Biodegradation of surimi processing and crab canning wastewater using indigenous bacteria consortium

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Abstract. Biological treatment of surimi and crab wastewater has been carried out using a consortium of indigenous bacteria isolated from the wastewater. The treatment carried out is by combining a consortium of proteolytic bacteria, lipolytic bacteria and proteo-lipolytic bacteria. The bacterial consortium was added to surimi and crab canning wastewater. The purpose of this study was to determine the optimum consortium of indigenous bacteria which can degrade protein and fat in the wastewater of surimi and crab canning. Liquid waste samples were taken from the initial sewerage of surimi processing and crab canning. Biodegradation of surimi processing wastewater and crab canning was carried out aerobically on a laboratory scale. The maximum growth rates of proteolytic bacteria were 0.020, 0.021 and 0.23 per hour, while lipolytic bacteria had a maximum growth rate of 0.0012 and 0.003 per hour. The decrease in dissolved protein content in surimi wastewater was higher than in crab canning wastewater. The dissolved protein content of surimi wastewater decreased from 11.38 to 3.28 mg/L using a proteo-lipolytic consortium, while there was a decrease of 3.19 to 2.64 mg/L in crab wastewater with the same consortium. The lipid content of crab canning wastewater decreased from 0.0623 to 0.0100 mg/L using a proteo-lipolytic consortium while there was a decrease of 0.0318 to 0.0120 mg/L in surimi wastewater with the same consortium. The highest percentage of degradation in dissolved protein was 46.73% in surimi wastewater using a lipolytic bacteria consortium while in crab canning wastewater was 12.48% with the same consortium of bacteria. The highest reduction rate of lipid content was 84% in crab canning wastewater using proteo-lipolytic consortium, while in surimi wastewater was 62.28% using te same consortium. Consortium bacteria in surimi wastewater tended to degrade protein higher than bacteria in crab canning wastewater.

1. Introduction

The fishing processing industry has several processing miniplans with adjacent locations. Generally processed is the same type as salting fish, canning rajungan and processing surimi whose process often occurs continuously or seasonally. Generally the processing industry whether it is fisheries or other agro-industry producing liquid waste containing the amount of contaminants in the form of dissolved, colloidal or particles. The degree of contamination depends on the existing process, e.g. low contaminants when only washing operations, medium contaminants in the fish filtration process and high contaminants occur in dirty water from the fish hatching process.

The high content of organic matter in fishery liquid waste can act as a food source for microbial growth. With an abundant food supply, microorganisms will multiply rapidly and reduce dissolved
oxygen in water. Normally, water contains approximately 8 ppm of dissolved oxygen. The minimum standard of dissolved oxygen for fish life is 5 ppm and below this standard will lead to the death of fish and other aquatic biota [1].

Central Java is an area that has a coastline that stretches along the north and south coasts, making it one of the sources of supply of Indonesian fisheries products. In 2019, the first quarter of January to April 2019, various fishery products both fresh and processed certified through Fish Quarantine Semarang were able to penetrate the market in 25 countries in the world. The five countries that rank highest are the United States, China, Japan, Malaysia and Taiwan. The United States topped the list with a volume of 1,041 tons worth 367 billion rupiah, while the lowest was Taiwan with an export volume of 981 tons, worth 45 billion rupiah. Variants of fishery products that are in demand by export markets in 25 countries include rajungan, tilapia, surimi, white shrimp and squid [2]. Surimi is a semi-finished product that is processed by crushing fish meat, then washed with cold water to remove the less interesting properties of organoleptis and after that separated the water [3].

Indonesia's rajungan exports to the U.S. (Import USA Crab Meat Indonesia) until December 2020, as much as 12,500 tons of pasteurized rajungan meat in cans. Average monthly export of rajungan as much as 1,000 tons [4]. The United States is the largest market share for the export of rajungan commodities from Indonesia. Captured in fish management areas (WWP) 711, 712, 713, and 714. The results of this rajungan catch are then processed in a number of areas, such as Medan, Lampung, West Java, Central Java, East Java, and Makassar. In addition to the United States, rajungan commodities are also exported to a number of countries, including: Hong Kong, China, Malaysia, Japan, Singapore, France and the United Kingdom.

Dissolved and suspended organic matter is very high in liquid waste fishery processing process, as well as nutrient content such as nitrogen and phosphate. The onset of foul odor is caused by the further decomposition of proteins rich in bersulfur amino acids (cysteine) producing sulfide acid, thiol group, and ammonia. Short-chain fatty acids resulting from decomposition of organic matter also cause a foul smell. Protein degradation led to the formation of simple peptides, free amino acids and then into ammonia compounds [5].

