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Isolation of Microorganisms and Development of Microbial Augmentation for Treatment of Industrial Wastewater containing Ammonium Nitrogen

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For effective treatment of wastewater containing ammonium nitrogen (NH_4-N), AT2, AT9, and AT12 strains, having high total organic carbon (TOC) removal capability, and FN47, possessing excellent ammonia nitrogen removal capability present in the activated sludge in the aeration tank of food wastewater treatment plants, were isolated and identified. The cells of these isolated strains were used for microbial augmentation with FIW-1 in the defatted rice bran as a medium to treat industrial wastewater. The investigation of the cultural characteristics of these isolated strains in the aeration tank showed that the affinities for substrate of the isolated strains were extremely high, of which AT12 (*Alcaligenes* sp. AT12) was the highest among the isolated strains. Ammonium nitrogen removal efficiency in the food wastewater was 71% in the isolated strain FN47 (*Microbacterium* sp. FN47) treatment group. When only activated sludge was added in the lab scale pilot using food wastewater during continuous culture experiment, the TOC removal efficiency was 63%. Meanwhile, the removal efficiency of 92% was obtained when the microbial augmentation FIW-1 for wastewater treatment was applied. In addition, the chemical oxygen demand (COD) level from the effluent wherein microbial augmentation FIW-1 was input for the initial three days in the wastewater treatment site experiment showed a treatment rate of about 43%, which was increased to 62% after an elapse of 5 days.

Key words : Ammonium nitrogen, microbial augmentation, pilot test, total organic carbon, wastewater treatment

Introduction

The organic wastewater generated from the industrial sites is composed of biodegradable organics, volatile organic compounds, recalcitrant organics, suspended solids, and nutrient salts (nitrogen and phosphorus). The objective of wastewater treatment is to reduce organics in the wastewater and to remove nutrient salts to reduce the contamination of surface water and groundwater through wastewater treatment process. In the treatment of organic wastewater, a method of artificially increasing the efficiency of water self-cleaning is widely used, which is called a biological treatment. In general, the biological treatment for wastewater treatment can be categorized into aerobic process like activated sludge method and biofilm process, and anaerobic process like digestion method. In many of the biological treatments, various microorganisms such as bacteria, fungi, protozoa, and micrometazoa are involved in the purification, while dozens or more kinds of microorganisms constitute a mixed culture system. On the other hand, wastewater containing various kinds of miscellaneous components is a highly complex multicomponent system, and biological treatment is a process of removing mixed substrates by mixed cultured microorganisms [5, 11, 16].

From the industrial wastewater, non-biodegradable or bio-toxic materials like synthetic organic compounds are detected along with biodegradable organics, which pose major factors affecting the deterioration of wastewater treatment efficiency and limiting the biological treatment [9, 19]. Removal of organic compounds by the biological treatment largely relies on the composition and treatment type of the wastewater and solid retention time. A typical aerobic biological treatment is known to remove soluble organic carbons until about 85%. Half of the organic compounds remained after biological treatment consists of humid acid, fulvic acid, and hymathomelanic acid. Meanwhile, easily degradable organics, like carbohydrates and protein, account for about 25% of the soluble organics [13].

Biological treatment, where microorganisms are used, is

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basically an economical method of treating wastewater by using metabolism; i.e., degradation, synthesis, and conversion of the organic matters presented in the wastewater. It is used with the objectives of removing organics, nitrogen, and phosphorus; reducing generated sludge; and collecting and treating natural resources. In the biological treatment, the physicochemical treatments such as precipitation, filtration, and coagulation are combined or used in parallel as a pretreatment or post-treatment to improve wastewater treatment efficiency.

Particularly, high concentrations of nitrogen included in the wastewater are not easily removed with general wastewater treatment, inflowing into the lakes and streams, thus inducing eutrophication to cause hyperplasia of hydrophytes, deficiency of dissolved oxygen in the water, bad order, and worsening water quality by accumulation of decomposed matter, thereby leading to serious environmental problems and ultimately making this nitrogen emerge as a new contaminant [10].

Physicochemical methods such as absorption and ion exchange, as well as the biological treatment method, are used to remove nitrogen in the wastewater. Though biological treatment method is friendlier to the environment, the efficiency is low in removing high concentrations of nitrogen; therefore, it is being used along with physicochemical method [2, 20].

