Thesis for the Degree of Master of Engineering

Field experiment and economic analysis of fish-processing wastewater fertilizer by biodegradation.

by

Jang Ho Kang

Department of Biotechnology

The Graduate School

Pukyong National University

February 2015

Field experiment and economic analysis of fish-processing wastewater fertilizer by biodegradation.

생물분해로 만든 수산 가공 폐액 비료의 현장시험과 경제성 분석

Advisor: Prof. Joong Kyun Kim

by

Jang Ho Kang

A thesis submitted in partial fulfillment of the requirements

for the degree of

Master of Engineering

in Department of Biotechnology, The Graduate School,

Pukyong National University

February 2015

Field experiment and economic analysis of fish-processing wastewater fertilizer by biodegradation.

A dissertation

by

Jang Ho Kang

Approved by:

____

In-Soo Kong

(Chairman)

____

Gwi-Taek Jeong

(Member)

____

Joong-Kyun Kim

(Member)

February 27, 2015

CONTENTS

LIST OF FIGURES AND TABLE

Field experiment and economic analysis of
fish-processing wastewater fertilizer
by biodegradation.

강 장 호

부경대학교 대학원 생물공학과

요 약

본 연구는 생 분해된 수산 가공 폐수의 비료적 가치와 경제성을 증명하였다. 액체비료로 되기 위한 수산 가공 폐액의 생 분해는 1 톤 파일롯 규모의 생물반응기에 11 종의 혼합 균주를 접종하여 80 시간 동안 수행하였다. 그 결과 수산 가공 폐액 비료(FPWF)는 상업 유기비료(COF)에 비해 낮은 중금속 농도 및 높은 아미노산 농도를 보였다. FPWF 와 COF 들을 사용한 배추(*Brassicarapa* L. ssp. *pekinensis*) 와 상추(*Lactucasativa* L.) 에서의 2 달 동안의 현장시험에서는 COF 들에 비해 FPWF 쪽이 식물 성장 파라미터가 향상 되었다. 또한 경제성분석을 수행한 결과 년간 24,931 달러의 긍정적인 현금흐름을 보였고 회수기간은 약 1.3 년으로 나타났다.

Field experiment and economic analysis of
fish-processing wastewater fertilizer
by biodegradation.

Jang Ho Kang

Department of Biotechnology, The Graduate School,
Pukyong National University

Abstract

The present study demonstrated that biodegraded fish-processing wastewater is capable of fertilizing-value and economic-feasibility. A scale-up biodegradation of fish-processing wastewater into liquid fertilizer was performed by inoculation of fish-processing wastewater of mixed 11-species microorganisms in a 1-tons pilot scale bioreactor for 80 h. As a result, fish-processing wastewater fertilizer (FPWF) showed high amino-acids content and low heavy metals concentration as compared to commercial organic fertilizers (COF). Chinese cabbage (*Brassicarapa* L. ssp. *pekinensis*) and lettuce (*Lactucasativa* L.) were cultivated using a FPWF and COFs in a field for 2 months, improved plant growth parameters (leaf number, leaf length, leaf size, leaf width and dry weight) as compared to COFs. Economic analysis showed positive cash flow of about 24931$/year and it has a payback time of about 1.3 years.

Ⅰ. INTRODUCTION

In 2012, Korea manufactured 1,885,427 tons of fish-processing products (Ministry of Ocean and Fisheries, 2013). It is increasing every year due to the consumer`s image, fostered by recent study, that fish is a healthy choice to other animal food (Muthukumaran and Baskaran, 2013).The fish-processing produces more than 60% waste, which includes bones, fins, head, scale and viscera. And only 40% fish products for human utilization.

Fish-processing wastes are treated by conversion into fishmeal and fish oils (Adeoti and Hawboldt, 2014 and Chalamaiah et al., 2012). But, this processing plant consumes large quantity of water and generates wastewater both in the processing and cleaning stages, characterized by high concentrations of organics including proteins, nutrient, oil and fats. In particular, the organic contaminants are in soluble, colloidal and particulate forms which vary largely between processing plant and fish type (Muthukumaran and Baskran, 2013).Thus, left unattended fish-processing waste and wastewater are usually disposed of landfill, burning, or by dumping into the sea. The landfill of fish-processing wastes has caused several problems such as rotten smells and leachate polluting ground and surface waters (Yun et al., 2000).

Also, dumping will then be prohibited in Korea according to the London

Convention (International Maritime Organization, 2006). But, in 2013, fish-processing waste and wastewater of 3,000 tons were loaded ocean dumping in Korea (Ministry of Ocean and Fisheries, 2013). These large quantities of fish-processing wastes from fisheries would create serious pollution and disposal problems in both developed and developing countries (Chalamaiah et al., 2012).

Thus, it is necessary to focus environmentally acceptable methods for reutilization of fish-processing waste. Several methods have already been performed. For example, production of omega-3 fatty acids (Nges et al., 2012), biodiesel (Wu et al., 2014 andGarcía-Moreno et al., 2014), esterase (Esakkiraj et al., 2012), fertilizer (Illera-Vives et al., 2013, Kim et al., 2007, 2010 and Dao and Kim, 2011) have been obtained from fish-waste.

Especially, reusing of fish-processing wastes such as organic wastes can provide nutrients for plant growth as well as improve soil health (Yadav and Gang, 2011).

Common and many use of N, P and K fertilizer such as chemical fertilizers has caused in soil quality and environmental degradation and heavy metals pollution (Brockmann et al., 2014, Carbonell et al., 2011 and Zhu et al., 2012).Therefore, there has been a recent resurgence of interest in environmentally friendly, sustainable and organic agricultural practices.

This system avoids or largely excludes the use of synthetic fertilizers, pesticides, growth regulators and livestock feed additives. Environmentally friendly organic agricultural systems depend heavily on bio-fertilization, green compost, farm manure, crop rotation, legumes, mineral-bearing rocks, and aspects of biological pest control to sustain soil productivity (Güneş et al., 2014).

Bio-fertilizers are products including living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Wu et al., 2005). A considerable number of fertilizer sources, mostly those associated with hormonal, organic or amino acid contents, promote plant growth. These ingredients render insoluble forms of plant nutrients into soluble forms through acidification and exchange reactions. These processes compensate for the higher cost of manufacturing fertilizers in industry and mobilize the fertilizers added to soil.