The presence of oil and fat on the surface of the water will inhibit biological processes in water and produce odorous gases [6]. The fat on the surface of the water will block the entry of light in the body of water so that the process of photosynthesis takes place is hampered thus oxygen levels will be low which will cause the aerobic organisms will die. In long-polluted environments and waste treatment ponds, it is possible that there are microbes degrading oil or lipid naturally, competing or consortium with other. Natural consortium is already in its natural habitat, namely wastewater, be it microbes degrading carbohydrate, microbes degrading fat or microbes degrading protein.

Wastewater treatment from fishery processing with the addition of a consortium of bacteria and heating treatment using autoclaves was able to reduce the total protein content by 36.11% and fat content by 34% in wastewater crab canning treatment Cirebon and surimi treatment Pekalongan [7]. Wastewater from product processing of fisheries generally has high protein and fat characteristics. Natural microbes in liquid waste will degrade existing proteins and lipids, but the degradation process is long. Therefore, it is necessary to know the growth of microbes in the liquid waste of the fishery to see the type of microbes and the number of microbes that play a role. This study used a consortium of bacteria derived from liquid waste processing fisheries as an aerobic agent of protein and lipid degradation.

2. Material and methods

2.1. Material

The wastewater of fishery products processing industry was used directly from two locations, namely crab canning in Cirebon (West Java) and surimi processing in Kendal (Central Java). Samples were taken using a sterile bottle of 500 mL volume. Wastewater is taken in the outlet part of the fish treatment unit that has not undergone treatment or pretreatment. The sample was put in a cool box
containing ice crushing, then taken to the Laboratory of the Research Center on Product Processing and Marine Biotechnology and Fisheries Jakarta.

2.2. Methods

2.2.1. Growth Curve Measurement. Determination of the growth curve against bacteria is done to know when the optimum bacteria are used as a bioremedian starter, which is when the bacteria are in the logarithmic phase. Cultivation has been modified by replacing the speed and temperature of the shaker incubator from 200 rpm at 15°C to 100 rpm at 30°C according to environmental conditions [8]. A total of 1 loop of bacteria from the selected colony is inoculated into a 100 ml nutrient broth (NB). Incubation is performed using a 100 rpm speed incubator shaker and a temperature of 37°C for 48 hours. The bacterial growth curve is determined by measuring (Optical Density/OD) at a wavelength of 600 nm using a spectrophotometer, every 2 hours to 48 hours.

2.2.2. Formulation of a consortium of protein and fat degrading bacteria. The basis of formula determination is the growth rate and Optical Density of proteolytic and lipolytic bacteria consortium obtained. The bacterial consortium formula added as a starter as much as 5% of the volume of waste in size (v/v) with density value (Optical Density/OD) at the beginning of the logarithmic phase of each bacterial isolate to be formulated. The bacterial consortium formula added there are 3 types, namely proteolytic bacteria consortium; lipolitik bacteria consortium and bacterial proteolipolitik consortium to 2 types of liquid wastes there are crab canning and surimi processing wastewater [9].

2.2.3. Degradation of dissolved protein and lipid. Liquid waste is observed for 54 hours to see degradation changes occurred [9]. The parameters measured are dissolved protein and lipid. Efficiency of the use of concentrations / proportions of bacteria to waste is also observed.

3. Result and Discussion
The fishery processing process has been known produced large amounts of organic waste and by products from inedible fish parts as well as shell parts from the stripping process. Liquid waste from fishery processing generally has a high content of protein and lipid compounds.

The logarithmic phase of the proteolytic bacterial isolate is estimated to start at the 9th hour and end at the 18th hour. The stationary phase of proteolytic bacterial isolate 2 occurs at the 24th hour which has a density value of 1.575 to the 48th hour. The highest Optical Density of proteolytic isolate bacterial was 1.551 from isolate proteo 2 (Figure 1). In cell growth patterns where the lag or adaptation phase is the phase in which new cells adjust to new environmental conditions [10,11]. After undergoing the adaptation phase, the cell begins to divide at a still low speed because the newly completed self adjustment stage is called the initial growth phase. The microbial logarithmic growth phase occurs rapid and constant division in which the increase in number follows the logarithmic curve. The speed of microbial growth is strongly influenced by the medium in which it grows such as pH and nutrient content as well as environmental conditions including temperature and humidity. In this phase the cell requires more energy compared to other phases, in addition the cells are most sensitive to environmental conditions. The maximum growth rate produced in selected proteolytic bacterial isolates is quite large. The maximum growth rate of animal cells ranges from 0.01–0.05/hour [11]. The determination of the specific growth rate is related to the exponential phase. In this phase, the specific growth rate is constant with a steady state of growth. Isolates of proteolytic bacteria that have the highest maximum growth rate are bacteria isolates 2 with a μ of 0.023/hour while proteolytic bacteria isolates 1 and 3 have specific growth rate of 0.02/hour and 0.021/hour.

