韓國環境復元技術學會誌 26(2) : 13~24(2023)
J. Korean Env. Res. Tech. 26(2) : 13~24(2023)
DOI : https://doi.org/10.13087/kosert.2023.26.2.13
ISSN 1229-3032

# 하수슬러지 처리에서 미생물과 메탄올 적용을 통한 암모니아 배출 감소 및 식물 성장 향상 연구*

김진원[1) ] · 양희건[1)†] · 양희종[2)] · 류명선[2)] · 하광수[1)] · 정수지[2)] · 이수영[1)] · 서지원[1)] · 정도연[3)]

[1)](재)발효미생물산업진흥원 연구원 · [2)](재)발효미생물산업진흥원 팀장 · [3)](재)발효미생물산업진흥원 원장

# Reducing Ammonia Emissions and Enhancing Plant Growth through Co-application of Microbes and Methanol in Sewage Sludge Treatment*

**Jin-Won Kim[1)] · Hee-Gun Yang[1)†] · Hee-Jong Yang[2)] · Myeong-Seon Ryu[2)] · Gwang-Su Ha[1)] · Su-Ji Jeong[2)] · Soo-Young Lee[1)] · Ji-Won Seo[1)] and Do-Youn Jeong[3)]**

[1)]Microbial Institute for Fermentation Industry (MIFI), Researcher,
[2)]Microbial Institute for Fermentation Industry (MIFI), Head of department,
[3)]Microbial Institute for Fermentation Industry (MIFI), Head of an organization.

**ABSTRACT**

Sewage sludge has been widely used as an organic fertilizer in agriculture. However, sewage sludge can cause serious malodor problems resulting from the decomposition of organic compounds in anaerobic conditions. The malodor of sewage sludge mainly occurs due to a low carbon to nitrogen ratio (C/N), high moisture, and low temperature, which are ideal conditions for ammonia emissions. Therefore, in this study, we investigated the reduction of the odor-causing ammonia nitrogen ($NH_3$-N) in sewage sludge by co-application of microbes and methanol (MeOH). The physico-chemical properties of the municipal sewage sludge showed that the odor was mainly caused by a higher $NH_3$-N content (2932.2 mg $L^{-1}$). Supplementation with MeOH (20%) as a carbon source in the sewage sludge significantly reduced the $NH_3$-N up to 34.2% by increasing C/N ratio. Furthermore, the sewage sludge

* This work was supported by a grant from the Establishment of Integrated Biobank for Agriculture, Food and Livestock Microbiome Project funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA).

**First author** : Jin-Won Kim, Microbial Institute for Fermentation Industry (MIFI), Researcher, Sunchang 56048, Korea, Tel : +82-63-650-2044, E-mail : kim.jinwon@srcm.kr

**Corresponding author** : Do-Youn Jeong, Microbial Institute for Fermentation Industry (MIFI), Head of an organization, Sunchang 56048, Korea, Tel : +82-63-653-9579, E-mail : jdy2534@korea.kr

**Received** : 4 January, 2023. **Revised** : 10 April, 2023. **Accepted** : 5 April, 2023

was treated with the $NH_3$-N reducing and plant growth promoting (PGP) bacteria *Stenotrophomonas rhizophila* SRCM 116907. The treatment with *S. rhizophila* SRCM 116907 significantly increased the seedling vigor index of *Lolium perenne* (10.3%) and *Chrysanthemum burbankii* (42.4%). The findings demonstrate that supplementing sewage sludge with methanol significantly reduces ammonia emissions, thereby mitigating malodor problems. Overall, the study highlights the potential of using a microbial and methanol approach to improve the quality of sewage sludge as an organic fertilizer and promote sustainable agriculture.

**Key Words:** *Ammonia nitrogen, Sewage sludge, Stenotrophomonas rhizophila, Methanol, Microbial community*

# I. INTRODUCTION

A higher amount of sewage sludge is generated every year from wastewater treatment plants (WWTPs), and its amount and treatment cost are gradually increasing worldwide (Sharma et al., 2017). Recently, many research on sewage sludge recycling has been reported as a fertilizer to generate profits. Sewage sludge can be used as a promising fertilizer in agriculture and afforestation because it contains many organic compounds, macronutrients, and micronutrients (Luukkonen et al., 2020).

However, its improper use in agriculture creates several problems, including causing serious malodor, pathogen problems and chemical contamination (Elloumi et al., 2016; Frišták et al., 2018). Generally, the odor pollution of sewage sludge occurs due to a low carbon to nitrogen ratio (C/N), high moisture, and low temperature, which are ideal conditions for ammonia emissions. An offensive malodor of sewage sludge can be harmful to the health of neighbors and plant workers. Therefore, proper management of sewage sludge is very important to reduce malodor emissions from the composting of sewage sludge. The use of sewage sludge as fertilizer in agriculture can have economic and environmental benefits, but malodor issues limit its widespread use. Thus, there is a need for effective and cost-efficient methods to reduce malodor emissions from sewage sludge.