Organic fertilizer, especially low grade, has received great attention as a source of plant growth-promoting bacteria (PGPB) and for use in agriculture. Natural organic foliar fertilizer is an effective method for feeding plants in soil that is deficient or contains unavailable forms of nutrients because plants absorb nutrients through their roots and foliage (Güneş et al., 2014).

Especially, amino acids are not only building blocks of proteins in addition to its structural role in proteins, amino acids functions as a precursor for essential biomolecules, such as vitamins and cofactors, antioxidants and many defense compounds. In young plants, amino acid biosynthesis is regulated by a compound metabolic network that links nitrogen assimilation with carbon metabolism (A´lvarez et al., 2010, Jander and Josh, 2010, Maeda and Dudareva, 2012, Miret et al., 2014 and Szabados and Savoure, 2010).

Therefore, the importance of bio-fertilizer is to provide socioeconomic and ecological benefits among which are improvements of soil quality, food quality and safety, human and animal health as well as environmental quality (Owamah et al., 2014).

During the past few decades, there has been increased interest in the use of beneficial microbial inoculations to improve plant and soil functions. Several microorganisms, such as plant growth-promoting bacteria (PGPB), have been widely studied (Figueiredo et al., 2010 and Calvoe et al., 2012).

PGPB were reported to increase the plant growth by reducing susceptibility to diseases caused by plant pathogenic fungi, bacteria, viruses, and nematodes and facilitate to grow under abiotic stress conditions such as drought and salinity (Khan et al., 2011, Nautiyal et al., 2013 and Siddikee et al., 2011).Reports are now accumulating on application of PGPB as elicitors for tolerance to abiotic stresses, such as drought, salt and nutrient deficiency in plants and raising possibility for incorporation of microbial genes into plant and diverse microbial species (Apse et al., 1999, Nautiyal et al., 2013 and Yang et al., 2009). Among the PGPB microorganisms, Bacillus spp. are widely used, mainly because they can survive as spores and can potentially alter the soil microbial composition. Bacillus spp. have a wide metabolic capability that allows them to play important roles in soil ecosystem functions and processes. Due to their heterotrophic nature, Bacillus spp. play an important role in the soil C cycle, soil N cycle, soil S cycle, and transformation of other soil nutrients (Mandic-Mulec and Prosser, 2011and Calvo et al., 2012). Furthermore, they act as biocontrol agents due to the wide range of antiviral, antibacterial, and antifungal compounds they produce, which can control pathogens and have an effect on other soil microorganisms (Chaabouni et al., 2012). Antibiotics are important metabolites that are produced by Bacillus spp. They not only can control pathogens but also confer a competitive advantage over other soil microorganisms (Stein, 2005).

Field experiment has many benefits. For example, farmers can test the recommendations of agricultural extension services in a wide range of production conditions. Farmers can verify if small-plot experiment station research applies to field scales under local environmental and management practices. Testing of equipment, systems or technology packages is also a benefit of on-farm trials. Apart from these benefits, the costs of field experiment trials are very low compared to the costs of experiments at a research institute (Meyer-Aurich et al., 2010).

Aerobic biodegradation is one of the most useful methods to convert fish waste into fertilizer by *bacillus* sp. Previous studies have examined fertilizer production from fish-waste (Kim et al., 2007, 2010 and Dao and Kim, 2011). But, most experiments have been of at a laboratory scale.In present study, bio-fertilizer was produced from fish-processing waste using a 1 m^3 pilot-scale. In addition, It was to investigate the effect of fish-processing waste for bio-fertilizer inoculation on field experiment of the Chinese cabbage(*Brassicarapa* L. ssp. *pekinensis*) and the lettuce (*Lactucasativa* L.). In addition, economic analysis of bio-fertilizer for sustainability was evaluated.

Ⅱ. MATERIALS AND METHODS

1. Microorganisms

The mixed microorganisms used in this study are comprised of eleven microorganisms: *Bacillus subtilis, Bacillus coagulans, Bacillus circulans, Bacillus anthracis, Bacillus agri, Bacillus licheniformis, Bacillus fusiformis, Bacillus cereus, Brevibacillusagri, Bacillus licheniformis and Brevibacillusparavrevis* (Kim et al., 2007 and 2010).They were isolated from humus, compost leachate and the viscera of earthworm. These were potential aerobically-degrading bacteria and there was no potential bacterial antagonist among them (Geun and Kim, 2012). Equal amounts of cells from each strain were separately cultivated and combined together. Then, the mixed microorganisms were used as an inoculum for the 1 m^3 pilot bio-reactor experiment. Each pure strain was maintained on 1.5% (w/v) Nutrient agar plate at 4˚C and subculture was transferred to a fresh agar plate every two weeks until used. At the same time, the potential degrading ability of each pure culture was periodically checked on 1% (w/v) skim milk agar and 3.215% (w/v) spirit blue agar. All agar plates were incubated at 45˚C until a change in color or a clear zone around each colony was observed.

2. Biodegradation of fish-processing wastewater

Fish-processing wastewater was obtained from a fish-meal processing plant located in Busan (Korea). These wastewater was boiled at 80 ℃ for 3 h for sterilization. It was pumped into reactor. The reactor was preliminarily washed with tap water that contains 5% (w/v) sodium hydroxide to remove microorganisms attached on the surface of the reactor wall. Then, the reactor was sterilized by steam equipment at $3.0 \cdot 10^5$ Pafor 30 min and it was filled with 600 L of hot fish-processing wastewater under steaming. When the reactor temperature cooled down to 45 ± 1 ℃, a 110 g of mixed microorganisms (wet weight basis) that contains equal weights of each eleven strain were inoculated into a reactor. Temperature of the reactor was maintained at 45 ± 3 ℃ by cooling system (25 L/min). Air supply was using 5-oxygen generator (25 L/min and 90 ± 3% purity) and booster (120 L/min). In order to avoid contamination from outer foreign microorganisms, discharging air or oxygen was passed through a 10-L flask that contained 10-M sodium hydroxide. The two flasks were put in ice to collect evaporated water from the discharging air. When severe foaming occurred during bioconversion, 10-fold diluted antifoam was used. Samples were periodicallytaken from the bioreactor every 6 h. The concentrations of DO, ORP and pH were measured for the every 2 h.

