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Eco-friendly waste management of mackerel wastewater and enhancement of its reutilization value

Hyun Yi Jung, Joong Kyun Kim*

Department of Biotechnology, Pukyong National University, Busan 608-737, Republic of Korea

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ABSTRACT

To reutilize mackerel wastewater (MWW) generated from mackerel processing plants, aerobic biodegradation of MWW was conducted in a 3-l reactor by using mixed microbes. During the first 24 h, biodegradation occurred actively, and the dissolved oxygen level, pH, and oxidation–reduction potential decreased with an increase in cell number. Then, the fishy smell started to disappear. The 42-h culture supernatant displayed high antioxidant activities, as indicated by the following results: 88.7% 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, 99.7% 2, 2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity, 96% hydroxyl radical scavenging activity, and 0.35 reducing power at A_{700}. The high antioxidant activities were attributed to specific amino acids, and this was confirmed using two-dimensional thin-layer chromatography. Moreover, the culture supernatants displayed antimicrobial and DNA protective activities and weak antifungal activity, enhancing the reutilization value of MWW. The remaining culture broth after MWW biodegradation was found to be phytotoxin-free, and it could be used as a biofertilizer on the basis of hydroponic culture of red bean and barley. Overall, advanced waste management of MWW was demonstrated.

1. Introduction

In 2012, the worldwide supply of fish from capture fisheries and aquaculture was about 158 million tons, and approximately 86% was consumed as food (http://www.fao.org/3/d1eaa9a1-5a71-4e42-86c0-f2111f07de16/i3720e.pdf). A major proportion of fishery products is used for human consumption, and a huge quantity of fishery wastes and by-products is being generated by fishery-processing industries. In addition, significant amounts of fishery wastes are also generated by the aquaculture industry, and these wastes pollute surface and ground water. The organic wastes contain nitrates and phosphates; they can stimulate algal blooms, resulting in oxygen depletion and destruction of aquatic life. Either the fishery wastes are used as low market-value products such as fishmeal, fish oil, and fertilizers or simply dumped into the sea, causing a serious environmental problem (Arvanitoyannis and Kassaveti, 2008). Therefore, appropriate management strategies for various fishery wastes are required.

Discarded wastes are rich sources of protein, and fish protein hydrolysates produced from fishery wastes by proteolytic hydrolysis are marketable as nutritional supplements, functional ingredients and flavor enhancers (Je et al., 2008). Therefore, underutilized fishery wastes could be used to produce value-added products such as proteins, amino acids, glycerol, fatty acids, meal, lactic acid and ethanol (Ramakrishnan et al., 2013). Fish proteins in fishery wastes can be hydrolyzed by chemicals in a relatively inexpensive and simple way. However, it is difficult to control the whole process specifically and leads to products with variable chemical composition and functional properties, limiting their use as food ingredients. Besides, hydrolysis performed at extreme temperatures and pH conditions may degrade the nutritional qualities of the products, resulting in poor functionality (Kristinsson and Rasco, 2000). Enzymatic hydrolysis was the preferred method for reutilization of fishery wastes because no residual organic solvents or toxic chemicals are present in the products. Under moderate conditions of pH and temperature, enzymatic hydrolysis is performed using endogenous or/and exogenous enzymes that influence the molecular size and hydrophobicity of the hydrolysate, but this process compromises the nutritive quality of the hydrolysate (Kristinsson and Rasco, 2000). Because of the low hydrolysis rate and high cost of enzymatic hydrolysis, microbial hydrolysis was introduced, particularly for the

* Corresponding author.
E-mail address: junekim@pknu.ac.kr (J.K. Kim).

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production of bioactive peptides (Fang, 2010). Microbial hydrolysis can produce a wide range of peptides that contain different amino acid sequences when it was performed by microorganisms possessing with extracellular proteases (Wang et al., 2015). The degree of hydrolysis depends upon the microbial species, substrate composition, and reaction conditions; thus, interesting bioactive properties can be observed.

Reutilization of fishery wastes would be a fruitful strategy, as it would address the environmental issue and result in economic gain (Awarenet, 2004). Fishery wastes are known to be valuable sources of raw materials such as bioactive compounds that play an important role in metabolic regulation and modulation (Sheriff et al., 2014). For instance, biologically inactive peptides that exist within the sequences of source proteins exhibited various physiological functions after being released by enzymatic hydrolysis (Vercruysse et al., 2005). This biofunctional activity was found to be dependent on the structural properties of the peptides; thus, their amino acid composition and sequences are required for diverse biological functions.

Use of antioxidants, especially food-derived natural antioxidants, has been recognized as feasible and potentially effective in overcoming radical-mediated damage, as synthetic antioxidants have restricted applications in food products because of safety and negative consumer perception (Park et al., 2001). Compared with synthetic antioxidants, antioxidants derived from marine resources are easy to obtain, cheap, and safe with no adverse effects. To date, antioxidant activity has been found in fish protein hydrolysates from diverse species: yellow stripe trevally (Klompong et al., 2007), yellowfin sole frame (Jun et al., 2004), herring (Sathivel et al., 2003), mackerel (Wu et al., 2003), muscles of threadfin bream and flyingfish (Shabeena and Nazeer, 2010), backbone of flyingfish (Shabeena and Nazeer, 2011), and backbones of great barracuda and hairtail (Nazeer et al., 2011). In addition, antimicrobial peptides secreted by fish have been known to play major roles in the innate immune system and provide protection against bacterial, fungal, viral, and other pathogenic infections (Smith et al., 2010). As fungal diseases have become a growing threat, many studies have been conducted in an attempt to isolate natural antifungal substances with potential pharmaceutical utility (Alcouloumre et al., 1993). Consequently, recovery of bioactive compounds from fishery wastes is significant because it would alleviate the problems related to the treatment of fishery wastes.