For the biological treatment used in the treatment of nitrogen containing wastewater, a considerable amount of research and development is continuing in terms of process. Researches are mainly advanced not only in the selection of new microorganisms that can treat high concentrations of nitrogen but also in improving wastewater treatment system, suiting the characteristics of microorganisms for process improvement and utilizing microorganisms in strain improvement and microorganism fixation [7-10].

In this study, microorganisms capable of degrading various organic materials and those that have excellent ammonium nitrogen removal capability were isolated and identified from the aeration tank which contained ammonium nitrogen in order to efficiently treat organic wastewater for environment purification. In addition, the isolated microorganisms were formulated and optimized in order to be utilized in treating the industrial wastewater that contains organic source and ammonium nitrogen.

Materials and Methods

Isolation of microorganisms in the aeration tanks

In order to isolate microorganisms codominant in the activated sludge in the aeration tank of food wastewater treatment plant, a sample from the aeration tank was set as an isolated sample and was diluted in the normal saline. The diluted wastewater was spread on the plate count agar (PCA) medium followed by culturing at 37℃ for 3 days. The population that formed the colony was isolated. The isolated strain was cultured in a liquid culture medium for the isolation of bacteria, while strain having exceptional cell growth was selected and used in the wastewater treatment efficiency test. The liquid culture medium for the isolation of bacteria was composed of 3.0 g glucose, 5.0 g yeast extract, and 3.0 g tryptone per 1 liter of medium. The pH of culture medium was adjusted to 7.0.

Isolation of ammonium nitrogen removal microorganisms

In order to isolate ammonium nitrogen (NH_4-N) removal microorganisms, samples from soil, rivers, and wastewater around food, paper, and leather manufacturing plants nationwide were diluted in normal saline, and 0.7 g/l of $(NH_4)_2SO_4$, as a single energy source, was spread on the solid medium wherein it was added. The strains were inoculated on the liquid culture medium for the isolation of bacteria and cultured at 30℃ for 3 days while the strain with excellent nitrogen removal capability was selected. The isolation medium was composed of 0.7 g $(NH_4)_2SO_4$, 0.8 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g $FeSO_4 \cdot 7H_2O$, and 3.0 g $CaCO_3$ per 1 liter of medium, with the pH adjusted to 7.0.

Identification and preservation of isolated strains

After investigating the morphological, physiological, and biochemical characteristics of the isolated strains, the identification of the strain was carried out according to Bergey's manual of determinative bacteriology [15] and biochemical tests for the identification of medical bacteria (2nd ed.) [12]. The DNA base composition of each strain (G+C contents) was analyzed by reversed-phase HPLC according to the method suggested by Tamaoka and Komagata [17]. The 16S ribosomal RNA gene is the most widely used marker gene in miocrobiology ecology. We therefor performed an additional experiment to demonstrate that sequencing of 16S rRNA gene for identification of bacteria. PCR amplification

of the 16S rRNA genes was performed with primers containing universal primers amplifying the V4 variable region (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTAC HVGGGTWTCTAAT) [3]. PCR products were pooled, column-purified, and size-selected through microfluidic DNA fractionation. Consolidated libraries were quantified by quantitative real-time PCR before loading into the sequencer. Sequencing was performed in a pair-end modality on the Illumina NextSeq 500 platform rendering 2×150 bp pair- end sequences. The isolated strains were preserved by subculture and kept in an ampoule after freeze-drying.

Preparation and characteristics of microbial augmentation

The strain with high ammonium nitrogen removal capabilities among the strains isolated from the samples of soil, stream, and wastewater around food, paper, and leather manufacturing plants was added into the strains which showed a high colony forming capability, total organic carbon (TOC), and chemical oxygen demand (COD) removal rate, among the microorganisms presented in the aeration tank of food wastewater treatment plant, in order to be used for microbial augmentation as a field application for industrial wastewater treatment. This complex microbial augmentation was named FIW-1, which was spread on the PCA medium to investigate the reproducibility of microorganisms and verify the viability of cells.

Measurement of cell mass

Cell mass was measured by washing the cell obtained by centrifugation of the culture solution of isolated strain twice, dried at 105℃ for eight hours, and then determined as dry cell weight (DCW).

Analysis of basic feature of wastewater

The total organic carbon concentration of the subjected wastewater was analyzed using TOC analyzer (Dohrman DC-180). NH_4-N was analyzed by liquid analyzer. Meanwhile, chemical oxygen demand (COD) was analyzed according to standard methods for the examination of water [1].