Previously, a range of physical and mechanical pretreatment methods were employed to enhance the digestion of sewage sludge and manage odors during the sewage sludge treatment process. These techniques included high pressure homogenization, ultrasonication, electro-kinetic disintegration, and chemical treatment, as noted by Nguyen et al. (2021). While these methods were commonly used and effective for digesting sewage sludge, they also had significant limitations, such as their high costs and the generation of secondary toxic by-products.

Microorganisms have important roles in sewage sludge composting processes by degrading organic materials. The understanding and characterization of the microbial community of sewage sludge could be helpful for successful composting and cost-effective operations because the characteristics of the microbial community highly depend on the physico-chemical properties of the sewage residue such as C/N ratio, pH, and temperature (Sharma et al., 2017; Robledo-Mahón et al., 2018).

The present investigation is aimed at reducing malodor emissions from sewage sludge by reducing

$NH_3$-N content. The physico-chemical property results of sewage sludge showed low carbon to nitrogen ratio (C/N) and higher $NH_3$-N content (2932.2 mg $L^{-1}$). Therefore, we tried to reduce C/N ratio of sewage sludge by adding MeOH as a carbon source. Methanol is already widely applied in water treatment plants to reduce $NH_3$-N due to its affordable and ease to buy than other carbon sources such as ethanol, glucose, and acetic acid. Furthermore, the plant growth-promoting bacteria (PGPB) was screened to inoculate the sewage sludge to enhancing reduce malodor and fertilizer properties. The *Stenotrophomonas rhizophila* SRCM 116907 strain, as an ammonia nitrogen ($NH_3$-N) reducing and PGPB strain, was used to treat the sewage sludge compost. Additionally, metagenomic analysis was performed to determine how the addition of methanol and the inoculation of *S. rhizophila* SRCM 116907 affected the microbial community change. The study provides insights into the microbial community changes in sewage sludge compost, which could be helpful for successful composting and cost-effective operations. The findings of this study can be applied in the field of agriculture and wastewater management to reduce malodor emissions and promote sustainable environmental practices.

## II. MATERIALS AND METHODS

### Sample collection and analysis

Sewage sludge (SS) and sewage sludge containing soil (CS) were supplied by the Do-kwang Company (Sejong-si, South Korea). Sewage sludge samples were collected from the municipal wastewater treatment plants in Gyeonggi-do. The SS and CS samples were passed through a 2 mm sieve for the physico-chemical property analysis. Physico-chemical properties such as pH, electrical conductivity (EC), organic matter (OM), moisture, and C/N ratio of the SS and CS were analyzed (Li et al., 2015). The pH and EC were measured at a 1:5 ratio (sewage sludge : water, wt/wt) using a pH (SevenEasy pH S20, Mettler Toledo) and EC (Orion 3-Star, Thermo Scientific, USA) meter following the official test method for soil (Cho et al., 2021). The OM content was measured using the Tyurin method (Šimanský et al., 2019). $NH_3$-N and $NO_3$-N concentrations were measured using the Standard Method (APHA et al., 1995); $H_2S$ concentration was determined by a colorimetric method based on Sugahara et al. (2016). To determine total mercaptans (RSH) concentration, the Gastec (model GV-100) gas sampling pump (Gastec Corp., Kanagawa, Japan) was used.

### Composting process

A schematic diagram of the enhanced sewage sludge composting process by adding MeOH and inoculation of bacteria for malodor reducing is shown in Figure 1. MeOH was injected directly into the SS samples for increasing C/N ratio and then composted for a week in lab scale (Figure 1C). Then, plant growth promoting microbes were inoculated in the composted SS (Figure 1D). The lab-scale reactor was operated at controlled temperature (30°C). A successfully composted SS can expect to have many positive effects on agriculture such as odor-reducing and plant growth-promoting (Figure 1F).

According to the previous report of Yang et al. (2017), the emission of $NH_3$-N occurerd mainly due to higher moisture and a lower C/N ratio. Therefore, we tried to reduce the malodor of the sewage sludge by increasing the C/N ratio using MeOH (5, 10, and 20%) as an additional carbon source and

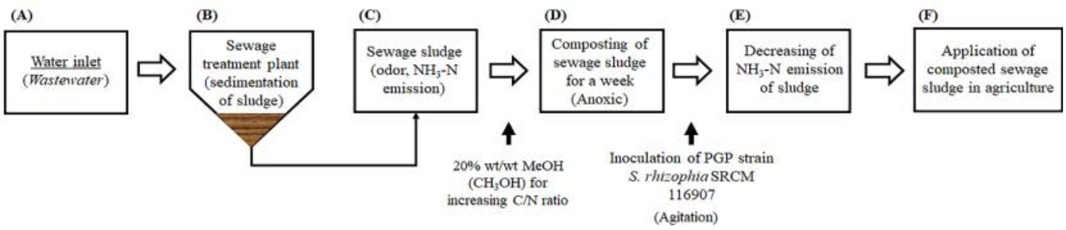


**Figure 1.** The graphical abstract of the sewage sludge composting process adding MeOH and PGPB for reducing malodor.

inoculating the sludge with ammonia nitrogen-reducing bacteria.

