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Chlorella Residue Functions as a Bio-Stimulant to Promote Plant Growth and Improve Soil Fertility

Asuka Okazaki[1], Toru Mizoguchi[2], Zakirul Islam[1], Quoc Thinh Tran[1], Motoki Kubo[1*]

[1]Laboratory of Bioengineering, Department of Biotechnology, Graduate School of Advanced Life Sciences, Ritsumeikan University, Shiga, Japan
[2]Production and Development Department, Sun Chlorella Co., Ltd., Kyoto, Japan
Email: *kubo@sk.ritsumei.ac.jp

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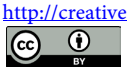


Abstract

Chlorella residues are currently underutilized. Therefore, in this study, we analyzed the nutritional components of *Chlorella* residue, and investigated its potential use as an organic fertilizer/bio-stimulant. Composition analyses revealed that the *Chlorella* residue contained a substantial amount of nitrogen (97,910 mg/kg), and significant quantities of secondary macronutrients, such as calcium (4300 mg/kg) and magnesium (9700 mg/kg), and micronutrients, such as iron (1850 mg/L) and manganese (359 mg/kg). The application of *Chlorella* residue to soil resulted in increased soil bacterial biomass. When *Chlorella* residue was added to the soil at a rate of 0.5% or 1.0% (w/w), the fresh weights of *Brassica rapa* and *Spinacia oleracea* were significantly increased. Furthermore, the application of *Chlorella* residue to the soil of *B. rapa* suppressed the reduction of the microbiome caused by clubroot disease and decreased the clubroot disease index. Therefore, *Chlorella* residue can be included in organic fertilizers that effectively improve soil nutrient contents, promote plant growth, and reduce the incidence of disease.

Keywords

Bacterial Biomass, Bacterial Diversity, *Chlorella* Residue, Clubroot Disease, Plant Disease

1. Introduction

The contents of major elements for plant growth such as nitrogen, phosphate, and potassium in soil are easily controlled through the addition of chemical fertilizers. Such fertilizers are usually applied in an inorganic form and are highly water

soluble, so their application is an efficient method to promote plant growth. The use of chemical fertilizers has significantly contributed to increases in the amounts of agricultural products [1] [2]. However, the excessive use of chemical fertilizers has led to a decline in soil fertility and water quality [3]-[5]. Furthermore, the mineral content in crops and vegetables has decreased in recent years because of the continuous use of chemical fertilizers [6] [7]. The long-term use of chemical fertilizers has gradually led to a decrease in the contents of secondary elements and micronutrients in agricultural fields.

In contrast, the application of organic fertilizers is believed to contribute to increased contents of secondary elements and micronutrients in the soil. However, in recent times, livestock feed has become dependent on crops cultivated with chemical fertilizers, leading to the depletion of secondary elements and micronutrients in livestock manure [8]. In fact, one study found that there was no significant difference in mineral content between vegetables grown using chemical fertilizers and those grown using organic fertilizers [9].

Members of the *Chlorella* genus are single-celled green algae that thrive in freshwater. They contain not only essential elements such as carbohydrates and proteins, but also minerals [10]. As a result, *Chlorella* is extensively used as a health supplement worldwide [11].

To enhance nutrient absorption for humans, beneficial components are extracted from cultured *Chlorella* cells [12]. This process generates a large amount of *Chlorella* residue, but most of it is discarded. In this study, we analyzed the composition of *Chlorella* residue and investigated its potential as a fertilizer to improve soil fertility.

2. Materials and Methods

2.1. Soil Preparation and *Chlorella* Residue Use

The base soil was prepared by mixing vermiculite (Kanuma Kosan, Tochigi, Japan), decomposed granite soil (Kohnan Shoj, Osaka, Japan), black soil (Tachikawa Heiwan Nouen, Tochigi, Japan), and peat moss (Kanuma Kosan) at a ratio of 5:3:1:1 (v/v) [13]. Cow manure, chicken manure, rice bran, oil cake, soybean meal, and bone meal were used as organic fertilizers, and the amounts added were 38.8 g, 3.9 g, 7.8 g, 1.9 g, 1.9 g, and 1.2 g, respectively, per kg base soil followed by previously developed fertilization method [13]. Chemical fertilizers (Kuki-hiryou, Mie, Japan) was added at a rate of 1.33 g per kg base soil. A specimen of *Chlorella* residue was purchased from Sun Chlorella Co., Ltd. (Kyoto, Japan), and was composed of four species of *Chlorella* (*Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlorella ellipsoidea*, and *Chlorella regularis*). The *Chlorella* residue was applied to both soil types at a concentration of 0%, 1%, 5 and 10%. The two types of prepared soil were adjusted to 30% (w/w) water content. The o soils were stored for 1 week at 23˚C prior soil bacterial biomass analysis. The soil added to cylindrical pots (10.5 cm in diameter, 22 cm in height) to a soil depth of 20 cm for plant cultivation.