3. Field experiment of fish-processing wastewater fertilizer

The field experiment was performed from March to May 2012 at the Nature Resource and Life Science attached farm of Pusan National University, using a cabbage (*Brassica rapa*L. ssp. *pekinensis*) and a lettuce (*Lactuca sativa* L.) field. Twenty-four plots (20 m × 12 m) were defined, and fertilized plots (fish-processing wastewater fertilizer and two commercial organic fertilizer) and non-fertilized plots were established in sandy-loam soil. Planting range and soil depth were 320 stocks at $10m^2$ and 15cm, respectively. To apply the recommended amount of two commercial organic fertilizer (each 1000-fold diluted), fish-processing wastewater fertilizer with an equivalent of 1000-fold diluted was applied. After 2 months, 30 samples were harvested in each plot. Themeasurements were conducted leaf length, number, width, area and fresh weight.

4. Economic analysis

Economic analysis is important factor for investment. To evaluate the feasibility of the system, a cost-benefit analysis of the fish-processing wastewater reutilization system was conducted. In this study the interest rate was set to 3.69% (The bank of Korea economic statistics system, 2014) and the project life, 10 years.Total cost was calculated by process parameters, which are cost of operation and others (Sánchez-Segado et al., 2012, Tufvesson et al., 2011 and 2013). The cost parameters were shown in table 1.

Cost benefit analysis originates from the premise that a project is only economically feasible if all incomes exceed the aggregate costs. In other words, according the Eq. (1) project have economically feasibility when net present value (NPV) wasmore thanto zero (Marenya et al., 2012).

$$NPV = \sum_{t=0}^{n} \frac{BFt\text{-}CFt}{(1+r)^t} - IC \qquad (1)$$

Where $BFt$and $CFt$are the benefit flow and cost flow at time t respectively, r is the interest rate, n is the project life, IC is the investment capital.

Table 1. Cost parameters used in estimate operation cost.

Cost parameters	Unit	Cost
Equipment cost		
Equipment	$[a]	25,411.76
Construction	$	762.35
Chemical substances		
Nutrient broth	$/kg	267.29
Skim milk	$/kg	82.82
Spirit blue	$/kg	946.82
Tributyrin	$/L	207.44
Polysorbate 80	$/L	142.86
Agar	$/kg	145.68
Sodium hydroxide	$/kg	27.78
Antifoam	$/kg	5.64
Utility		
Process water	$/m^3	0.21[b]
Electricity	$/kWh	0.06[c]
Other cost		
Maintenance	$/year	522.74
Labor	$/year	38,180.45
Insurance	$/year	1,879.7
Taxes	$/year	93.98

[a] 1 $= 1063.7 ₩ (Korea exchange bank, 2014)

[b] Process water price referenced by Korea water resource corporation, 2014.

[c] Electricity price referenced by Korea electric power corporation, 2014.

5. Analytical methods

The concentrations of Na^{+}, NH_4^{+}, NO_3^{-}, PO_4^{3-} and SO_4^{2-} were analyzed by ion chromatography (Metrohm 792 Basic IC, Switzerland). The columns used in these analyses were Metrosep C2-150 (150 × 4.0 mm) and Metrosepsupp 5-150 (150 × 4.0mm) for anions and cations respectively. The concentrations of chemical oxygen demeand (COD-Cr) and total nitrogen (TN) were analyzed by a Water-quality analyzer (Humas Co., Ltd, Korea). The dry-sludge weight (DSW) was determined by weighing 5 ml in an aluminum dish after being dried in an oven at 105 ℃ for 12 h. The viable cells number expressed as colonies forming units (CFU) were estimated with a proper dilution factor by counting colonies formed on the agar plate of the nutrient broth medium containing 1.5% (w/v) agar, which were expressed as CFU per ml of sample. Amino-acid composition, N, P and K content were determined by Life Science Lab Korea Basic Science Institute (Korea). Concentration of the heavy metals As, Cd, Cr, Cu, Hg, Ni, Pb and Zn were determined by Foundation of Agri, Tech, Commercialization and Transfer (Korea).

Ⅲ. RESULTS AND DISCUSSION

1. Fish-processing wastewater fertilizer analysis

Operation of the reactor was continued until spore of microorganisms formed. Fig. 1 and Fig. 2show the characteristics of biodegraded fish-processing wastewater during the total 80h biodegradation period.

Fig. 1(a) presents the amount of COD-cr and TN in the FPWF was found to be reduced by 74.3% and 68.9% respectively. The carbon/ nitrogen ratio of the FPWF was from 9.75 to 7.86. As seen in Fig. 1(b), the amount of DSW reduced 54.2%. The cell number increased from 1 x 10^6 CFU/ml to 7.13 x 10^6 CFU/ml. The pH decreased steadily to 5.69. The drop in pH was due to production of amino acids, which was also seen in our previously studies involving fish-waste (Kim at al., 2007, 2010, Dao and Kim, 2011, Geun and Kim, 2012). The results mean that the cell grew on degraded fish-processing wastewater. Fig. 2(a) represents the changes in the concentrations of cations. The concentrations of Na^+ did not change significantly until 80 h. But, the concentration of NH_4^+ increased until 80 h, and the maximum concentration was 770.67 mg/l. NH_4^+ is produced through the biodegradation of protein-rich material (Nges et al., 2012). Fig. 2(b) represents the changes in the concentrations of anions. The concentration of NO_3^-, PO_4^{3-}, SO_4^{2-} increased until 80 h, and the maximum concentrations were 116.81

mg/l, 1080 mg/l and 179.13 mg/l respectively. Since these concentrations reduced near the end, the anions must have been at least incompletely utilized by the microorganisms (Yamamoto et al., 2005).

Amino-acids are an necessary part of the active portion of organic matter in a fertilizer. The growth of plants depends finally upon the availability of a suitable balance of amino-acids. The amino-acids composition of the FPWF is shown in Table 2. During biodegradation, the amino-acids total content in the FPWF increased from 4.16 g/100g to 14.66 g/100g. The total amino-acid was higher than the previous content of results (Kim at al., 2007, 2010, Dao and Kim, 2011, Geun and Kim, 2012). In addition, the content of total amino-acids of FPWF was higher in comparison with that of COFs.