## Measurement of NH3-N removal capacity

The $NH_3$-N stock solution was prepared using $NH_4Cl$ (Extra Pure grade, Daejung Chemical & Metals, Co., Ltd, South Korea) and MeOH ($CH_3OH$, 99.8%) was purchase from Daejung Chemicals & Metals Co., Ltd., (South Korea). To confirm the influence of initial MeOH concentration on the removal of $NH_3$-N in the sewage sludge samples, sewage sludges were composted for 7 days at 30°C with different MeOH concentrations (0, 5, 10, and 20% wt/wt) without additional agitation.

To select superior $NH_3$-N removal bacteria, the microbials were grown in an M9 minimal medium (15 g $L^{-1}$ glucose, 4 g $L^{-1}$ $MgSO_4 \cdot 7H_2O$, 30 g $L^{-1}$ $K_2HPO_4$, 15 g $L^{-1}$ $KH_2PO_4$, 2 g $L^{-1}$ citric acid, 0.5 g $L^{-1}$ NaCl, 1.5 ml $L^{-1}$ trace elements solution) containing 1000 mg $NH_3$-N $L^{-1}$ (Yari et al., 2012). A log-phase seed culture of microbes was inoculated into 100 ml of M9 minimal medium and incubated for 72 h. Microbial growth was measured spectrophotometrically at 600 nm using a spectrophotometer (Tecan Spark 10 M, Tecan Trading AG, Switzerland).

## Extracellular enzyme isolation and plant growth experiment

Four strains (SRCM 116890, 116907, 121335, and 121341) were isolated from paddy soil in order to apply in composting process of sewage sludge. The extracellular enzyme activities (amylase, protease, cellulase, and lipase) and plant growth promoting (PGP) factors (IAA, Siderophore, and Nitrogen fixation) of selected microbes were analyzed using agar well diffusion and spectrophotometric method, respectively. To determine the extracellular enzyme actives of isolates, 100 uL culture supernatants were added to the agar wells according to Abe et al., 2015 and production of PGP factors was determined according to Shim et al. (2014).

To identify the microorganisms, 16S rRNA gene analysis was performed by Macrogen (Macrogen Inc., Seoul, South Korea) using the 27 F (5’-AGAGTTTGATCATGGCTCAG-3’) and 1492 R (5’-GGTTACCTTGTTACGACTT-3’) universal primers. Among the isolates, the partial 16S rRNA of the *S. rhizophila* SRCM 116907 (GenBank Accession No. OP710251) was deposited in the GenBank database.

A plant growth experiment was conducted to investigate the effectiveness of using microbial treatment to improve the fertilization properties of sewage sludge and its potential application for vegetation restoration. Plant growth experiments were performed with *Lolium perenne* and *Chrysanthemum burbankii* on a lab-scale.

**Table 1.** Chemical properties of the experiment raw sewage sludge (SS) and sewage sludge containing soil (CS)

| Samples | pH (1:5) | EC (dS m-1) | OM (%) | Moisture (%) | T-C (%) | T-N (%) | C/N | NH3-N (mg L-1) | NO3-N (mg L-1) | H2S (mg L-1) | Mercaptan (mg L-1) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SS | 7.48±0.05 | 0.31±0.03 | 7.28±0.08 | 58±3.5 | 4.22±0.08 | 0.22±0.03 | 19.2 | 2932.2±20 | ND* | ND | ND |
| CS | 7.55±0.03 | 0.28±0.04 | 8.1±0.35 | 35±2.6 | 4.7±0.21 | 0.77±0.02 | 6.1 | 75.3±1.61 | ND | ND | ND |

*ND: not detected.

Hoagland's salt medium (Sigma, St. Louis, MO) containing 0.5% agar for plant growth was prepared. Seeds were sterilized with 70% ethanol (1 min) and 5% NaOCl (20 min) and then washed with deionized water three times. Plants were grown for 2 weeks in a growth chamber (25°C) with a 16/8 h dark/light cycle. The germination rate, length of shoot, and root of *Lolium perenne* and *Chrysanthemum burbankii* were measured. The seedling vigor index of plants was calculated based on Zhao et al., 2021. All statistical analysis of the plant experiment data was performed with SAS 9.1 program (SAS, Cary, NC, USA). A statistical test was conducted using a one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

### DNA extraction and next generation sequencing (NGS)

Total DNA was extracted from the sewage sludge, MeOH (20%) containing sewage sludge, and bacteria inoculated sewage sludge using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The yield and purity were measured using Invitrogen Qubit 4 Fluorometer (Invitrogen Inc., Carlsbad, CA, USA) and NanoDrop spectrophotometer (Thermo Fisher scientific, 5225 Verona Rd, USA), respectively. Amplicon libraries were constructed with next generation sequencing by targeting the hypervariable V3-V4 region of the 16S rRNA gene using 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') primers (Pacwa-Płociniczak et al., 2020). Libraries were prepared with Nextera XT index Kit v2 (Illumine, REF, 15052163). Sequencing was performed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA).