2.2. Plant Cultivation

In this study, the plant species *Brassica rapa* var. periviridis and *Spinacia oleracea* L. were used to observe the effect of *Chlorella* residue on plant growth. The seeds were purchased from Takii & Co., Ltd. (Kyoto, Japan) and were sown in red clay soil. Seven days of 2 seedlings were transplanted in each pot at different application rate (0%, 0.5%, and 1%, v/v) of *Chlorella* residue and cultivated for 4 weeks in a plant growth chamber (23˚C, light: 12 hours, dark: 12 hours) [14]. The water content of soil was maintained at 30% during cultivation period. After 4 weeks of cultivation, plant growth was measured in terms of fresh shoot weight. The experiment was conducted at triplicates.

2.3. Preparation Pathogenic Soils and Plant Cultivation

To prepare pathogenic soil, resting spores of *Plasmodiophora brassicae* were obtained from the infected roots of *B. rapa*, and a spore suspension was prepared to create pathogenic soil. Infected *B. rapa* roots were carefully extracted and washed, and the galls were collected. The galls were then crushed at a ratio of 1 g galls:1.5 mL water using a mixer. The resulting suspension was filtered through 500- and 100-mesh sieves, and then the filtrate was centrifuged (500 rpm for 5 min) to separate the debris. The supernatant was subjected to a second centrifugation step (1000 rpm for 10 min.) and the pellet containing resting spores was suspended in distilled water. This purification process was repeated three times to ensure spore purity. Storage buffer (Hoagland's solution) was added to the final pellet to inhibit spore germination. The number of resting spores in the suspension was determined using a hemocytometer under a microscope (BX-50, Olympus, Tokyo). The spore suspension (4.0×10^8 spores/mL) was stored at 4˚C.

Pathogenic soils containing chemical and organic fertilizers were created by adding the resting spore suspension. The pathogenic soils were prepared with a spore concentration of 5×10^5 spores/g-soil. Chlorella residue was added a concentration of 0.5% (v/v) in both chemical and organic pathogenic soil. Seven days of 2 seedlings of *B. rapa* were transplanted in each pot and cultivated for 4 weeks in a plant growth chamber (23˚C, light: 12 hours, dark: 12 hours). The water content of soil was maintained at 30% during cultivation period.

2.4. Evaluation of Disease Index

Disease indexes (DI) were evaluated based on the infection status of root (gall formation) to assess the severity of clubroot disease. The disease status of the roots was categorized into five classes (Class 0: no symptoms, Class 1: small galls only on the lateral roots, Class 2: small galls on the taproot, Class 3: large galls on the taproot, but lateral roots unaffected, Class 4: galls on the entire root).

The *DI* was calculated for each soil treatment using the following equation:

$$DI = \frac{\left(1n_1 + 2n_2 + 3n_3 + 4n_4\right)}{4N_t} \times 100$$

where n_1 - n_4 is the number of plants in each class, and N_t is the total number of plants in the treatment.

2.5. Analytical Methods

The TC (total carbon) concentration was determined using the total organic carbon analyzer (TOC-VCPH, Shimadzu, Kyoto, Japan). To determine TN (total nitrogen), TP (total phosphorus), and TK (total potassium) contents, soil samples were extracted using $CuSO_4 \cdot 5H_2O$, H_2SO_4, and H_2O_2, respectively, at 420˚C [15]. Subsequently, the TN and TP concentrations were assessed followed by our previous method [8]. The TK, TNa (total sodium), TCa (total calcium), TMg, (total magnesium), TFe (total iron), TMn (total manganese), TZn (total zinc), and TCu (total copper) concentrations were measured by atomic absorption spectrophotometry using a Z2300 instrument (Hitachi, Tokyo, Japan). The contents of ammonia nitrogen (NH_4-N) and nitrate nitrogen (NO_3^- - N) were analyzed according to our previous study [16] after extracting soil samples with 1 M KCl. Total bacterial biomass was determined by quantifying environmental DNA (eDNA) extracted using the slow-stirring method [17] [18]. Following eDNA separation by agarose gel electrophoresis, the eDNA band was quantified using Kodak 1D Image Analysis Software (Kodak, Rochester, NY, USA).

