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Enhancing Quality of Ultisols through Phototropic Bacteria

*Aidee Kamal Khamis[1], Ramlan Aziz[1], Siti Nazrah Zailani[2] and Firdausi Razali[2] *,*

[1] *Institute of Bio-Product Development, Universiti Teknologi Malaysia, 81310 Johor, Malaysia.*

[2] *Department of Bioprocess Engineering, Faculty of Chemical Engineering, University Teknologi Malaysia, 81310 Johor, Malaysia.*

* *Correspondent author: firdaus@cheme.utm.my*

Abstract

The agriculture sector in Malaysia relies heavily on chemical fertilizers to forecast their production yields. Use of the chemical fertilizers increases production cost, and prolonging their use destroys the natural soil ecosystem and deteriorates soil health. In this study, an attempt to improve the fertility of Malaysian soils known as Ultisols was done by applying phototropic bacterium, *Rhodopseudomonas palustris. R. palustris* is considered to be a "versatile bacterium". The bacterium has the ability to fix carbon dioxide to form biomass, converts nitrogen to ammonia, and produces hydrogen (as a by-product of nitrogen fixation). Experiments were carried out by introducing amounts of 1, 2.5 and 5 % v/w of *R. palustris* to the Malaysian Ultisols (*Bungor)* and they were allowed to ferment for 30 days under an anaerobic condition. Deionized water was poured to the soils to increase the soil moisture to have approximately 30% humidity. During the experiment, light exposure, temperature and moisture of soil were controlled. After the fermentation period, the elementary analysis was performed to quantify the C/N ratio, soil pH, cation exchange capacity (C.E.C) and soil electrical conductivity (EC). The analysis of CO_2 for O_2 uptake rate (OUR) was also recorded to monitor the *R. palustris* respiration in soil. Based on the findings, the optimum results obtained were pH 5.5, C/N ratio 3, C.E.C 38.62 meq/100g, EC 314 µS and OUR 1.59 x 10^{-5} moles of O_2/mass of soil (kg) x Time (h). Experimental results from this study have proven that the use of *R. palustris* had positively improved the Malaysian Ultisols quality in terms of nutrient content uptake capability.

Keywords: Ultisols, *R. palustris*, C/N ratio, C.E.C, Malaysia.

1. Introduction

Ultisols is one of the upland soil categories that is largely cultivated in Malaysia for use in the planting of crops such as palm oil, rubber, cocoa and orchard. Ultisols are naturally deeply weathered, acidic and have low buffering capacities. Although Ultisols have a good physical make-up, they are typically devoid of major nutrients such as calcium and potassium and have a low cation exchangeable capacity (1). They can be easily exhausted, and require more careful nutrient management as compared to other soils. Usually, a high amount of urea is used to increase the acidity of the Ultisols which is done by leaching base cations and nitrate (NO_3^-) and increasing active aluminum. Theoretically, a nitrification process occurs in the soil where the oxidation of 1 mol

NH_4^+ or NH_3 from urea would lead to the release of 2 mol H^+ into the soils (2).

$$NH_3 + O_2 \text{ ----> } NO_2^- + 3H^+ + 2e^- \quad (1)$$

$$NH_3 + O_2 + 2H^+ + 2e^- \text{ ----> } NH_2OH + H_2O \quad (2)$$

$$NH_2OH + H_2O \text{ ----> } NO_2^- + 5H^+ + 4e^- \quad (3)$$

$$NO_2^- + H_2O \text{ ----> } NO_3^- + 2H^+ + 2e^- \quad (4)$$

One of the advantages of *R. palustris* bacterium is that they can survive in both aerobic and anaerobic conditions. The bacterium is able to fix carbon dioxide to form biomass, converts nitrogen to ammonia and produces hydrogen (as a by-product of nitrogen fixation). The potential of *R. palustris* to produce hydrogen has also been reported (3) (4). The focus of this paper is the application of *R. palustris* bacterium in soils and their effects on the nutrient uptake. The chemical fertility of the soils monitored included carbon/nitrogen (C/N) ratio, cation exchange capacity (C.E.C), electrical conductivity (EC) and oxygen uptake rate (OUR).

Table 1. Characteristics of *Bungor* soil series.