## III. RESULTS AND DISCUSSION

### Physico-chemical properties of the sewage sludge

The SS sample showed a high $NH_3$-N (2932.2 mg $L^{-1}$) and moisture (58%) content (Table 1). However, nitrate nitrogen, hydrogen sulfide, and mercaptan were not detected in both the SS and CS samples. This result suggested that the main reason for the malodor of the samples was due to a higher $NH_3$-N emission, in part. The C/N ratio of the SS and CS was 19.2 and 6.1, respectively. The C/N ratio is one of the important factors in the composting process because it can be affected the rate of organic matter decomposition (Palaniveloo et al., 2020). A C/N ratio of around 25~35 is considered optimal for the composting process, as it provides enough carbon for microbial growth and energy while allowing for efficient nitrogen utilization (Zhou, 2017).

**$NH_3$-N removal capacity of MeOH and**

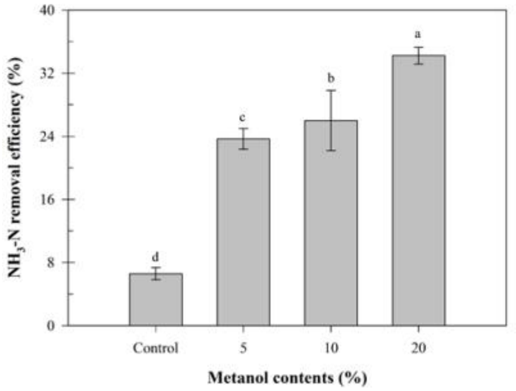


**Figure 2.** Influence of initial methanol concentration on the NH3-N removal efficiency in the sewage sludge ($p < 0.05$, $n = 3$).

**microorganisms**

The $NH_3$-N removal capacity gradually increased with the increasing MeOH content (Figure 2). Thus, 34.2% of the $NH_3$-N in the sewage sludge was removed by adding 20% MeOH as a carbon source. Previous studies have suggested MeOH and acetate can be effectively used as biodegradable carbon sources and it is commonly applied for denitrification of activated sludge (Lu et al., 2014). Nitrification and denitrification mechanisms involving MeOH were expressed to equations (1) and (2), respectively.

Nitrification：

$$NH_4^+ + 2O_2 \rightarrow 2H^+ + H_2O + NO_3^- \quad (1)$$

Denitrification：

$$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 6OH^- + 5CO_2 + 7H_2O \quad (2)$$

where MeOH ($CH_3OH$) was involved as an electron donor in the denitrification process. This mechanism showed external electron donor is needed to improve denitrification (Fu et al., 2022).

Additionally, the *S. rhizophila* SRCM 116907 strain exhibited a superior ammonia reducing property (Figure 3B). By 24 h, the $NH_3$-N removal efficiency reached 24.2%. As shown in Figure 3A, stationary phase was reached in the order SRCM 116907 > SRCM 121335 > SRCM 121341 > SRCM 116890. Among the isolates, the *S. rhizophila* SRCM 116907 strain had the highest growth and $NH_3$-N removal efficiency in M9 minimal medium containing $NH_3$-N. The denitrification process of nitrate ion using MeOH as an electron donor by microorganism are given in equation (3).

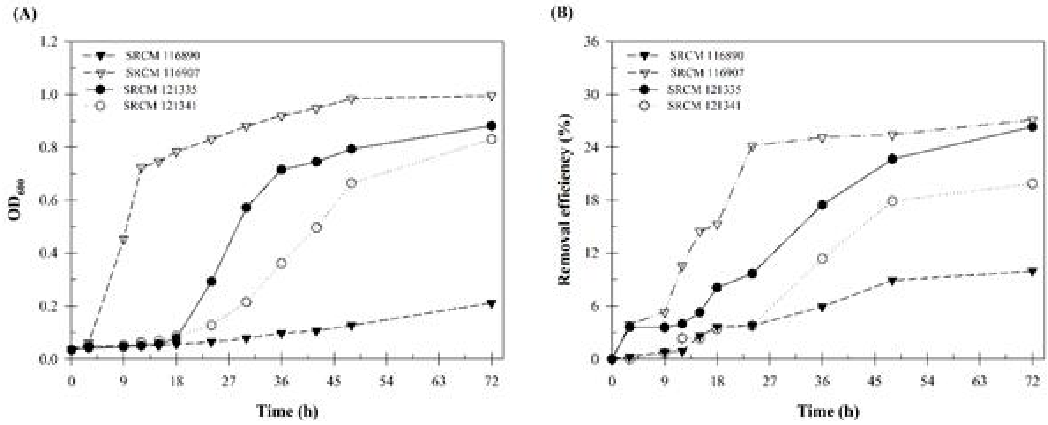


**Figure 3.** Growth curves (A) and $NH_3$-N concentration change (B) of selected microbes in the M9 minimal medium containing 1000 mg $NH_3$-N $L^{-1}$.