2.6. PCR-DGGE Analysis

PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis) was conducted to investigate the effect of chlorella residue on bacterial diversity in the pathogenic chemical and organic soil. The 16S rRNA bacterial gene was amplified using the primer pair DGGE-F (5'-CGCCC GCCGC GCCCC GCGCC CGTCC CGCCG CCCCC GCCCG CCTAC GGGAG GCAGC AG-3') and DGGE-R (5'-CCGTC AATTC CTTTG AGTTT-3') [19] [20]. The PCR reaction mixture (50 μL) consisted of 0.01 ng/μL of DNA template, 1.5 U rTaq DNA polymerase, 5.0 μL 10× buffer, 5.0 μL 2 mM dNTPs, 3.0 μL $MgCl_2$, and 2.0 μL of each 10 mmol/L primer. The DNA polymerase, dNTPs, and PCR buffer were obtained from TOYOBO (Osaka, Japan), and all primers were synthesized by Sigma-Aldrich (Tokyo, Japan). The thermal cycling program for PCR consisted of initial denaturation at 95˚C for 1 min, followed by 35 cycles of denaturation at 95˚C for 1 min, primer annealing at 55˚C for 30 s, and extension at 72˚C for 1 min, with a final extension at 72˚C for 5 min. Subsequently, the amplified 16S rRNA bacterial genes were subjected to denaturing gradient gel electrophoresis (DGGE) analysis using the D Code System (BioRad Laboratories Inc., Hercules, CA, USA). A total of 20 μL PCR product was loaded onto an 8% (w/v) polyacrylamide gel with a denaturing gradient ranging from 27.5% to 67.5%. The gel was run in 1× Tris-acetate EDTA buffer at a constant voltage of 70 V at 60˚C for 15 hours. After electrophoresis, the gel was stained with ethidium bromide for 30 min and then rinsed with distilled water.

3. Results

3.1. Analysis of *Chlorella* Residue

To investigate the effects of *Chlorella* residue as a bio-stimulant, its composition was analyzed (Table 1). The *Chlorella* residue contained a range of nutrients. The concentrations of the primary macronutrients such as TN, TP, and TK were high at 97,910 mg/kg, 11,760 mg/kg, and 5500 mg/kg, respectively. However, the concentrations of inorganic nitrogen, such as NO_3^--N (92 mg/kg) and NH_4-N and (160 mg/kg), were relatively low. These results indicate that *Chlorella* residue contains a large amount of organic nitrogen.

The concentrations of TMg and TCa were 4300 mg/kg and 9700 mg/kg, respectively. The *Chlorella* residue was also rich in micronutrients; the TFe concentration was 1850 mg/L and the TMn concentration was 359 mg/kg. The low C/N ratio (5.1) confirmed the suitability of *Chlorella* residue as an organic nitrogen fertilizer. Furthermore, the *Chlorella* residue contained substantial amounts of secondary macronutrients and micronutrients.

3.2. Effects of *Chlorella* Residue on Soil Microorganisms

The addition of organic materials to soil affects the microbial community. We analyzed the bacterial biomass in the organic soil after adding *Chlorella* residue (Table 2), and found that the bacterial biomass was increased by 2.44 times after addition of Chlorella residue at 1% w/w, and by 2.13 times after addition of Chlorella residue at 5% w/w. However, the addition of Chlorella residue at a higher concentration (10% w/w) did not lead to an increase in bacterial biomass. Thus, the lower concentrations of *Chlorella* residue had positive effects on the bacterial biomass in the soil.