Pilot test for treating industrial wastewater

A continuous culture test employing a lab scale pilot reactor with acryl was carried out in order to investigate the treatment efficiency of the food industrial wastewater. The wastewater was input into the reactor as continuous reaction was induced to the control group, into which only activated sludge was added, and the test group, into which microbial augmentation was applied to measure wastewater treatment efficiency.

The pilot test for food wastewater treatment efficiency was adjusted for its hydraulic retention time of 24 hr, while the treatment efficiency changes of the control with only activated sludge and treatment block and with microbial augmentation were observed over time. An inoculum of 200 mg/l was added daily.

Results and Discussion

Isolation of microorganism in the aeration tank

The codominant microorganisms in the aeration tank degrade organic sources symbiotically or competitively to use a self-proliferation process and to discharge water and carbon dioxide as a final decomposition product. In order to select a codominant microorganism in the activated sludge in the aeration tank in the food wastewater treatment plant, a sample was spread on the PCA medium and cultured for 3 days. The 7 dominant strains that formed the colony were selected.

Among the strains isolated from the aeration tank, AT2, AT9, and AT12 strains were isolated as dominant microorganisms. When the AT2, AT9, and AT12 strains were added, the treatment efficiency for the total organics in the influent water of the subjected wastewater was higher in the TOC removal activities compared with those of the control group wherein only an activated sludge was added as shown in Table 1. Particularly, in the case of the isolated AT12, the TOC removal rate was 81%, which was the highest treatment efficiency as well as the highest COD removal efficiency. These isolated strains (AT2, AT9, and AT12) were used to prepare the microbial augmentation for biological wastewater treatment.

Isolation of nitrogen removal microorganisms

In order to isolate the ammonium nitrogen (NH_4-N) removal microorganisms, the strains having excellent nitrogen removal capability among the 92 strains were isolated from the soil, stream, and wastewater around food, paper, and leather manufacturing plants nationwide. First, 12 strains with excellent colony forming capabilities when cultured on the solid culture medium containing ammonium nitrogen

Table 1. Selection of microorganisms for application test in the wastewater treatment site

Strains	TOC concentration (mg/l)								
	0 hr	4 hr	Removal rate (%)	8 hr	Removal rate (%)	24 hr	Removal rate (%)	48 hr	Removal rate (%)
AT 2	751	374	50	298	60	203	73	165	78
AT 5	732	428	42	395	46	303	59	271	63
AT 9	725	279	62	269	63	184	75	174	76
AT12	742	311	58	194	74	163	78	140	81
AT16	728	385	47	340	53	284	61	254	65
AT19	745	439	41	379	49	275	63	249	67
AT23	736	412	44	368	50	272	63	235	68
Control	743	445	40	393	47	349	53	267	64

*Control: Only activated sludge was added. TOC: total organic carbon.

for isolation of bacteria were selected. Among the isolated strains, strain FN47, which has exceptional strain growth and ammonium nitrogen removal capability, was finally selected. Isolated FN47 was found to be excellent not only for its ammonium nitrogen removal capability but also for its organic removal activity.

Identification of isolated strains

The microorganisms in the aeration tanks of the wastewater treatment plant were examined, isolated, and investigated for their morphological, physiological, and biochemical characteristics for each strain (AT2, AT9, and AT12) (data not shown). The isolated strain AT2 was a gram-negative bacillus and facultative aerobic bacteria that had mobility. It showed a positive response to the catalase and oxidase, producing acid in the glucose, but showed a negative response toward Voges-proskauer (VP) test. It likewise showed a positive reaction to urease. The G+C content was 60%. These results exhibited similar characteristics with *Pseudomonas* species. It was found that the strain AT2 was a species which was very close to *Pseudomonas sagittaria*, when 16S rRNA of the isolated strain AT2 was analyzed further for more accurate identification (Table 2). Therefore, the isolated strain AT2 was finally named *Pseudomonas* sp. AT2.

The AT9 strain was a gram-negative bacillus and had mobility. It showed a positive response to catalase and negative response to oxidase, forming acid by reacting with glucose. When strain AT9 was used as a carbon source, it could not be fermented. Meanwhile, the strain AT9 could not use nitrate and showed a negative response to urease. The G+C content of DNA was 67%, showing similar characteristics with the *Acinetobacter* species. It was found that the strain AT9 was a species which was very close to *Acinetobacter baumannii*, when 16S rRNA of the isolated strain AT9 was analyzed further for more accurate identification. Therefore, the isolated strain AT9 was finally named *Acinetobacter* sp. AT9.