Parameters	Amount
pH	4.10
C/N ratio	9.42
Cation exchange capacity (C.E.C), meq/100g	10.14
Electrical conductivity (EC), μS	100.2
Clay, %	47.0
Silt, %	9.0
Coarse sand, %	6.0
Fine sand, %	38.0

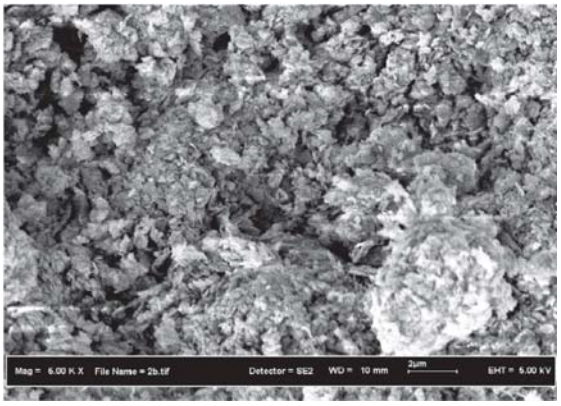


Figure 1. Field Emission Scanning Electron Microscopy (FESEM) image of *Bungor* soil with magnification view of surface 5.00K X.

2. Methodology

250 g *Bungor* soil (soils based on the Malaysian soil classification) was placed in a 1*L* conical flask. The number of colony forming unit (CFU) of *R. palustris* used in this experiment was 6 X 10^7 which was freshly prepared before being used. A composition of *R. palustris* with 1, 2.5 and 5% (v/w) was added to the soil in different conical flasks having the same aerobic condition. 100 ml of deionized water was added to each conical flask, to achieve a soil humidity of 30-40%. At the top of the conical flask, a set of respirometer was set up (Figure 2). This experiment was carried out for 30 days, with and without the presence of light. The light exposure is referred to a 60W light exposure in the laboratory. The soil pH, C/N ratio, C.E.C, and EC were analyzed after 30 days. During the experiments, OUR was consistently monitored and the results were collected daily.

2.2 Bacterium

The bacterium, *R. palustris* DSM 131 was purchased through DSM. The culture was stored in a container at temperature -80^{O}C. A typical length for this bacterium measures at 0.6-0.9 μm with optimum growth pH of between 6.5 and 7.5. The culture can reproduce

itself through a asymmetrical polar cell division in a budding mode. This culture is grown in an incubator at 30°C with white neon light for 2 days and shaken at 150 RPM. In this process, Nutrient broth was used as a growth media. (5).

2.3 Determination of soil pH

A pH meter (Model : Hanna HI8424) was used to measure the pH of soil suspension with soil to water ratio of 1:2.5 (w/v) (6).

2.4 Determination of soil C/N ratio

Soil C/N ratio was measured by using a CHN analyzer (Model: Thermo Finnigan, Flash EA 1112). 2.5-3.0 mg of samples were used for each measurement including the standard.

2.5 Determination of soil cation exchange capacity (C.E.C)

The soil CEC was determined using cations displacement by 1 M NH_4OAc buffered solution at pH 7. Air-dry soil (0.5 g) was shaken with 20 ml of NH_4OAc and centrifuged at 5000 rpm for 15 min in a 50 ml centrifuge tube. The supernatant was discarded, and the extraction was repeated. The sample was then washed twice with 10 ml methanol to remove the remaining ammonium. The samples were then mixed with 20 ml of 0.5 M $CaCl_2$ to return the ammonium held on the exchange sites. Following that, the solution was centrifuged at 5000 rpm for 10 min and the supernatant was poured into a 100 ml volumetric flask. This extraction was repeated and the volume of supernatant was increased to 100 ml by adding deionised water. The ammonium concentration was nally measured with an ammonium electrode (Jenway 3045 Ion Analyser, Jenway Ltd, England). In this study, it is assumed that the amount of NH_4^+ corresponds to the amount of negative charge occupied previously by K^+, Na^+, Mg^{2+}, Ca^{2+} and H^+ (7).

2.6 Determination of soil electrical conductivity (EC)

The soil electrical conductivity meter (Model: Camlab CW6220) was used to measure the EC of soil suspensions of a 1:5 soil to water ratio (8).