3.3. Effect of *Chlorella* Residue on Plant Growth

The effects of *Chlorella* residue on plant growth were investigated using *B. rapa* and *S. oleracea* (Figure 1 and Figure 2). When *Chlorella* residue was added to the soil at 0.5% or 1.0% (w/w), the fresh weight of *B. rapa* was increased by 1.64 times and 1.75 times, respectively (Table 3), and the fresh weight of *S. oleracea* was increased by 1.25 times and 1.55 times, respectively (Table 4). These results indicate that *Chlorella* residue can function as an organic nitrogen fertilizer and/or biostimulant in soil.

3.4. Inhibition of Clubroot Disease by *Chlorella* Residue

The inhibitory effects of *Chlorella* residue on clubroot disease were investigated (Figure 3). First, we compared the DI of clubroot disease between plants grown in soil with chemical fertilizer and those grown in soil containing organic fertilizer. In the plants growing in soil with chemical fertilizer, the DI was 100%, while in those growing in soil with organic fertilizer, the DI was 40%. This difference was related to differences in microbial biomass between the two soils. A similar

Table 1. Composition of *Chlorella* residue.

Component	Concentration (mg/kg of dry *Chlorella* residue)
Total carbon (TC)	502,460
Total nitrogen (TN)	97,910
Total phosphorus (TP)	11,760
Total potassium (TK)	5500
C/N ratio	5.13
Nitrate nitrogen (NO_3^- - N)	92
Ammonia nitrogen (NH_4-N)	160
Phosphoric acid (H_3PO_4)	5430
Sodium (Na)	423
Calcium (Ca)	9700
Magnesium (Mg)	4300
Iron (Fe)	1850
Manganese (Mn)	359
Zinc (Zn)	13.4
Copper (Cu)	3.1

Table 2. Effect of addition of *Chlorella* residue on soil bacterial biomass.

Chlorella residue (%)	Bacterial biomass ($\times 10^8$ cells/g)
0	9.9
1	24.2
5	21.1
10	12.3

Table 3. Effect of addition of Chlorella residue on plant growth (*B. rapa*).

Chlorella residue (%)	Fresh weight (g)	Ratio of fresh weight (%)
0	70.3 ± 4.7	100
0.5	90.7 ± 4.2	125
1	110.1 ± 4.9	155

Different superscript letters within a column indicate significant differences (Tukey's post-hoc test; $p < 0.05$) ($n = 3$).

Table 4. Effect of addition of Chlorella residue to soil on plant growth (*S. oleracea*).

Chlorella residue (%)	Fresh weight (g)	Ratio of fresh weight (%)
0	45.8 ± 3.2	100
0.5	75.2 ± 4.1	164
1	80.3 ± 3.9	175

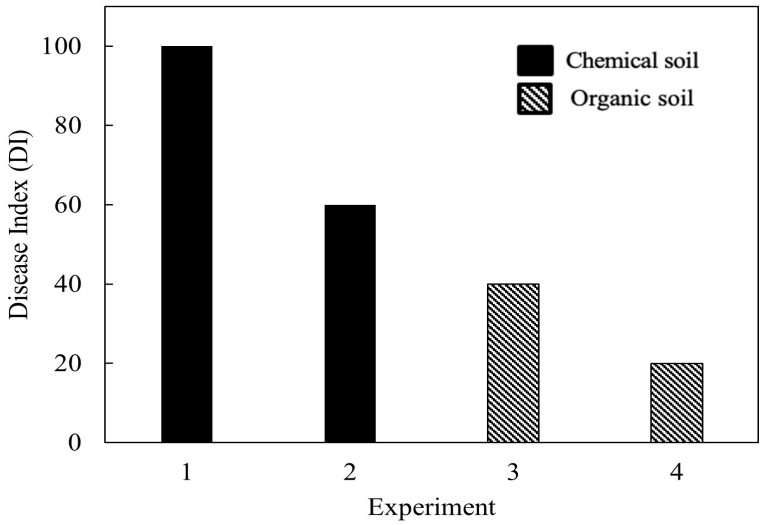
Different superscript letters within a column indicate significant differences (Tukey's post-hoc test; $p < 0.05$) ($n = 3$).

A: 0%, B: 0.5%, C: 1.0%.

Figure 1. Effect of addition of *Chlorella* residue to soil at three concentrations on plant growth (*B. rapa*).

