atmosphere. On the result, the production of CO₂ is proportional with the nitrogen mineralization. It is considerable to use the CO₂ production for estimating the nitrogen mineralization since the relation between the amount of CO₂ and nitrogen mineralization during incubation has been given off. The advantage of this procedure to be the fact that there is no need to extract the soil to determine the amount of nitrogen.

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