2.7 Determination of soil oxygen uptake rate (OUR)

A study of microbial activity of the soil was carried out using the respirometry method. A simple customized respirometer as shown in Figure 2 was designed to measure the O_2 uptake rate (OUR) or CO_2 released during respiration by bacterium. The respirometer consists of a sealed container with a linked specimen of the soil. The container is plugged with soda lime pellet which is connected to potassium hydroxide (KOH). The container is connected to a simple U-tube/manometer. The purpose of soda lime pellet is to adsorb the CO_2 released during respiration, while the U-tube/manometer measures the changes of the volume of gas produced during the activity. When the organism takes in O_2, it gives off an equal amount of CO_2. As CO_2 is absorbed by the soda lime, air is sucked from the U-tube/manometer to keep the pressure constant, hence displacing the liquid (8).

The relation of pressure and height of displacement of fluid in U-tube/manometer can be written as,

$$P = \rho g h \quad (5)$$

By assuming the ideal gas behavior for O_2, the ideal gas equation to determine the number of moles of O_2 consumed by microbes can be employed.

$$PV = nRT \quad (6)$$

where P is pressure (N/m^2), V is volume occupied by O_2 (m^3), n is number of moles of O_2 (gmol), R is gas constant, and T is temperature during the respiration. The fluid volume in the U-tube/manometer, V can be calculated by the displaced volume in the U-tube/manometer

$$V = \pi r^2 h \quad (7)$$

where r^2, is the square internal radius, and h is the height of the fluid level. By rearranging equations (6) and (7), with assumption 1 mole O_2 uptake equals 1 mol CO_2 emit, OUR is equivalent to

Moles of O_2/mass of soil (kg) x Time (h) (8)

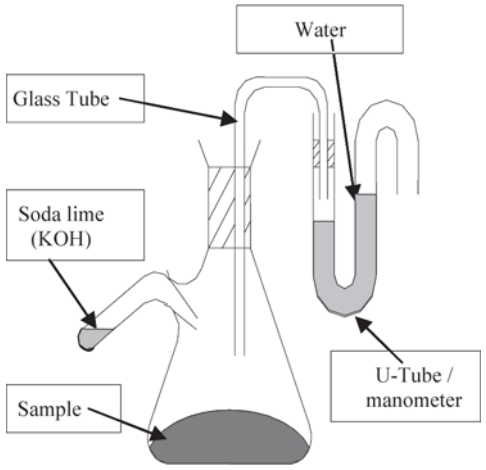


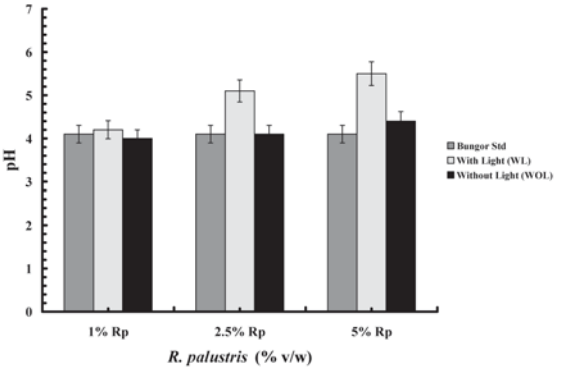
Figure 2. 2D-Schematic diagram of the respirometer.

3. Results and Discussion

3.1 Effect of *R. palustris* on soil pH

Figure 3 shows the soil pH with application of *R. palustris* after 30 days. The experiments were carried out with and without light exposure. The value of pH for the control soil (without the bacterium and without exposing to light) was not significantly different after being left for 30 days. Even with the addition of 1 % *R. palustris*, with and without light presence, the soil pH did show have any substantial difference from the control soil. However, a significant reduction of acidity (from pH 4.1 to 5.1) was observed for the soil sample with 2.5 % (v/w) *R. palustris* after having been exposed to light. The best condition to increase the pH of the soil towards neutralization (pH 5.5) would be to leave the soil for 30 days with an addition of 5% (v/w) *R. palustris* and have the soil exposed to light.

The results in Figure 3 show that with the presence of light, *R. palustris* is effective for converting H^+ to hydrogen gas as a result of H^+ from soil. As the H^+ is reduced, the pH value is increased, resulting in the reduction of acidity (10).