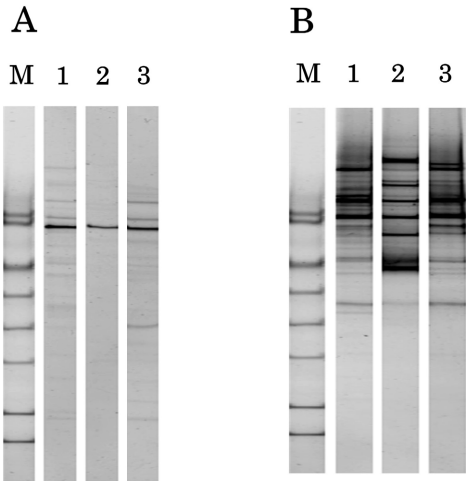
A: 0%, B: 0.5%, C: 1.0%.

Figure 2. Effect of addition of *Chlorella* residue to soil at three concentrations on plant growth (*S. oleracea*).



1: Chemical soil, 2: Chemical soil + *Chlorella* residue, 3: Organic soil, 4: Organic soil + *Chlorella* residue.

Figure 3. Effect of addition of *Chlorella* residue to soil on disease index of clubroot disease in *B. rapa.*



M: Marker, 1: Non-pathogenic soil, 2: Pathogenic soil, 3: Pathogenic soil with *Chlorella* residue.

Figure 4. Bacterial diversity after addition of *Chlorella* residue in chemical (A) and organic (B) soil.

trend was observed in the organic soil, with a decrease in the DI from 40% to 20% upon addition of *Chlorella* residue. These results suggest that *Chlorella* residue positively affects the microbial community in the soil.

Next, we determined the effect of adding *Chlorella* residue on bacterial diversity in soils with chemical and organic fertilizers (**Figure 4**). The bacterial diversity is reduced in both pathogenic chemical and organic soil. When *Chlorella* residue was added to both soils, the bacterial diversity returned to almost the same level as in non-infected soils. These results show that the addition of *Chlorella* residue, *i.e.*, organic matter rich in nitrogen and mineral components, to the soil led to increased microbial biomass and bacterial diversity. The addition of *Chlorella* residue also contributed to increased plant yield and the suppression of clubroot disease.

4. Discussion

Chlorella is used as a food source worldwide because it is rich in nutrients [21]. However, most of the residue left after extracting valuable components from *Chlorella* is discarded, and few studies have explored its composition and potential applications.

Our results show that *Chlorella* residue contains a significant amount of nitrogen (approximately 15%). Additionally, the C/N ratio of *Chlorella* residue is 5.1, indicating that it has a significantly higher proportion of nitrogen than other materials commonly used as organic fertilizers such as cow manure (C/N = 17.7) and soybean waste (C/N = 12.5) [22] [23]. The application of *Chlorella* residue to the soil as an organic fertilizer resulted in a notable increase in soil bacterial biomass, and significantly improved nitrogen circulation activity. These results indicate that the nitrogen components in *Chlorella* residue are suitable for the growth of soil bacteria, thereby contributing to increased bacterial biomass in soil. The

increased bacterial biomass appeared to activate bacteria associated with nitrification in the soil.

Our analyses indicate that *Chlorella* residue also contains significant amounts of secondary elements and micronutrients, such as calcium, magnesium, iron, and manganese. Thus, *Chlorella* residue has the potential to supply not only major elements, but also secondary elements and micronutrients, to the soil. The use of *Chlorella* residue to supplement soils with various nutrient deficiencies can improve the photosynthetic activity, enzymatic activity, and mineral contents in agricultural products.

In the present study, the application of *Chlorella* residue to the soil suppressed clubroot disease in *B. rapa*. Some bacterial species inhabiting the soil or roots produce antibacterial compounds that inhibit the pathogen causing clubroot disease [24]. The increase in microbial biomass resulting from addition of *Chlorella* residue to the soil suggests that microorganisms antagonistic to clubroot disease were able to proliferate. In a similar way, *Chlorella* residue may also reduce the incidence of other plant diseases such as *Fusarium* and *Verticillium* wilt [25] [26].

Chlorella residue contains not only nitrogen but also significant amounts of other elements and micronutrients. Incorporating *Chlorella* residue into organic fertilizers is a promising strategy to promote plant growth, improve soil fertility, and reduce the incidence of plant diseases.