**Table 2.** Extracellular enzymes activities and plant growth promoting properties analysis of selected microbes

| Strain No. | Identification | Amylase (mm) | Protease (mm) | Cellulase (mm) | Lipase (mm) | IAA (mM) | Siderophore (mm) | Nitrogen fixation (+/-) |
|---|---|---|---|---|---|---|---|---|
| SRCM 116890 | V. soli | - | 6.20 | 5.2 | - | 0.01 | - | - |
| SRCM 116907 | S. rhizophila | - | 6.18 | 4.8 | - | 0.01 | 8.30 | + |
| SRCM 121335 | R. skierniewicense | - | - | 5.0 | - | 0.05 | 8.20 | + |
| SRCM 121341 | E. adhaerens | - | 5.80 | 5.0 | - | 0.01 | - | + |

$$NO_3^- + 1.08CH_3OH + 0.24H_2CO_3 \rightarrow 0.06C_5H_7O_2N + 0.47N_2 + 1.68H_2O + HCO_3^- \quad (3)$$

where $C_5H_7O_2N$ is biomass of microorganism and the theoretical MeOH requirement for heterotrophic denitrification is 2.47 mg MeOH $mg^{-1}$ $NO_3$-N (Ahn, 2006). These findings provide important insights into the potential use of MeOH and *S. rhizophila* SRCM 116907 strain in the treatment of sewage sludge and highlight the importance of understanding the microbial processes involved in the composting of sewage sludge.

Furthermore, *S. rhizophila* SRCM 116907 showed relatively diverse enzymatic (protease and cellulase) and PGP (IAA, siderophore, and nitrogen fixation) properties compared to other microorganisms (Table 2). These productions of extracellular enzymes by microbes have an important role in the composting process of sewage sludge due to it can help degradation of specific substrates such as cellulose (Nikaeen et al., 2015).

## Plant growth

The length of the *Lolium perenne* shoots (9.3%) and roots (35.2%) and the seedling vigor index (19.2%) were significantly increased by the inoculation of *V. soli* SRCM 116890 ($p < 0.05$) (Fig 4A and 4C). However, the shoot length, root length, and seedling vigor index of *Chrysanthemum burbankii* were highly influenced by the inoculation of *S. rhizophila* SRCM 116907 and *E. adhaerens* SRCM 121341 (Figure 4B and 4D). Especially, when inoculated with *S. rhizophila* SRCM 116907 strain, the seedling vigor index of *Chrysanthemum burbankii* increased by 42.4% compared with the control. These results suggest that *S. rhizophila* SRCM 116907 can effectively be used for $NH_3$-N removal and plant growth promotion. A similar plant growth promoting effect by inoculation of the *Stenotrophomonas rhizophila* was previously reported (Majeed et al., 2015).

*L. perenne* is a type of perennial ryegrass commonly used as a forage crop for livestock, due to its fast growth rate and high yield. It is also used for turf and erosion control (Woo and Jeon, 2005). *C. burbankii*, on the other hand, is a type of ornamental plant commonly used for landscaping and decoration due to its attractive flowers (Park and Shim, 2018). Since these plants germinate quickly, they are mainly used for slope and forest restoration (Cho et al., 2015). Therefore, the increase in growth due to the application of

**Table 3.** Number of sequences, alpha-diversity of different sewage sludge treatments

| Sample | OTUs | Alpha-diversity | | | |
|---|---|---|---|---|---|
| | | Chao | Shannon | Simpson | Good's coverage of library (%) |
| Control | 1024 | 1214.5 | 4.40 | 0.04 | 99.7 |
| MeOH (20%) | 780 | 972.5 | 3.38 | 0.09 | 99.7 |
| SRCM116907 | 850 | 990.1 | 3.98 | 0.07 | 99.8 |

microorganisms can be an important key in the application of forest restoration technology.

### Comparative metagenomic analysis of the microbial community

A total of 1024 OTUs (operational taxonomic units) were found in the sewage sludge sample, and Good's coverage values of >99.7% were obtained for each sample (Table 3). The order of the OTUs and Chao and Shannon indices were determined as Control > SRCM 116907 > MeOH, which demonstrates that the bacterial diversity richness could be reduced by adding MeOH due to solvent toxicity (Dyrda et al., 2019). The Chao and Shannon indices show the diversity richness and alpha-diversity of the samples, respectively.

As shown in Table 4, the genus level of the microbial composition of the sewage sludge was determined as *Bdellovibrio* > *Sphingobacterium* > *Advenella* > *Taibaiella* > *Ochrobactrum*. A similarity was observed between the microbial composition of the *S. rhizophila* SRCM 116907 inoculated sewage sludge sample and the sewage sludge sample. These microbes may play crucial roles in composting process, which contributes to the degradation of organic matters in sewage sludge (Siebielec et al., 2018). However, the *S. rhizophila* SRCM 116907 inoculated sewage sludge sample showed a significant increase in the *Stenotrophomonas* composition. These results suggest that the *S. rhizophila* SRCM 116907 can be successfully applied to composting process of sewage sludge.

Additionally, the *Bdellovibrio* and *Taibaiella* compositions were drastically decreased in the sewage sludge containing MeOH (20% MeOH). A relative abundance of the top 9 species of the microbes was presented in a stacked histogram and heat map (Figure 5A and 5B). Among the species, *Advenella kashmirensis* and *Ochrobactrum pseudintermedium* were significantly dominant at MeOH treatment compared with control. Previously, *Advenella* sp. have been reported that they can use methanol as energy source, and some *Advenella* species have PGP property (Poroshina et al., 2015; Kuzmina et al., 2022).