Meanwhile, the isolated strain AT12 was a gram-positive bacillus and had mobility. The strain AT12 showed a positive response towards both catalase and oxidase. However, AT12 could not produce acid with the glucose. AT12 used nitrate and showed a negative response to urease. The G+C content of AT12 was 58%. These results exhibited similar characteristics with *Alcaligenes* species. It was found that the strain AT12 was a species which was very close to *Alcaligenes aquatilis*, when 16S rRNA of the isolated strain AT12 was analyzed further for more accurate identification. Therefore,

Table 2. BLAST search results of isolated strains

Strains	Description	Max score	Total score	Query cover	Expect value	Percent identity
AT 2	*Pseudomons sagittaria* strain CC-OPY-1 16S rRNA, partial sequence	2639	2639	100%	0.0	99.59%
AT 9	*Acinetobacter baumannii* strain 16S rRNA, partial sequence	2497	2497	99%	0.0	98.12%
AT12	*Alcaligenes aquatilis* strain LMG 22996, 16S rRNA, partial sequence	2501	2501	99%	0.0	98.11%
FN47	*Microbacterium diaminobutyricum* strain RZ 63 16S rRNA, partial sequence	2438	2438	100%	0.0	98.28%

*BLAST : basic local alignment search tool

the isolated strain AT12 was finally named *Alcaligenes* sp. AT12.

The ammonium nitrogen degrading strain FN47 was a gram-positive bacillus having the size of 0.5~0.6×1.2~2.7 μm and possessing mobility. The FN47 colony had a light-yellow color and it was facultative aerobic bacteria that could grow well under aerobic, as well as anaerobic condition. The strain FN47 showed a negative response to urease and well-degraded starch and cellulose. The strain FN47 produced acids by reacting with saccharides like glucose, fructose, and lactose. Its G+G contents of DNA were 71%. These results exhibited similar characteristics with *Microbacterium* species. It was found that the strain FN47 was a species which was very close to *Microbacterium diaminobutyricum*, when 16S rRNA of the isolated strain FN47 was analyzed further for more accurate identification (Table 2). Therefore, the isolated strain FN47 was finally named *Microbacterium* sp. FN47.

Incubation characteristics of isolated strains in the aeration tank

In order to investigate the incubation characteristics of isolated strains *Pseudomonas* sp. AT2, *Acinetobacter* sp. AT9, and *Alcaligenes* sp. AT12, the wastewater itself was used as a culture medium. After adding nitrogen, phosphorus, and micronutrients of 3.0 g $(NH_4)_2SO_4$, 0.3 g K_2HPO_4, 0.02 g $FeSO_4$, 0.02 g $MgSO_4$, and 0.01 g $MnSO_4$ per 1 liter of medium, the growth activities of the isolated strains were investigated. The wastewater was used without sterilization so that the isolated strains could be used in actual wastewater generation sites. Therefore, wastewater had a contamination rate at around 10^5-10^6 CFU/ml in the influent wastewater. Still, the isolated strains occupied most of the wastewater after culture so that affinity for substrate by the isolated strains was elevated. Particularly, when the culture test was conducted according to the nutrient source for isolated strain *Alcaligenes* sp. AT12, high culture characteristics were exhibited in the test group wherein nitrogen, phosphorus, and micronutrients were added compared with the test group with organic nitrogen. The optimum activity of the strain was exhibited after 30 hours of culture (Fig. 1).

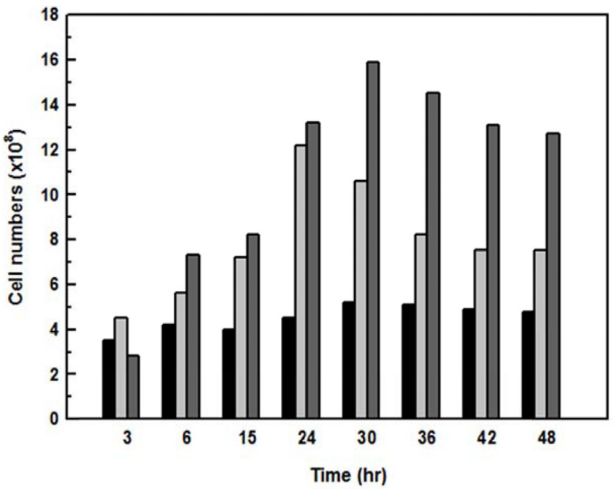

Fig. 1. Culture test using the isolated strain AT12 in the aeration tank of food wastewater. The symbols ; (■) activated sludge, (□) isolate AT12+organic nitrogen source (0.1 g yeast extract), (▣) isolate AT12+3.0 g $(NH_4)_2SO_4$, 0.3 g K_2HPO_4, 0.02 g $FeSO_4$, 0.02 g $MgSO_4$, and 0.01 g $MnSO_4$ per 1 liter of medium.