5. Conclusion

The application of *Chlorella* residue increased the concentrations of soil nutrients and soil bacterial biomass. In addition, the activation of antagonistic bacteria against the pathogen *P. brassicae* resulted in reduced severity and incidence of clubroot disease in *B. rapa*. Our results indicate that the nutrient-rich components abundant in *Chlorella* residue can stimulate the growth of soil microorganisms and help to suppress soil pathogens. Together, these findings highlight the biostimulant role of *Chlorella* residue when used as an organic fertilizer.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z. and Lin, W. (2019) The Effects of Chemical and Organic Fertilizer Usage on Rhizosphere Soil in Tea Orchards. *PLOS ONE*, **14**, e0217018. https://doi.org/10.1371/journal.pone.0217018

[2] Islam, Z., Tran, Q.T., Koizumi, S., Kato, F., Ito, K., Araki, K.S., *et al.* (2022) Effect of Steel Slag on Soil Fertility and Plant Growth. *Journal of Agricultural Chemistry and Environment*, **11**, 209-221. https://doi.org/10.4236/jacen.2022.113014

[3] Kumar Bhatt, M., Labanya, R. and Joshi, H.C. (2019) Influence of Long-Term Chemical Fertilizers and Organic Manures on Soil Fertility—A Review. *Universal Journal of Agricultural Research*, **7**, 177-188. https://doi.org/10.13189/ujar.2019.070502

[4] Kumar, R., Kumar, R. and Prakash, O. (2019) The Impact of Chemical Fertilizers on

Our Environment and Ecosystem. In: *Research Trends in Environmental Sciences*, AkiNik Publications, 71-86.

[5] Lal, R. (2009) Soil Degradation as a Reason for Inadequate Human Nutrition. *Food Security*, **1**, 45-57. https://doi.org/10.1007/s12571-009-0009-z

[6] Honetschlägerová, L., Škarohlíd, R., Martinec, M., Šír, M. and Luciano, V. (2018) Interactions of Nanoscale Zero Valent Iron and Iron Reducing Bacteria in Remediation of Trichloroethene. *International Biodeterioration & Biodegradation*, **127**, 241-246. https://doi.org/10.1016/j.ibiod.2017.10.009

[7] Workinger, J.L., Doyle, R.P. and Bortz, J. (2018) Challenges in the Diagnosis of Magnesium Status. *Nutrients*, **10**, Article 1202. https://doi.org/10.3390/nu10091202

[8] Tran, Q.T., Araki, K.S. and Kubo, M. (2021) An Investigation of Upland Soil Fertility from Different Soil Types. *Annals of Agricultural Sciences*, **66**, 101-108. https://doi.org/10.1016/j.aoas.2021.07.001

[9] Li, B.Y., Zhou, D.M., Cang, L., Zhang, H.L., Fan, X.H. and Qin, S.W. (2007) Soil Micronutrient Availability to Crops as Affected by Long-Term Inorganic and Organic Fertilizer Applications. *Soil and Tillage Research*, **96**, 166-173. https://doi.org/10.1016/j.still.2007.05.005

[10] Fenton, O. and Ó hUallacháin, D. (2012) Agricultural Nutrient Surpluses as Potential Input Sources to Grow Third Generation Biomass (Microalgae): A Review. *Algal Research*, **1**, 49-56. https://doi.org/10.1016/j.algal.2012.03.003

[11] Tian, S., Khan, A., Zheng, W., Song, L., Liu, J., Wang, X., *et al*. (2022) Effects of Chlorella Extracts on Growth of *Capsicum annuum* L. Seedlings. *Scientific Reports*, **12**, Article No. 15455. https://doi.org/10.1038/s41598-022-19846-6

[12] Chang, Y., Tsai, W. and Li, M. (2015) Characterization of Activated Carbon Prepared from Chlorella-Based Algal Residue. *Bioresource Technology*, **184**, 344-348. https://doi.org/10.1016/j.biortech.2014.09.131

[13] Pholkaw, P., Muraji, A., Maeda, K., Kawagoe, T., Kubota, K., Sanpa, S., *et al*. (2019) Utilization of Wood Biomass for Organic Soil Based on the Soil Fertility Index (Sofix). *Journal of Agricultural Chemistry and Environment*, **8**, 224-236. https://doi.org/10.4236/jacen.2019.84017