## Ⅳ. CONCULSIONS

The study concludes that the treatment of MeOH and *S. rhizophila* SRCM 116907 on municipal sewage sludge is appropriate for the removal of $NH_3$-N. The MeOH (20% wt/wt) treatment effectively removed 34.2% of the $NH_3$-N in the sewage sludge. Among the isolates, finally *S. rhizophila* SRCM 116907 was selected for application in the sewage sludge composting

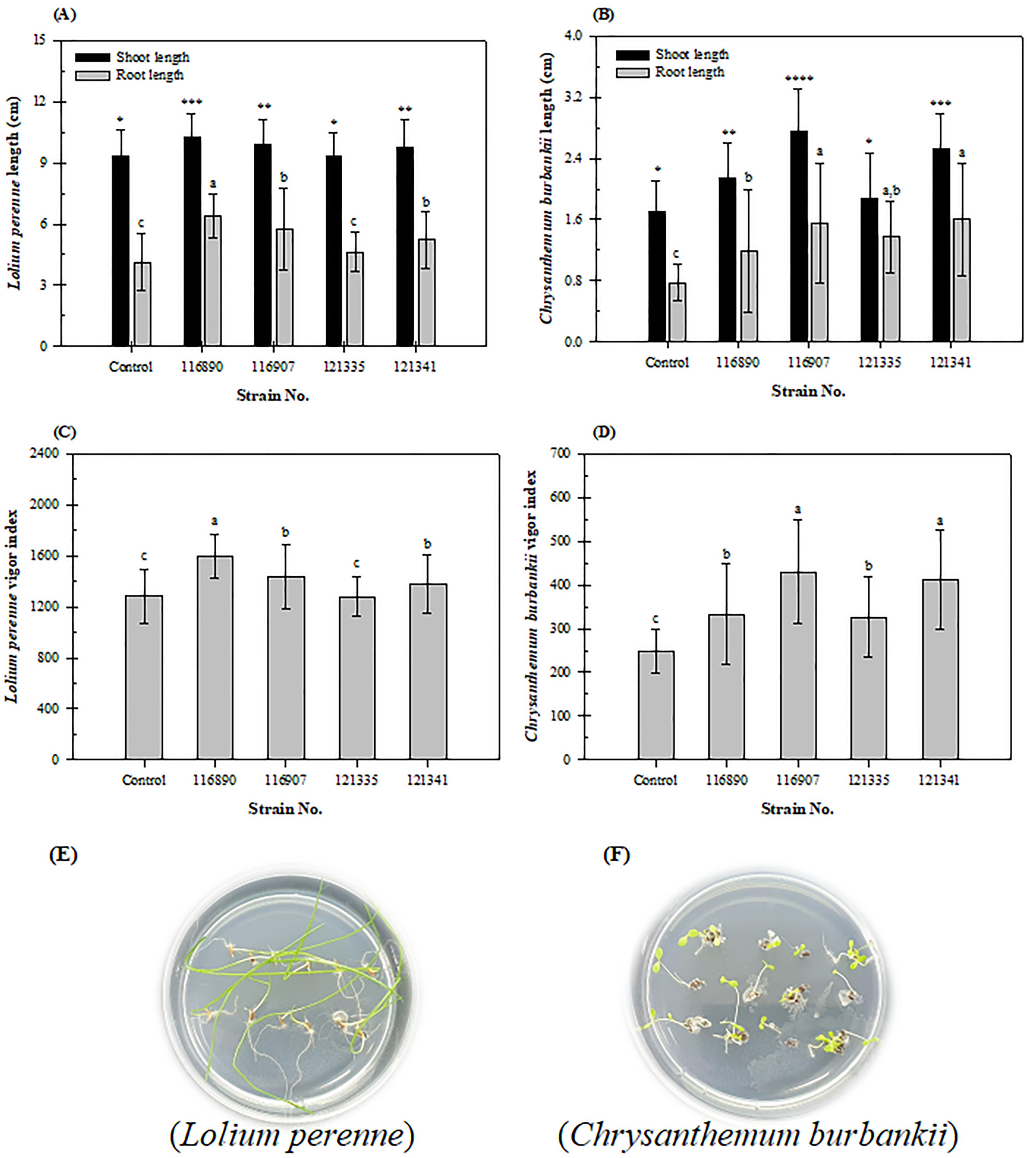


**Figure 4.** The growth of the *Lolium perenne* (A), *Chrysanthemum burbankii* (B), it's seedling vigor index (C, D), and plant growth monitoring image (E, F). Bars represent standard deviations and different letters are indicating significant differences among the treatments according to the Duncan's multiple range test ($p < 0.05$, $n = 10$).

process, which showed superior enzymatic and PGP properties. *S. rhizophila* SRCM 116907 was removed 27.1% of $NH_3$-N within 72 h in M9 minimal medium containing 1000 mg $NH_3$-N $L^{-1}$. Additionally, *S. rhizophila* SRCM 116907 had a positive effect on the growth of *Lolium perenne* and *Chrysanthemum burbankii*. The effect of treatment of the MeOH and *S. rhizophila* SRCM 116907 on