Table 3 shows the concentrations of N, P, K and heavy metals. The concentrations of heavy metals were lower than COFs. But, the concentrations of N, P and K were 2.26%, 0.87% and 0.65% respectively, which were half levels than COFs.

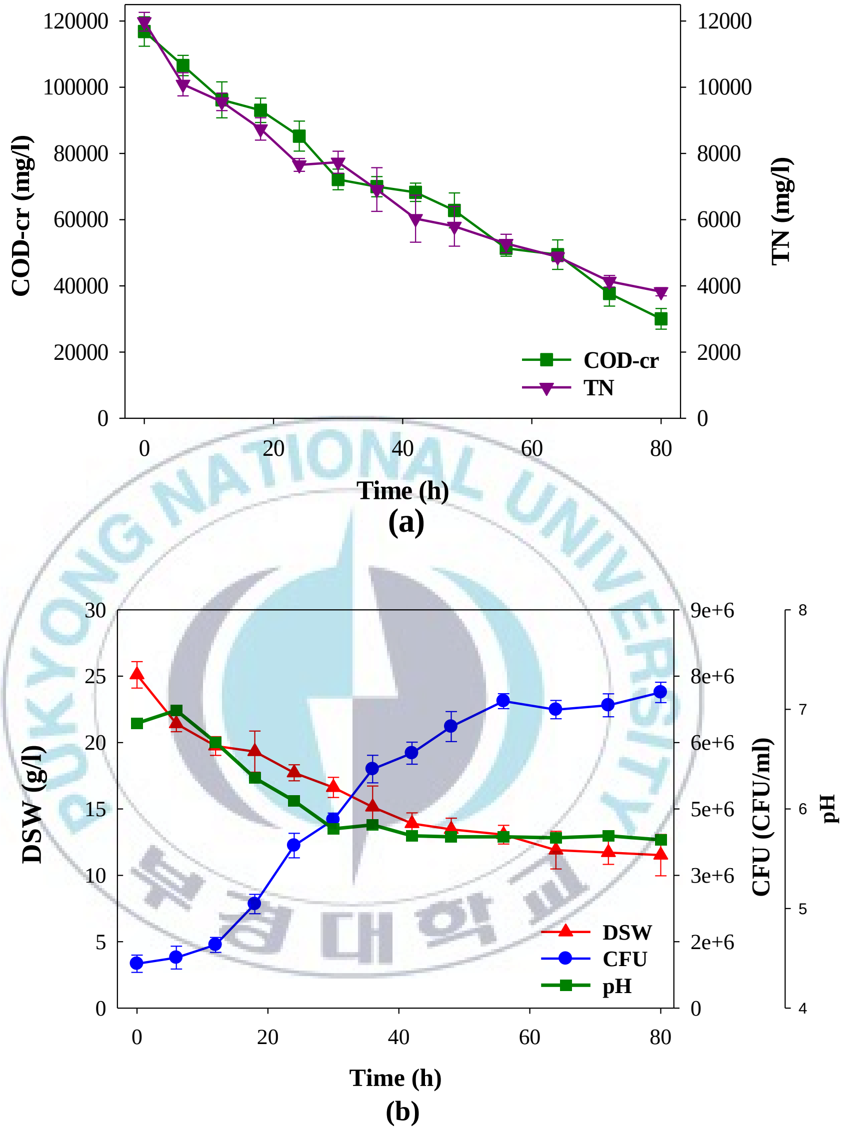

Fig. 1. Changes of COD-cr, TN (a), DSW, CFU and pH (b) during the biodegradation of fish-processing wastewater. Error bars represent the standard deviations of the mean values of three replicates.

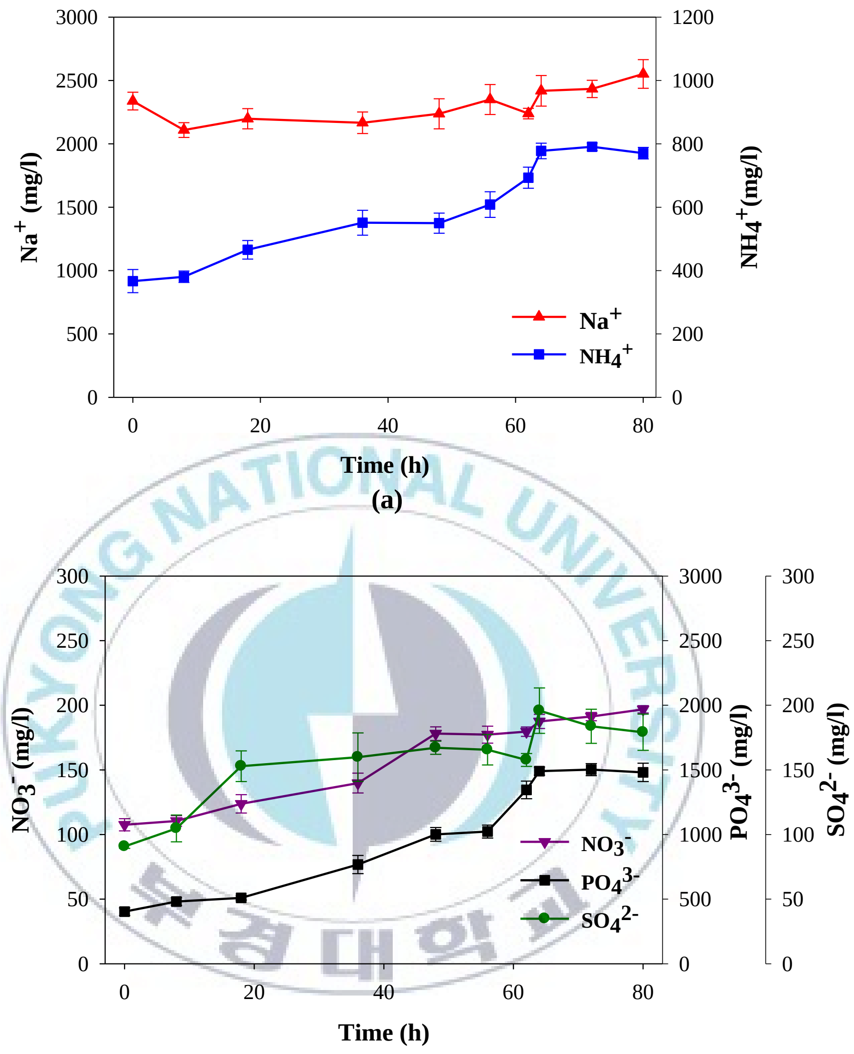

Fig. 2. Changes of the concentrations of cations (a) and anions (b) during the biodegradation of fish-processing wastewater. Error bars represent the standard deviations of the mean values of three replicates.