Ammonium nitrogen removal efficiency in the industrial wastewater

In order to remove ammonium nitrogen contained in the industrial wastewater, the food wastewater having a high nitrogen concentration was adopted for the test (Table 3). The ammonium nitrogen removal rate was depleted in the control where only activated sludge was added, suggesting that the active microorganisms inside the aeration tank suppressed dominance of the nitrification bacteria, which ultimately dropped total nitrogen removal efficiency. However, the ammonium nitrogen removal rate reached 71% in the treatment group with *Microbacterium* sp. FN47, which was excellent compared with that of the control. From the above results, it can be inferred that *Microbacterium* sp. FN47 is an effective ammonium nitrogen removal strain in the wastewater treatment site and is expected to improve water quality.

Table 3. Treatment efficiency of *Microbacterium* FN47 in the food wastewater containing ammonium nitrogen

	Component	Influent	Control	Removal rate (%)	FN47	Removal rate (%)
Food wastewater	NH_4-N (mg/l)	515	310	40	149	71
	T-N (mg/l)	624	356	43	168	73
	TOC (mg/l)	723	129	82	108	85

*Control: only activated sludge was added. T-N: total nitrogen, TOC: total organic carbon.

Preparation of microbial augmentations and their characteristics

In order to investigate the wastewater treatment efficiency of the strains isolated from the aeration tank, the culture broth of the isolated strains *Pseudomonas* sp. AT2, *Acinetobacter* sp. AT9, and *Acaligenes* sp. AT12, as well as the culture broth of ammonium nitrogen degrading strain *Microbacterium* sp. FN47 with the defatted rice bran as a medium, were used to prepare the microbial augmentation FIW-1 for food wastewater treatment.

Optimum media were developed for growing cells of the isolated strains in the batch liquid culture. Each cell culture liquid was mixed in the defatted rice bran at a ratio of 2:8 and then solid phase fermentation was induced, followed by air drying at room temperature to prepare the microbial augmentation with the four strains. These four types of microbial augmentations were mixed at an equal ratio, with a complex microbial augmentation finally prepared. The complex microbial augmentation was prepared by the mixing of each strain in the defatted rice bran at a similar ratio. The nutrient solution of 0.5% yeast extract was added at 5% (v/w) in the rice bran, and combined with nitrogen, phosphorous source, and mineral nutrients Ca^{2+} and Fe^{2+}. Ca^{2+} was added to fill alkalinity and Fe^{2+} was added to relax the absorption of microorganisms in the form of $CaCl_2$ and $FeSO_4$. AMP, ADP, $CaCl_2$, and $FeSO_4$ were also added at a weight ratio of 10% (W/W) by combining 1:1:2.0:0.5, respectively. The combined complex microbial augmentation was named FIW-1, and in the subsequent experiments, FIW-1 was added to the experimental group to examine its activity. The probiotic activity of the strains contained in the complex microbial augmentation FIW-1 was maintained at about 10^9 cells without significant change within 45 days of room temperature storage. The moisture content of the complex microbial augmentation containing four types of strains was measured 28.5%.

For the isolated strains, an optimum medium composition was established for cell growth in the batch liquid culture, and each culture broth was mixed with the defatted rice bran at a ratio of 2:8, followed by solid phase fermentation for 2 days. The fermented cell was air-dried to make each microbial preparation with five types of strains. These five strains were mixed for the microbial augmentation. All the five strains for the microbial augmentation maintained a cell mass higher than 10^9 CFU/g. In the case of a microbial augmentation FIW-1 where five microbial strains were mixed, the cell mass was 2.4×10^{10} CFU/g, the density was 0.305 g/cm^3, and the moisture content was 28.5% (Table 4). There was difficulty in wastewater treatment in the actual wastewater treatment plants due to the mixing of various materials including recalcitrant organics. The microorganisms could be made as a formulation or immobilized, and then inoculated so that the density of the population can be raised for a biodegradation process in the wastewater treatment plants [4].