[14] Matsumiya, Y., Taniguchi, R. and Kubo, M. (2012) Analysis of Peptide Uptake and Location of Root Hair-Promoting Peptide Accumulation in Plant Roots. *Journal of Peptide Science*, **18**, 177-182. https://doi.org/10.1002/psc.1435

[15] Pholkaw, P., Tran, Q.T., Kai, T., Kawagoe, T., Kubota, K., Araki, K.S., *et al*. (2020) Characterization of Orchard Fields Based on Soil Fertility Index (Sofix). *Journal of Agricultural Chemistry and Environment*, **9**, 159-176. https://doi.org/10.4236/jacen.2020.93014

[16] Kai, T. and Kubo, M. (2020) Chemical and Biological Properties of Apple Orchard Soils under Natural, Organic, Hybrid, and Conventional Farming Methods. *Journal of Agricultural Chemistry and Environment*, **9**, 134-146. https://doi.org/10.4236/jacen.2020.93012

[17] Aoshima, H., Kimura, A., Shibutani, A., Okada, C., Matsumiya, Y. and Kubo, M. (2006) Evaluation of Soil Bacterial Biomass Using Environmental DNA Extracted by Slow-Stirring Method. *Applied Microbiology and Biotechnology*, **71**, 875-880. https://doi.org/10.1007/s00253-005-0245-x

[18] Adhikari, D., Jiang, T., Kawagoe, T., Kai, T., Kubota, K., Araki, K., *et al*. (2017) Relationship among Phosphorus Circulation Activity, Bacterial Biomass, pH, and Mineral Concentration in Agricultural Soil. *Microorganisms*, **5**, Article 79.

https://doi.org/10.3390/microorganisms5040079

[19] Muyzer, G., de Waal, E.C. and Uitterlinden, A.G. (1993) Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA. *Applied and Environmental Microbiology*, **59**, 695-700. https://doi.org/10.1128/aem.59.3.695-700.1993

[20] Matsumiya, Y., Wakita, D., Kimura, A., Sanpa, S. and Kubo, M. (2007) Isolation and Characterization of a Lipid-Degrading Bacterium and Its Application to Lipid-Containing Wastewater Treatment. *Journal of Bioscience and Bioengineering*, **103**, 325-330. https://doi.org/10.1263/jbb.103.325

[21] Bito, T., Okumura, E., Fujishima, M. and Watanabe, F. (2020) Potential of Chlorella as a Dietary Supplement to Promote Human Health. *Nutrients*, **12**, Article 2524. https://doi.org/10.3390/nu12092524

[22] Cai, L., Gong, X., Ding, H., Li, S., Hao, D., Yu, K., *et al*. (2022) Vermicomposting with Food Processing Waste Mixtures of Soybean Meal and Sugarcane Bagasse. *Environmental Technology & Innovation*, **28**, Article 102699. https://doi.org/10.1016/j.eti.2022.102699

[23] Islam, Z., Tran, Q.T. and Kubo, M. (2023) Development of a Small-Scale Cherry Tomato Cultivation Method Using Organic Soil. *Organic Agriculture*, **13**, 237-246. https://doi.org/10.1007/s13165-023-00424-7

[24] He, Y., Zhu, M., Huang, J., Hsiang, T. and Zheng, L. (2019) Biocontrol Potential of a *Bacillus subtilis* Strain BJ-1 against the Rice Blast Fungus *Magnaporthe oryzae*. *Canadian Journal of Plant Pathology*, **41**, 47-59. https://doi.org/10.1080/07060661.2018.1564792

[25] Mulero-Aparicio, A., Cernava, T., Turrà, D., Schaefer, A., Di Pietro, A., López-Escudero, F.J., *et al*. (2019) The Role of Volatile Organic Compounds and Rhizosphere Competence in Mode of Action of the Non-Pathogenic *Fusarium oxysporum* FO12 toward Verticillium Wilt. *Frontiers in Microbiology*, **10**, Article 1808. https://doi.org/10.3389/fmicb.2019.01808

[26] Li, X., Wang, X., Shi, X., Wang, B., Li, M., Wang, Q., *et al*. (2020) Antifungal Effect of Volatile Organic Compounds from *Bacillus velezensis* CT32 against *Verticillium dahliae* and *Fusarium oxysporum*. *Processes*, **8**, Article 1674. https://doi.org/10.3390/pr8121674