Table 2. Comparison of amino acid composition between FPWF, COF1 and COF2.

Amino acid	Source[a]			
	Initial FPWF[b]	FPWF	COF1[c]	COF2[c]
Cystein	0.02	0.04	n.d.	0.02
Aspartic acid	0.02	0.34	0.72	0.59
Glutamic acid	0.09	1.11	0.49	0.89
Glutamine	0.08	0.21	1.08	n.d.
Glycine	0.06	1.68	2.36	1.25
Threonine	0.18	0.69	0.18	0.22
Alanine	0.27	1.03	0.27	0.99
Serine	0.06	0.21	n.d.	0.3
Proline	0.33	0.40	0.96	0.61
Tyrosine	0.11	0.14	0.33	0.05
Valine	0.86	1.12	0.51	0.24
Methionine	0.08	1.55	1.26	n.d.
Isoleucine	0.22	1.02	0.38	0.14
Leucine	0.68	1.08	0.22	0.26
Phenylalanine	0.31	1.46	0.68	0.18
Tryptophan	n.d.[d]	0.97	0.31	n.d.
Lysine	0.79	1.43	0.82	0.53
Argine	n.d.	0.18	n.d.	0.15
Total	4.16	14.66	10.57	6.42

[a]Unit of amino acid composition is g/100g dry weight.

[b]Fish-processing wastewater fertilizer

[c]Thisis one of the well-selling organic fertilizers in Korea.

[d] Not detected.

Table 3. Comparison of concentrations of N, P, K and heavy metals between the FPWF, COF1 and COF2.

Component	FPWF[a]	COF1[b]	COF2[b]
Heavy metals (mg/kg)			
Cd	n.d.[c]	n.d.	n.d.
Cu	1.06	14.62	2.72
Ni	n.d.	0.37	2.16
Pb	n.d.	n.d.	0.33
Zn	2.89	71.30	6.97
Hg	n.d.	n.d.	n.d.
Cr	0.79	17.57	3.30
As	0.22	4.71	2.94
N, P, K (%)			
N	2.26	3.67	1.57
P_2O_5	0.87	2.89	1.95
K_2O	0.65	1.46	2.19

[a]Fish-processing wastewater fertilizer

[b]Thisis one of the well-selling organic fertilizers in Korea.

[c] Not detected.

2. Field experiment by fish-processing wastewater fertilizer

Results of the statistical analysis of the effect of FPWF in lettuce and Chinese cabbage growth are shown in Fig. 3. Among the field experiments, FPWF showed the best promoting effects on the growth of the lettuce and the Chinese cabbage. For instance, the lettuce of leaf number, leaf length, leaf size, leaf width and dry weight in the FPWF group increase by 23.75%, 25.78%, 29.39%, 30.74% and 28.41% over non-fertilizing plot (control group), respectively. The Chinese cabbage increased by 40.86%, 24.86%, 29.34%, 23.01% and 23.62% over non-fertilizing plot, respectively. And, growth of plants were some better than COFs.

Improving the growth of plants are the primary functions of fertilizer. FPWF produced positive effect on plant growth and the availability of water-soluble nutrients, which are easily absorbed by plants. FPWF biodegraded by 11-species Microorganisms using fish-processing wastewater contained many amino-acids. This FPWF has important potential application as a non-toxic, high efficiency and environmentally friendly liquid fertilizer.

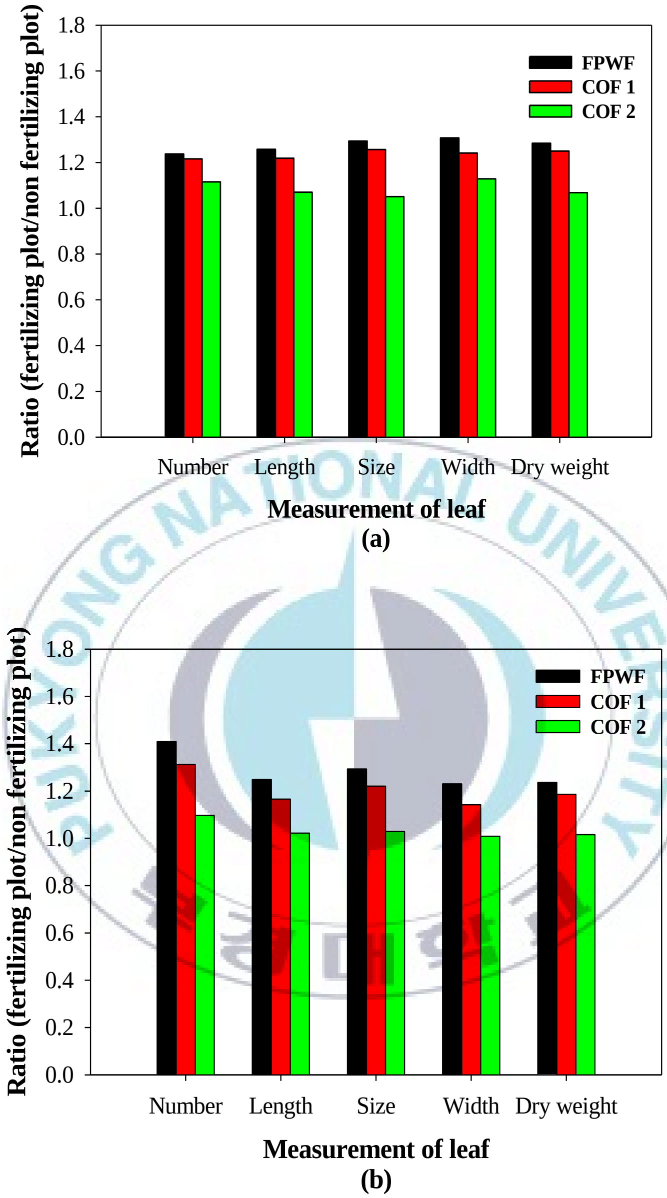

Fig. 3. Comparison of growth of lettuce (a) and Chinese cabbage (b) between FPWF, COF 1 and COF 2.