**Table 4.** 16S rRNA reads analysis result in genus level at different treatments

| Taxon | Proportion (%) | | |
|---|---|---|---|
| | Control | MeOH (20%) | SRCM116907 |
| *Bdellovibrio* | 17.28 | 0.01 | 22.33 |
| *Advenella* | 4.64 | 23.65 | 5.45 |
| *Ochrobactrum* | 3.61 | 16.34 | 3.93 |
| *Sphingobacterium* | 8.80 | 8.46 | 4.03 |
| *Flavobacterium* | 4.72 | 4.86 | 4.20 |
| *Bordetella* | 0.81 | 8.28 | 0.48 |
| *Stenotrophomonas* | 1.94 | 0.59 | 3.86 |
| *Acinetobacter* | 0.59 | 5.70 | 0.08 |
| *Taibaiella* | 4.12 | 0 | 2.20 |
| *ETC* | 53.49 | 32.12 | 53.45 |

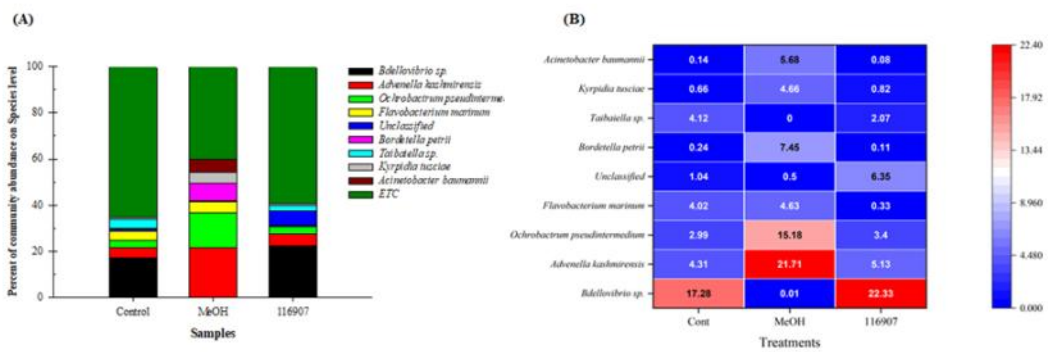


**Figure 5.** Stacked histogram (A) and heatmap (B) illustrate the bacterial relative abundance of taxa at the species level in the sewage sludge samples.

the sewage sludge was determined using microbial community composition analysis. These results suggest treatment of MeOH and *S. rhizophila* SRCM 116907 could effectively reduce $NH_3$-N emission from the municipal sewage sludge.

The study has several significant findings that have implications for environmental restoration efforts. By demonstrating that the treatment of MeOH and *S. rhizophila* SRCM 116907 can effectively remove $NH_3$-N from municipal sewage sludge, the study provides a potential solution for reducing environmental pollution caused by sewage waste. However, real-world testing is needed to fully understand effectiveness and limitations. Hence, further analysis of the construction method's field applicability is necessary.

## References

Abe CAL·Faria CB·de Castro FF·de Souza SR·dos

Santos FC·da Silva CN·Tessmann DJ and Barbosa-Tessmann IP. 2015. Fungi isolated from maize (*Zea mays* L.) grains and production of associated enzyme activities. Int. J. Mol. Sci. 16(7): 15328-15346.

Ahn YH. 2006. Sustainable nitrogen elimination biotechnologies: a review. Process Biochem. 41(8): 1709-1721.

APHA AWWA, WEF. 1995. Standard methods for the examination of water and waste water. American Public Health Association/ American Water Works Association/Water Environment Federation, 19th ed. Washington DC, USA.

Cho SR·Kim JH and Shim SR. 2015. Pratical Use of Several Ground Covers on a Slope Revegetation Construction - *Miscanthus sinensis* var. *purpurascens*, *Festuca arundinacea*, *Pennisetum alopecuroides*, *Zoysia japonica* -. J. Korean Env. Res. Tech. 18(3): 97-107.

Cho SR·Lee YJ·Lee CW·Hyun BG·Song YS·Ryu CH·Kim YM and Lee SB. 2021. The effect of livestock and food waste compost on rice yield and nutrient utilization efficiency according to different nitrogen fertilizer treatments rates. Korean J. Soil Sci. Fert. 54(4): 558-566.

Dyrda G·Boniewska-Bernacka E·Man D·Barchiewicz K and Słota R. 2019. The effect of organic solvents on selected microorganisms and model liposome membrane. Mol. Biol. Rep. 46: 3225-3232.

Belhaj, D·Elloumi N·Jerbi B·Zouari M·Abdallah FB·Ayadi H and Kallel M. 2016. Effects of sewage sludge fertilizer on heavy metal accumulation and consequent responses of sunflower (*Helianthus annuus*). Environ. Sci. Pollut. Res. 23: 20168-20177.

Frišták V·Pipíška M and Soja G. 2018. Pyrolysis treatment of sewage sludge: a promising way to produce phosphorus fertilizer. J. Clean. Prod. 172: 1772-1778.