Table 4. Characteristics of microbial augmentation FIW-1

Characteristics	Contents
Total viable count	2.1×10^{10} CFU/g
Bulk density	0.305 g/cm^3
Water content	28.5%
Partical size	60~90 mesh

Pilot test for the effectiveness of industrial wastewater by microbial augmentation FIW-1

The wastewater treatment test for the subjected wastewater from the food industry was conducted in the lab scale reactor to confirm the applicability of the microbial augmentation FIW-1 for industrial wastewater treatment.

The treatment efficiency test was carried out for the control in which only activated sludge from the test site was added and for test group into which microbial augmentation FIW-1 was added in the pilot for continuous treatment. The 1st treated water was set as an influent during five days before the inputting of the microbial augmentation FIW-1 and before the treated supernatant of aeration tank was set as an effluent. The TOC, COD, and ammonium nitrogen concentration were measured after making the reaction continue for 10 days in the culture.

In the case of control wherein only activated sludge was added, the TOC was removed at a rate of 63%, while the COD removal rate was 64%. However, when microbial augmentation FIW-1 was applied, the TOC and COD removal rates were increased to 92% and 85%, respectively. In the case of ammonium nitrogen removal rate, it was 37% in the control, and 82% of removal rate was observed from the test group with microbial augmentation FIW-1 (Table 5).

Field Application Test

A test was carried out to ascertain if the microbial augmentation of FIW-1 can be applied in the wastewater treatment site. The average COD in the influent water for 5 days

Table 5. Effectiveness of wastewater treatment using microbial augmentation FIW-1 on bench scale pilot

Treatment Group		TOC (mg/l)	COD (mg/l)	NH_4-N (mg/l)
Activated sludge (control)	Influent	716	986	521
	Effluent	265	355	328
	Remaval rate (%)	63	64	37
Microbial augmentation, FIW-1	Influent	723	1,034	538
	Effluent	58	155	97
	Removal rate (%)	92	85	82

*TOC: total organic carbon, COD: chemical oxygen demand.

before field test was 985 mg/l and in the effluent water 621 mg/l, showing that the COD removal rate of the aeration tank was merely 37%. However, during 3 days after inputting of the microbial augmentation FIW-1, a constant treatment rate (43%) was observed, followed by 62% of the treatment rate with a COD value of 374 mg/l after an elapse of 5 days.

These results indicated that the microbial augmentation FIW-1 was composed of a dominant microorganism, and ammonium nitrogen removal microorganism can establish an effective biological treatment system not only in the food wastewater which was the subjected wastewater in the study but also in the paper mill wastewater and leather wastewater containing ammonium nitrogen if it is used through a site adaption process.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