3. Economic analysis

Table 4 shows a summary of the process evaluation and the factors used for calculating the operation cost. As shown in Table 4, the total operating cost of FPWF is 70,613.18 $/year. And, according to Table 1 initial investment capital (equipment cost) is 26,174.11 $. The income for sale the fertilizer was determined to 1.88 $/l and it is the average price of the COFs that was used for in this study.

A conventional net present value (NPV) analysis is showed in Fig. 4. As a result, generates a positive cash flow of about 24,931 $/year and it has a payback time of about 1.3 years.

Table 4. Calculation of the operating cost for production of FPWF.

Cost factor	Cost ($[a]/year)
Chemical substances	25,223.64
Utility	4,712.74
Other cost	40676.87
Total	70,613.18

[a] 1 $= 1063.7 ₩ (Korea exchange bank, 2014).

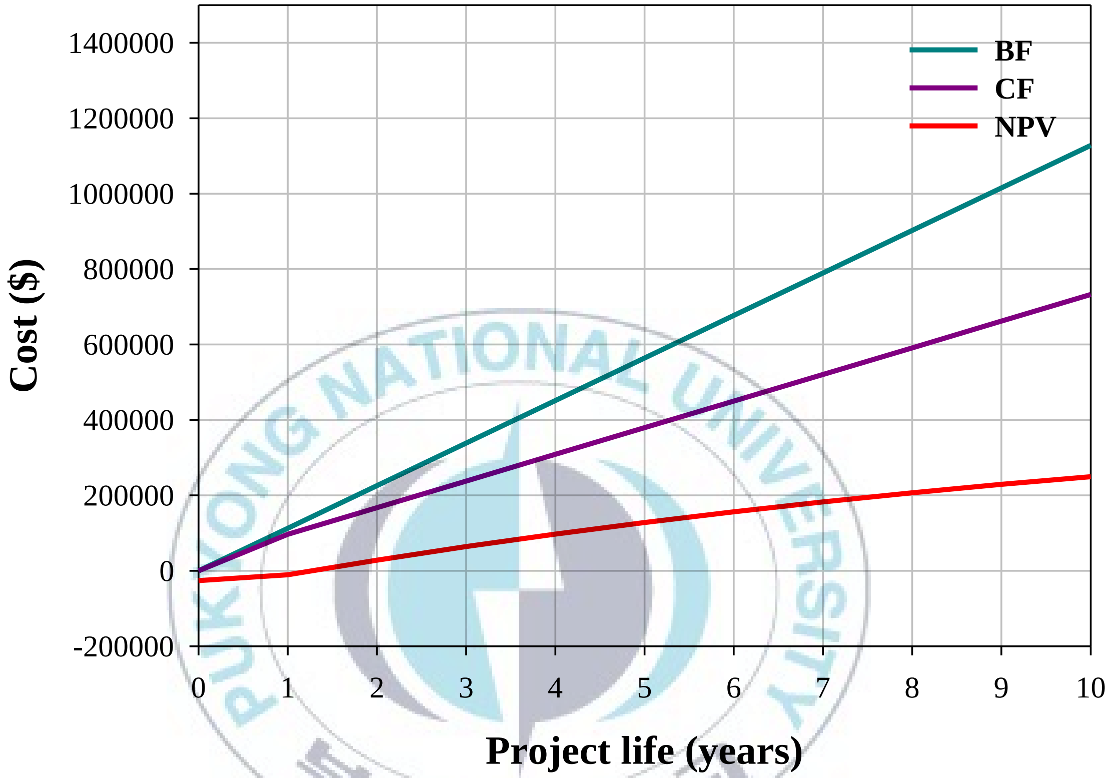

Fig. 4. Analysis of the change of benefit flow, cost flow and net present value.

Ⅳ. CONCLUSION

Experimental comparison of biodegraded fish-processing wastewater fertilizer with a commercial organic fertilizer shows that the formers have better fertilizing value than the latter despite this is a marketed product.

In addition, biodegraded fish-processing wastewater can be considered viable as fertilizers from an economic point of view, according to the obtained results of economic analysis.

Ⅴ. ACKNOWLEDGEMENTS

무엇보다도 항상 아버지처럼 많은 격려와 따뜻함으로 보살펴 주신 우리 김중균 교수님께 감사의 말씀을 드립니다. 석사 생활 동안 많은 우여곡절이 있었지만 이렇게 무사히 졸업을 할 수 있었던 건 교수님을 비롯해 실험실 동료들 덕택이라고 생각됩니다. 졸업논문을 마무리하는 이 시점에서도 현실에 부딪혀서 힘겨워 하고 있는 저 이지만 모두다 즐거웠고 모두다 힘들었었던 지난날을 돌이켜보며 다시금 힘을 얻고 있습니다.

오랫동안 함께했던 우리 실험실 캡틴 은정아! 선배가 많이 챙겨주고 힘이 되어줘야 했는데 이런 저런 일들과 나 살기가 바빠서 많이 못 도와줬던게 아쉽구나... 항상 열심히 하고 잘 해내는 너의 모습을 보면서 자극도 많이 되고 많은걸 배웠다. 앞으로도 그렇게 멋있고 당찬 모습 보여주면서 무사히 졸업했으면 좋겠어.

그리고 1 학년부터 지금까지 인연이 되어온 경환이! 우리 벌써 10 년 지기 친구가 되었네 경환아~ 서로 힘들 때 대연역 근처 포장마차에서 한잔 하면서 이야기 했던 때가 엊그제 같은데 벌써 우리 나이 30 이

되었네 앞으로도 좋은 친구로 남길 바라며 다시금 그때 포장마차로 가서 술 한잔 기울이자.

현이랑 다솜이는 마무리 잘하고 있겠지? 내가 졸업이 늦어져서 같이 졸업하는구나 좀 더 친해지고 동네 오빠, 듬직한 선배 역할을 못한 것 같아서 아쉽네 그래도 이게 끝은 아니니깐 연락하면서 지내자.