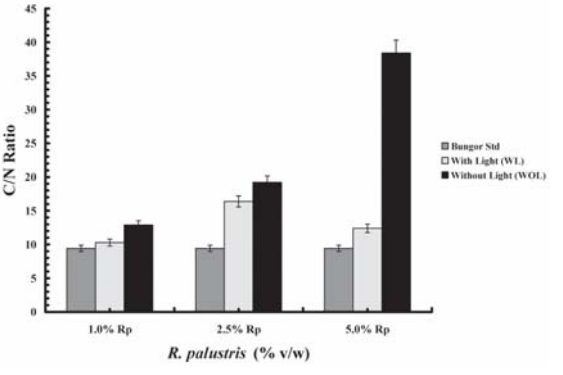


#Note : Results are the averages of three independent experiments ± SD. P<0.05 compared to (+) *Bungor* Std soil.

Figure 3. Effect of *R. palustris* on soil pH.

3.2 Effect of *R. palustris* on soil C/N ratio

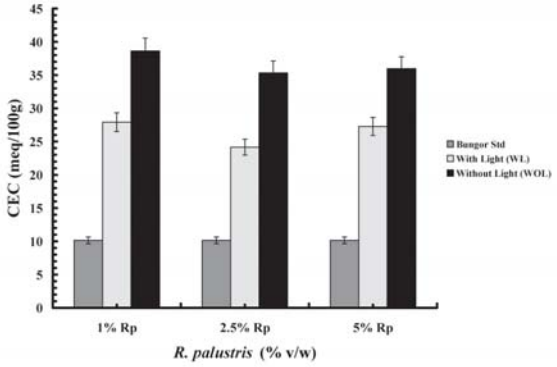
The C/N ratio values of fermented soil are shown in Figure 4. The value of C/N ratio with 1% *R. palustris* added to the soil, had slightly increased from 9.42 to 10.30 after the exposure to light (WL), and rose from 9.42 to 12.90 without the exposure to light (WOL). The amount of C/N ratio gradually increased with the increasing amounts of *R. palustris* from 1% to 2.5% v/w. The C/N ratio for soils with light exposure (WL) increased from 10.3 to 16.4 and had 12.9 to 19.2 for soils without light exposure (WOL).



#Note : Results are the averages of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.

Figure 4. Effect of *R. palustris* on soil C/N ratio.

Analysis of the results from the C/N ratio has led to a conclusion that *R. palustris* could adjust the soil acquisition ratios according to the composition of resources consistently without the presence of light. This soil acquisition ratio is represented as the general soil nutrient cycling of Carbon (C), Nitrogen (N), and Phosphorus (P) in the soil. With the amount of C/N ratio 9.42 existing in *Bungor* soil, the *R. palustris* is capable of using the carbon and nitrogen as a food sources to survive. An increase of C/N ratio is good for maintaining the community of *R. palustris* itself and access of the C/N ratio can be used by other microorganism communities in the soil (11). By adding 5% of *R. palustris* and without any light exposure (WOL) condition, the optimum C/N value achieved was 38.8.



#Note : Results are the averages of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.

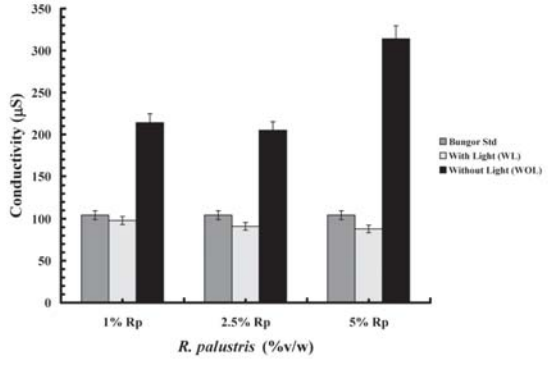
Figure 5. Effect of *R. palustris* on soil C.E.C.

3.3 Effect of *R. palustis* on soil cation exchange capacity (C.E.C)

The C.E.C of *Bungor* soil increased tremendously from 10.14 meq/100g to 27.92 meq/100g with the exposure to light (WL) and increased from 10.14 meq/100g to 38.62 meq/100g for soils without exposure to light (WOL) after the addition of 1% *R. palustris* (Figure 5). The C.E.C rise was related with the reaction of *R. palustris* with a variety of charges mineral such as Ca^{+}, Mg^{2+}, K^{+} and Na^{+}. However, there were no significant or different increments of C.E.C values in this experiment, even though the amount of *R. palustris* was increased from 2.5% to 5%, and placed in environments with or without the presence of light.