References

1. APHA, AWWA, and WEF. 1992. Standard methods for the examination of water and wastewater. 18th eds., APHA, Washington.
2. Chung, G. T., Park, S. H., Park, J. H., Lim, E. T., Bang, S. H. and Park, D. H. 2009. Effect of factors of nitrification process in wastewater treatment. *Kor. J. Biotech. Bioeng.* **24,** 296-302.
3. Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D, Lozupone, C. A., Turnbaugh, P. J., Fierer, N. and Knight, R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci.* **108,** 4516-4522.
4. Haggblom, M. M., Nohyjek, L. J. and Salkinoja-Salonen, M. S. 1988. Degradation *o*-methylation of chlorinated phenolic compounds b *Rhodococcus* and *Mycobacterium* strains. *Appl. Environ. Microbiol.* **54,** 3040-3052.
5. Kennedy, J. F. and Bradshow. 1984. Progress in industrial *microbiology*. Bushell, M. E. (ed.) Elsevier. **19,** 319-365.
6. Layton, A. C., Karanth, P. N., Lajoie, C. A., Meyer, A. J., Gregory, I. R., Stapleton, R. D., Tayler, D. E. and Sayler, G. S. 2000, Quantification of hypomicrobium populations in activated sludge from an industrial wastewater treatment system as determined by 16 rRNA analysis. *Appl. Environ. Microbiol.* **66,** 1167-1174.
7. Lazarova, V. and Manem, J. 1995. Biofilm characterization and activity analysis in water and wastewater treatment. *Wat. Res.* **29,** 2227-2245.
8. Lee, S. L., Yoo, S. Y., Chung, S. Y., Park, C. S. and Choi, Y. L. 2003. Microbial immobilization, characterization and isolation of nitrogen oxidizing bacteria. *Appl. Biol. Chem.* **46,** 1-6.
9. Leisinger, T., Cook, A. M., Hutter, R. and Nuesch, J. 1981. Microbial degradation of xenobiotics and recalcitrant compounds. Academic Press. Zurich.
10. Liu, Y. and Capdeville, B. 1996. Specific activity of nitrifying biofilm in water nitrification process. *Wat. Res.* **30,** 1645-1650.
11. Loosdrecht, V. and Heijnen, S. J. 1993. Biofilm bioreactors for wastewater treatment. *Trends Biotechnol.* **11,** 117-121.
12. MacFaddin, J. F. 1984. Biochemical tests for identification for medical bacteria. 2nd eds., Williams and Wilkins Co., Baltimore, Maryland.
13. Manka, J., Rebhun, M. and Mandelbaum, A. 1974. Characterization of organics in secondary effluents. *Environ. Sci. Technol.* **8,** 1017-1020.
14. Paniagua-Michel, J., Franco-Rivera, J. A., Cantera, J. L. and Stein, L. Y. 2005. Activityof nitrifying biofilms constructed on low-density polyester enhances bioremediation of acoastal wastewater effluent. *World J. Microbiol. Biotechnol.* **21,** 1371-1377.
15. Peter, H. A. S., Nicholas, S. M., Sharpe, M. E. and Holt, J. F. 1986, Bergey's manual of systematic bacteriology, Williams and Wilkins Co., Baltimore, Maryland.

16. Shin, S. B. and Ichikawa, K. 1980. Wastewater treatment by microorganism. *Kor. J. Appl. Microbiol.* **66,** 5201-5205.
17. Tamaoka, K. and Komagata, K. 1984. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25,** 125-128.
18. Tanaka, K., Tada, M., Kimata, T., Harada, S., Fuji, Y., Mizuguchi, T., Mori, N. and Emori, H. 1994. Development of new nitrogen removal system bacteria immobilized in synthetic resin pellets. *Wat. Sci. Tech.* **1,** 681-690.
19. Van Loosdrecht, M. C. M. and Heijnen, S. J. 1993. Biofilm bioreactors for wastewater treatment. *Trends Biotechnol.* **11,** 117-121.
20. Wagner, M. and Loy, A. 2002. Bacterial community composition and function in sewage treatment systems. *Curr. Opin. Biothechnol.* **13,** 218-227.

초록 : 암모니아성 질소함유 산업폐수처리를 위한 미생물의 분리 및 복합미생물제제의 개발

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암모니아성 질소(NH_4-N) 함유폐수의 효과적인 처리를 위하여 암모니아성 질소가 함유되어있는 식품폐수처리장의 폭기조 활성오니에 우점적으로 존재하는 미생물 중 TOC (total organic carbon) 제거율이 높은 AT2, AT9, AT12 균주와 암모니아성 질소 제거능이 우수한 FN47을 분리, 동정하였다. 이들 분리균주의 균체를 탈지강을 담체로하여 산업폐수처리용 미생물제제 FIW-1 을 제조하였다. 폭기조 분리균주들의 대상으로 폐수자체를 배양기질로 하여 배양학적 특성을 조사하였을 때 분리균주들의 기질친화성이 매우 높은 것으로 나타났으며, 분리균주 중 AT12 균주(*Alcaligenes* sp. AT12)가 가장 높은 기질친화성을 나타내었다. 식품폐수 중의 암모니아성 질소 제거효율은 분리균주 FN47 (*Microbacterium* sp. FN47)의 처리구에서 71%의 제거율을 나타내었다. 실험실규모의 반응조에서 식품폐수를 이용한 연속배양 실험에서 활성슬러지만을 첨가한 경우 63% TOC제거효율을 나타내었으며, 폐수처리용 미생물제제 FIW-1을 첨가 시에는 92%의 제거효율을 나타내었다. 또한 폐수처리용 미생물제제 FIW-1의 폐수처리장 현장실험에서는 미생물제제 FIW-1을 투입한 초기 3일간의 유출수COD (chemical oxygen demand) 값은 43% 내외의 처리율을 나타내었으나, 5일이 경과하였을 때 유출수의 COD값은 62%의 처리효율을 나타내었다.