성은이는 쫄래쫄래 따라다니면서 인사하던 1 학년 때가 아직도 기억나는데 벌써 석사 2 년차가 되는구나 속도 깊고 뭐든지 열심히 하는 모습 아주 보기 좋아 다만 너무 많은것들 생각하지 말고 팍팍 밀고 나가면 더 좋은 성은이가 될거야

희진아 방장하면서 속 많이 상했지? 내가 연락도 잘 안하는 바람에 골머리 썩었을거야 미안하다. 안그래도 힘들텐데 도와주지 못할 망정 오히려 힘만 들게 해서 면목이 없다. 항상 똘똘하고 당찬 모습 유지하고 남은 생활 뜻있게 마무리 했으면 한다.

우리 실험실 막내 자영이는 항상 과묵해서 생각보다 친해지지 못한 것 같아서 아쉽네 연애도 많이 해보고 공부도 열심히 하는 자영이가 되었으면 좋겠다.

그리고 대학생활 동안 만난 가장 친한 친구 중 한명인 정기형님 어쩔 때는 옆집 형처럼 어쩔 때는 둘도 없는 친구처럼 항상 힘이 되어줘서 고마워요. 앞으로도 이렇게 지냈으면 좋겠고 항상 고맙습니다.

마지막으로 없는 살림에 항상 응원해 주시는 어머니, 외할머니 말로는 잘 표현 못하는 무뚝뚝한 아들, 손자지만 제 마음에는 항상 누구보다도 최고로 사랑한다는 것 알아주시고 멋지고 자랑스러운 아들이 되겠습니다.

비록 큰 결실은 맺지 못한 것 같지만 모두를 만나서 즐거웠고 힘이 되었습니다. 앞으로 살아가는 원동력이 되어 주셔서 감사하고 이게 끝이 아닌 서로의 시작으로 삼아서 더 나은 자신을 찾아가길 기원합니다. 감사합니다.

Ⅵ. REFERENCES

1. Adeoti, I., A., Hawboldt, K., 2014. A review of lipid extraction from fish processing by-prorduct for use as a biofuel. Biomass Bioenergy. 63, 330-340.
2. Á lvarez, C., Á ngelesBermúdez, M., Romero, L.C., Gotor, C., Garc ía, I., 2012. Cysteine homeostasis plays an essential role in plant immunity. New Phytol. 193, 165-177.
3. Apse, M., P., Ahron, G., S., Snedden, W., A., Blumwald, E., 1999. Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+antiport in Arabidopsis. Science. 285, 1256-1258.
4. Brockmann, D., Hanhoun, M., Négri, O., Hélias, A., 2014. Environmental assessment of nutrient recycling from biological pig slurry treatment – Impact of fertilizer substitution and field emissions. Bioresour. Technol. 163, 270-279.
5. Calvo, P., Watts, D.B., Ames, R.N., Kloepper, J.W., Torbert, H.A., 2012. Microbial-based inoculants impact nitrous oxide emissions from an incubated soil medium containing urea fertilizers. J. Environ. Qual. 42. 704-712.
6. Carbonell, G., Miralles de Imperial, R., Torrijos, M., Delgado, M., Rodriguez, J.A., 2011. Effects of municipal solid waste compost and mineral fertilizer amendments on soil properties and heavy metals distribution in maize plants (Zea mays L.). Chemosphere. 85, 1614-1623.
7. Chaabouni, I., Guesmi, A., Cherif, A., 2012. Secondary metabolites of

Bacillus: Potentials in biotechnology. In: Sansinenea, E. (Eds.), Bacillus thuringiensis biotechnology. Springer, Dordrecht, the Netherlands. 347–366.

8. Chalamaiah, M., Dinesh kumar, B., Hemalatha, R., Jyothirmayi, T., 2012. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. Food chem. 135, 3020-3038.
9. Dao, V.T., Kim, J.K., 2011. Scaled-up bioconversion of fish waste to liquid fertilizer using a 5 L ribbon-type reactor. J. Environ. Manage. 92. 2441-2446.
10. The bank of Korea economic statistics system, 2014. Market interest rates. <http://ecos.bok.or.kr> (accessed October 2014).
11. Esakkiraj, P., Usha, R., Palavesam, A., Immanuel, G., 2012. Solid-state production of esterase using fish processing wastes by *Bacillus altitudinis* AP-MSU. Food Bioprod. Process. 90, 370-376.
12. Figueiredo, M.V.B., Seldin, L., Araujo, F.F., Mariano, R.L.R., 2010. Plant growth promoting rhizobacteria: Fundamentals and applications. In: Maheshwari, D.K. (Eds), Plant growth and health promoting bacteria. Springer, Berlin. p. 21–43.
13. García-Moreno, P.J., Khanum, M., Guadix, A., Guadix, E.M., 2014. Optimization of biodiesel production from waste fish oil. Renewable Energy. 68, 618-624.
14. Geun, B.K., Kim, J.K., 2012. Feasibility study on production of liquid

fertilizer in a 1 m^3 reactor using fishmeal wastewater for commercialization. Environ. Eng. Res. 17(1), 3-8.

15. Griffin, T., Dobbins, C.L., Vyn, T., Florax, R.J.G.M., Lowenberg-DeBoer, J., 2008. Spatial analysis of yield monitor data: case studies of on-farm trials and farm management decision-making. Precis. Agric. 9, 269–283.

16. Güneş, A., Turan, M., Güllüce, M., Şahin, F., 2014. Nutritional content analysis of plant growth-promoting rhizobacteria species. Eur. J. Soil Biol. 60, 88-97.

17. IMO, 2006. London Convention and Protocol, Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter.<http://www.imo.org/OurWork/Environment/LCLP/Pages/default.aspx> (accessed July 2014).

18. Illera-Vives, M., Labandeira, S.S., López-Mosquera, M.E., 2013. Production of compost from marine waste: evaluation of the product for use in ecological agriculture. J. Appl. Phycol. 25, 1395-1403.

19. Jander, G., Josh, V., 2010. Recent Progress in Deciphering the Biosynthesis of Aspartate-Derived Amino Acids in Plants. Mol. Plant. 3, 54-65.)