3.4 Effect of *R. palustris* on soil electrical conductivity (EC)

Figure 6 shows that the applications of 1, 2.5 and 5% of *R. palustris* gave significantly different results of EC values as compared to the EC values of the standard soils for experiments conducted without the presence of light. The application of *R. palustris*, even at low percentage, was able to ameliorate the *Bungor* soil electrical conductivity under the condition where there is an absence of light. The highest conductivity, 314 mS was achieved on day 30 after the application of 5% of *R. palustris*. These conductivity results show that increasing amounts of organic acid were secreted by *R. palustris* (13, 14).



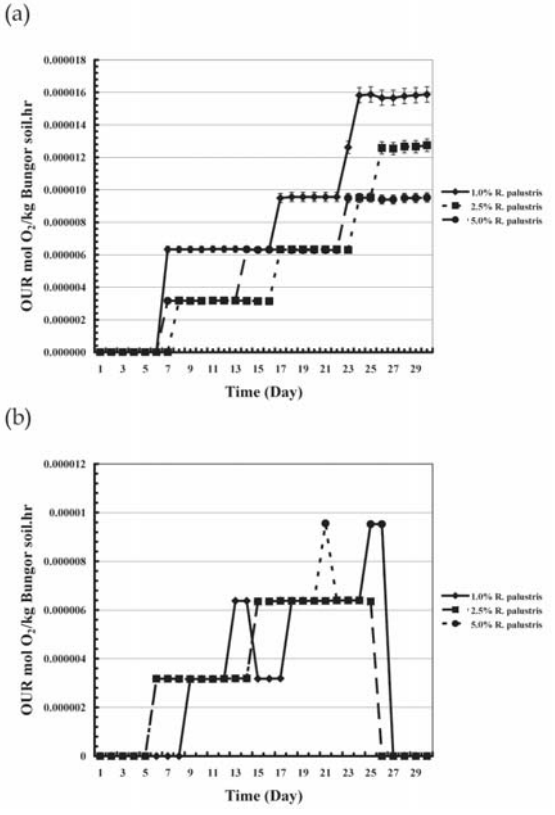
#Note : Results are the averages of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.

Figure 6. Effect of *R. palustris* on soil electrical conductivity (EC).

3.5 Effect of *R. palutris* on soil oxygen uptake rate (OUR)

The results of the respiration of the bacterium in *Bungor* soils in relation to time and bacteria treatment are presented cumulatively over a 30 day period as shown in Figure 7 (a) shows that by increasing the amount of *R. palustris* from 1 to 5%, with the presence of light, the OUR

was 40% lower after the 30 day duration. Figure 7 (b) shows that with the absence of light, the highest OUR was achieved when 5% *R. palustris* was used, and the respiration activity appeared to have been completed within 27 days. From this result, it can be concluded that the best condition used for *R. palustris* to have a complete respiration process on the field is by using an amount of 5% v/w with the absence of light condition.



#Note : Results are the averages of three independent experiments ± SD. P<0.01 compared to (+) *Bungor* Std soil.

Figure 7. Effect of *R. palustris* on soil oxygen uptake rate (OUR). (a) With exposure to light, WL (b) Without exposure to light, WOL.

The OUR results show that *R. palustris* without the presence of light, was able to provide better and higher respiration activities as compared to the experiment that had the presence of light. This respiration activity was parallel and in accordance with the results of C/N ratio in the soil (see Figure 4). The bacterium grew until carbon and/or nutrients limited further growth and reached a maximum peak as reported elsewhere (15, 16).

4. Conclusion

All the experimental results have demonstrated that *R. palustris* has the capability to alter the chemical fertility of *Bungor* soil. The effect on pH of the soil condition class was an upgrade from 4.1 (extremely acidic) to 5.5 (strongly acidic). The C.E.C value of the soils was elevated from a low (6-12 meq/100g) to high range (25-40 meq/100g). The higher values of C/N ratio, EC and OUR obtained signified that the nutrient contents have been improved as well as nutrient uptake capability of the soils. The effect of the light exposure studied in this work may indicate as to whether the situation of day or night will affect the function of *R. palustris* which would alter the specific soil parameters. The research has shown that the soil treatment using this bacterium has a great potential as it could be used to utilize and improve fertility of Ultisols.

5. Acknowledgement

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