Fu X·Hou R·Yang P·Qian S·Feng Z·Chen Z·Wang F·Yuan R·Chen H and Zhou B. 2022. Application of external carbon source in heterotrophic denitrification of domestic sewage: A review. Sci. Total Environ. 153061.

Kuzmina LY·Gilvanova EA·Galimzyanova NF·Arkhipova TN·Ryabova AS·Aktuganov GE·Sidorova LV·Kudoyarova GR and Melent'ev AI. 2022. Characterization of the Novel Plant Growth-Stimulating Strain *Advenella kashmirensis* IB-K1 and Evaluation of Its Efficiency in Saline Soil. Microbiology. 91(2): 173-183.

Li Y·Liu B·Zhang X·Gao M and Wang J. 2015. Effects of Cu exposure on enzyme activities and selection for microbial tolerances during swine-manure composting. J. Hazard. Mater. 283: 512-518.

Lu H·Chandran K and Stensel D. 2014. Microbial ecology of denitrification in biological wastewater treatment. Water Res. 64: 237-254.

Luukkonen T·Prokkola H and Pehkonen SO. 2020. Peracetic acid for conditioning of municipal wastewater sludge: Hygienization, odor control, and fertilizing properties. Waste Manage. 102: 371-379.

Majeed A·Abbasi MK·Hameed S·Imran A and Rahim N. 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front. Microbiol. 6: 198.

Nguyen VK·Chaudhary DK·Dahal RH·Trinh NH·Kim J·Chang SW·Hong Y·La DD·Nguyen XC·Ngo HH·Chung WJ and Nguyen DD. 2021. Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. Fuel 285:

119105.

Nikaeen M·Nafez AH·Bina B·Nabavi BF and Hassanzadeh A. 2015. Respiration and enzymatic activities as indicators of stabilization of sewage sludge composting. Waste Manage. 39: 104-110.

Pacwa-Płociniczak M·Biniecka P·Bondarczuk K and Piotrowska-Seget Z. 2020. Metagenomic functional profiling reveals differences in bacterial composition and function during bioaugmentation of aged petroleum-contaminated soil. Front. Microbiol. 11: 2106.

Palaniveloo K·Amran MA·Norhashim NA·Mohamad-Fauzi N·Peng-Hui F·Hui-Wen L·Kai-Lin Y·Jiale L·Chian-Yee MG·Jing-Yi L·Gunasekaran B and Razak SA. 2020. Food waste composting and microbial community structure profiling. Processes 8(6): 723.

Park JA and Shim SR. 2018. Germination percentage and speed under international seed testing association conditions of main herbaceous plants used on the slope. J. Korean Env. Res. Tech. 21(3): 47-56.

Robledo-Mahón T·Aranda E·Pesciaroli C·Rodríguez-Calvo A·Silva-Castro GA·González-López J and Calvo C. 2018. Effect of semi-permeable cover system on the bacterial diversity during sewage sludge composting. J. Environ. Manage. 215: 57-67.

Poroshina MN·Doronina NV·Kaparullina EN and Trotsenko YA. 2015. *Advenella kashmirensis* subsp. methylica PK1, a facultative methylotroph from carex rhizosphere. Microbiology 84: 73-79.

Sharma B·Sarkar A·Singh P and Singh RP. 2017. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. Waste Manage. 64: 117-132.

Shim J·Kim JW·Shea PJ and Oh BT. 2015. IAA production by *Bacillus* sp. JH 2-2 promotes Indian mustard growth in the presence of hexavalent chromium. J. Basic Microb. 55(5): 652-658.

Siebielec G·Siebielec S and Lipski D. 2018. Long-term impact of sewage sludge, digestate and mineral fertilizers on plant yield and soil biological activity. J. Clean. Prod. 187: 372-379.

Šimanský V·Juriga M·Jonczak J·Uzarowicz Ł and Stępień W. 2019. How relationships between soil organic matter parameters and soil structure characteristics are affected by the long-term fertilization of a sandy soil. Geoderma 342: 75-84.

Sugahara S·Suzuki M·Kamiya H·Yamamuro M·Semura H·Senga Y·Egawa M and Seike Y. 2016. Colorimetric determination of sulfide in microsamples. Anal. Sci. 32(10): 1129-1131.

Woo KJ and Jeon GS. 2005. A Study on Revgetation Character for Environment Factor of Slope. J. Korean Env. Res. Tech. 8(5): 47-55.

Yang F · Li G · Zang B and Zhang Z. 2017. The maturity and $CH_4$, $N_2O$, $NH_3$ emissions from vermicomposting with agricultural waste. Compost Sci. Util. 25(4): 262-271.

Yari K·Fatemi SSA and Tavallaei M. 2012. High level expression of recombinant BoNT/A-Hc by high cell density cultivation of *Escherichia coli*. Bioproc. Biosyst. Eng. 35: 407-414.

Zhao X·Joo JC and Kim JY. 2021. Evaluation of heavy metal phytotoxicity to *Helianthus annuus* L. using seedling vigor index-soil model. Chemosphere 275: 130026.

Zhou, JM. 2017. The effect of different C/N ratios on the composting of pig manure and edible fungus residue with rice bran. Compost Sci. Util. 25(2): 120-129.