20. Joshi. V., Joung, J.G., Fei, Z., Janer, G., 2010. Interdependence of threonine, methionine and isoleucine metabolism in plants: accumulation and transcriptional regulation under abiotic stress. Amino Acids. 39, 933-947.

21. Khan, N., Mishra, A., Chauhan, P.S., Nautiyal, C.S., 2011. Induction of *Paenibacilluslentimorbus* biofilm by sodium alginate and $CaCl_2$ alleviates drought stress in chickpea. Ann. App. Biol. 159, 372-386.

22. Kim, J.K., Dao, V.T., Kong, I.S., Lee, H.H., 2010. Identification and characterization of microorganisms from earthworm viscera for the conversion of fish wastes into liquid fertilizer. Bioresour. Technol. 101, 5131-5136.

23. Kim, J.K., Kim, J.B., Cho, K.S., Hong, Y.K., 2007. Isolation and identification of microorganisms and their aerobic biodegradation of fish-meal wastewater for liquid-fertilization. Int. Biodeterior. Biodegradation. 59, 156-165.

24. Korea electric power corporation, 2014. Electricity price. <http://cyber.kepco.co.kr/ckepco/front/jsp/CY/E/E/CYEEHP00201.jsp> (accessed October 2014).

25. Korea exchange bank, 2014. The won-dollar exchange rate. <http://www.keb.co.kr> (accessed October 2014).

26. Korea water resource corporation, 2014.Tap water price. <http://kwater.or.kr/> (accessed October 2014).

27. Lu, L., Tan, H., Luo, G., Liang, W. 2012. The effects of *Bacillus subtilis* on nitrogen recycling from aquaculture solid waste using heterotrophic nitrogen assimilation in sequencing batch reactors. Bioresour. Technol. 124, 180-185.

28. Maeda, H., Dudareva, N., 2012. The shikimate pathway and aromatic amoni acid biosynthesis in plats. Annu. Rev. Plant Biol. 63, 73-105.

29. Mandic-Mulec, I., Prosser, J.I., 2011. Diversity of endospore-forming bacteria in soil: Characterization and driving mechanisms. In: Logan, N.A., De Vos, P. (Eds.), Endospore-forming soil bacteria. Springer, London. pp. 31–59.

30. Marenya, P., Nkonya, E., Xiong, W., Deustua, J., Kate, E., 2012. Which policy would work better for improved soil fertility management in sub-Saharan Africa, fertilizer subsidies or carbon credits? Agric. Syst. 110, 162-172.

31. Meyer-Aurich, A., Griffin, T.W., Herbst, R., Giebel, A., Muhammmad, N., 2010. Spatial econometric analysis of a field-scale site-specific nitrogen fertilizer experiment on wheat (*Triticumaestuvum L.*) yield and quality. Comput. Electron. Agric. 74, 73-79.

32. Ministry of ocean and Fisheries, 2013. Major statistics of ocean and fisheries, in: Information Management Officer (Eds.), Ministry of Ocean and Fisheries. Korea, pp. 33-106.

33. Miret, J.A., Munné-Bosch, S., 2014. Plant amino acid-derived vitamins: biosynthesis and function. Amino Acids. 46, 809-824.

34. Muthukumaran, S., Baskaran, K., 2013. Organic and nutrient reduction in a fish processing facility – A case study. Int. Biodeterior. Biodegrad. 85, 563-570.

35. Nautiyal, C.S., Srivastava, S., Chauhan, P.S., Seem, K., Mishra, A., Sopory, S.K., 2013. Plant growth-promoting bacteria *Bacillus*

amyloliquefaciens NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol. Biochem. 66, 1-9.

36. Nges, I.A., Mbatia, B., Björnsson, L., 2012. Improved utilization of fish waste by anaerobic digestion following omega-3 fatty acids extraction. J. Environ. Manage. 110, 159-165.
37. Sanchez-Segado, S., Lozano, L.J., de los Rios, A.P., Hernandez-Fernandez, F.J., Godinez, C., Juan, D., 2012, Process design and economic analysis of a hypothetical bioethanol production plant using carob pod as feedstock. Bioresour. Technol. 104, 324-328.
38. Szabados, L., Savoure, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89-97.
39. Siddikee, M.A., Glick, B.R., Chauhan, P.S., Yima, W.J., Sa, T., 2011. Enhancement of growth and salt tolerance of red pepper seedlings (Capsicum annuum L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant physiol. Biochem. 49, 427-434.
40. Stein, T., 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. Mol. Microbiol. 56, 845–857.
41. Tufvessona, P., Ekmana, A., Sardarib, R.R.R., Engdahla, K., Tufvesson, L., 2013, Economic and environmental assessment of propionic acid production by fermentation using different renewable raw

materials.Bioresour. Technol. 149, 556-564.

42. Tufvesson, P., Lima-Ramos, J., Nordblad, M., Woodley, J.M., 2011, Guidelines and cost analysis for catalyst production in biocatalytic processes. Org. Proc. Res. Dev., 15, 266-274.
43. Wu, S.C., Cha, Z.G., Cheung, K.C., Wong, M.H., 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma. 125, 155-166.
44. Wu, Y., Li, T., Yang, L., 2012. Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: A review. Bioresour. Technol. 107, 10-18.
45. Wu, Y.P., Huang, H.M., Lin, Y.F., Huang, W.D., Huang, Y.J., 2014. Mackerel biodiesel production from the wastewater containing fish oil. Energy. 70, 43-48.
46. Yadav, A., Gang, V.K., 2011. Recycling of organic wastes by employing *Eiseniafetida*. Bioresour. Technol. 102, 2874-2880.
47. Yang, J., Kloepper, J.W., Ryu, C.M., 2009. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14, 1-3.
48. Yun, Y.S., Park, J.I., Suh, M.S., Park, J.M., 2000. Treatment of food wastes using slurry-phase decomposition. Bioresour. Technol. 73, 21–27.
49. Zhu, H.J., Sun, L.F., Zhang, Y.F., Zhang, X.L., Qiao, J.J., 2012. Conversion of spent mushroom substrate to biofertilizer using a stress-tolerant phosphate-soulubilizing*Pichia farinose* FL7. Bioresour. Technol. 111, 410-416.