Mackerel (*Scomber scombrus* or *Scomberomorus niphonius*) is one of three fish (mackerel, squid, and hairtail) consumed by most people in Korea (http://portal.nfrdi.re.kr/upload/all/all_2010_07.pdf). Of the three fish, protein content is the richest in mackerel (http://portal.nfrdi.re.kr/upload/all/all_2010_07.pdf). Mackerel has been known to contain approximately 15.57% protein in the whole body; 12.3%, head; 17.17%, skin; 14.16%, frame; 3.92%, bone; and 12.18%, viscera (Li et al., 2013; Ramakrishnan et al., 2013). Mackerel is processed to enhance its food value: frozen mackerel is thawed, sorted, eviscerated, washed, salted, and finally washed (http://chickenofthesea.com/company/know-your-seafood/mackerel). Similar to most processing industries, mackerel processing produces a large volume of wastewater that contains organic contaminants, oils, etc. (Garcia-Sanda et al., 2003). Recently, the Ministry of the Environment in Korea changed the paradigm for waste policy and emphasized resource recycling with zero emission (Mathews, 2012), urging ecologically acceptable waste management.

The objective of this study was to demonstrate a new advance in waste management of mackerel wastewater (MWW) for the enhancement of its reutilization value. For the advanced waste management of MWW, it was first degraded by mixed microbes possessing protease, lipase, and carbohydrate-degrading enzymes. Culture broths containing metabolites produced from MWW biodegradation were examined for antioxidant, antifungal, antibacterial, and DNA protective activity in order to find the reutilization value of MWW. To pick out specific amino acids affecting antioxidant activity among those produced from the MWW biodegradation, two-dimensional thin-layer chromatography (2D-TLC) was used. Finally, possibility of use of the remaining culture broth as a biofertilizer was tested using hydroponic culture in order to find out an advanced way to reutilize waste culture broth.

2. Materials and methods

2.1. Design for advanced waste management

Advanced waste management was designed to reutilize MWW (Fig. 1). Sterile MWW was fed into a 3-l bioreactor and degraded using the mixed microbes. Bioactive compounds produced after appropriate biodegradation were extracted from the culture broth and tested for use as antioxidant, antifungal, antibacterial, and DNA protective agents. The remaining culture broth, including the mixed microbes, was tested for use as a biofertilizer for plant growth. Use of this design for advanced waste management led to no additional generation of wastes during the whole process and enhancement of the reutilization value of MWW.

2.2. Microorganisms and culture

The mixed microbes used in this study were composed of two patented bacteria, *Pknufermbacteria* (patent No. 10-0822239) and *Wormbacteria* (patent No. 10-1058245). *Pknufermbacteria* is a mixture of seven bacteria: *Bacillus subtilis* (DQ219358), *Bacillus licheniformis* (AY468373), *Bacillus coagulans* (AF466695), *Bacillus circulans* (Y13064), *Bacillus anthracis* (AY138279), *Bacillus fusiformis* (AY548950), and *Brevibacillus agri* (AY319301). *Wormbacteria* is a mixture of four bacteria: *Bacillus licheniformis* (EF113324, 99% similarity), *Bacillus cereus* (DQ923487, 99% similarity), *Brevibacillus agri* (AJ586388, 99% similarity), and *Brevibacillus parabrevis* (AB215101, 99% similarity). It was confirmed that there was no bacterial antagonist among them. For the preparation of seed culture, equal amounts of cells from each strain were first cultivated in a 100-ml flask containing 3-fold diluted MWW. Each cell was transferred to 0.5-fold diluted MWW and cultivated to adapt the cells to MWW to the maximum extent. Then, each cell proliferated until the late-log phase and was separately harvested and combined together for use as an inoculum for MWW degradation. Each pure strain was maintained on 1.5% nutrient agar plates at 4 °C until use and transferred to a fresh agar plate every two weeks.

2.3. Preparation of MWW

MWW was obtained from D company (Jangnim, Busan, Korea), which produces processed foods from mackerel. MWW (per liter) had the following characteristics: 29800 ± 2800 mg, chemical oxygen demand (COD_{Cr}); 3900 ± 350 mg, total nitrogen (TN); and 0.3 ± 0.01%, salt. When MWW was used in the biodegradation experiment, initial pH was adjusted to 7, and MWW was autoclaved at 121 °C for 15 min.

2.4. Biodegradation of MWW

The autoclaved MWW was aseptically transferred to a sterile 3-l bioreactor. Degradation of MWW was initiated just after 10% (v v^{-1}) seed culture of the mixed microbes was used for inoculation. Air (5 l min^{-1}) generated from an air compressor was supplied into the bioreactor through a 0.2-μm filter. The biodegradation was