The stationary growth phase in which the number of bacterial populations remains due to the number of bacteria growing is equal to the number of dead bacteria. The size of the cells in this phase becomes smaller because the cells remain split even though the nutrients have started to run out. Due
to lack of nutrients, it is likely that the cells have a different composition to the cells that grow in the logarithmic phase. In this phase the cells become more resistant to extreme conditions such as heat, cold, radiation and chemicals.

![Figure 1](image1.png)

**Figure 1.** Optical Density of isolates proteolytic bacteria

The bacterial death phase occurs because a portion of the bacterial population begins to die due to several causes because a) nutrients in the medium are exhausted; b) the reserve energy in the cell runs out. The number of dead cells is getting more and more and the rate of death is influenced by nutrient conditions, environment and microbial types [10].

Measurement of the rate of decrease in dissolved protein levels in each isolate of proteolytic bacteria aims to know how quickly each isolate is able to lower the total and dissolved protein levels so that it is known the ability of each bacteria when formulated in liquid waste.

![Figure 2](image2.png)

**Figure 2.** Optical Density of isolates lipolytic bacteria
Lipolytic bacterial isolate 3 has the highest Optical Density value of 1.3225 which is the end of the logarithmic phase at the 18th hour while the beginning of the logarithmic phase is 0.1245 at the 3rd hour. The stationary phase of lipolytic bacterial isolate 3 occurs at the 30th hour which has a density value of 1.261 to the 48th hour. Lipolytic bacterial isolate 1 has the lowest Optical Density value of 1.064 at the 18th hour while the beginning of the logarithmic phase is 0.1685 at the 9th hour. The stationary phase of lipolytic bacterial isolate 1 occurs at the 30th hour which has a Optical Density value of 1.118 at the 48th hour (Figure 2). Isolates of lipolytic bacteria that have the highest maximum growth rate are bacteria isolates 2 and 3 with a μ of 0.003/hour while bacteria isolates 1 have the lowest specific growth rate of 0.0012/hour.

Measurement of the rate of decrease in lipid content in each isolate of lipolitik bacteria aims to know how quickly each bacteria is able to lower fat levels so that it will be known the ability of each isolate when formulated in liquid waste.

Crab canning wastewater comes from the boiling process at a temperature of 80-100°C for 15-25 minutes depending on the number of crabs being processed. This also affects the dissolved protein levels in wastewater. Because the crab canning processing time does not exceed 45 minutes, there may not be a decrease in dissolved protein during processing. this can be seen from the Figure 3. A decrease in dissolved protein will occur when heating at a temperature of 100°C for 45 minutes or more. There were some stable proteins left from treated wastewater [12].

The use of mixed cultures (microbial consortium) in the handling of fishery wastewater reduces several parameters of the water quality of the treated waste. Mixed culture is more efficient at reducing pollutants from wastewater compared to using pure culture. Degradation of dissolved protein in crab canning and surimi processing wastewater can be seen in Figures 3 and 4. It can be seen that surimi wastewater has a higher protein content than crab processing wastewater. There was a decrease in the dissolved protein content in the surimi wastewater with the addition of a consortium of bacteria. the proteo-lipolytic bacteria consortium showed a higher ability to degrade protein possibly due to the mixed culture of proteolytic and lipolytic bacteria.
The use of a consortium of bacteria by 75% using inoculum by 38% resulted in a decrease in fat levels in liquid waste processing processes, which occurs due to metabolic induction [13]. In a batch process where both the starter is included, the microorganisms have sufficient time to degrade the solids in the waste. So that if the dissolved solids content is higher, the efficiency of the time allowance will also be longer [14]. The presence of bacterial extracellular proteolytic enzymes causes casein to be hydrolyzed into soluble peptides and amino acids. Bacillus sp is very efficient in breaking down various long-chain carbohydrates, lipids and proteins into short-chain units or simpler compounds [15].
Lipid is an example of organic matter. Material organic matter is naturally easier to decompose than inorganic material. Lipolytic enzymes can degrade lipid into simpler substrates. In the biodegradation process that takes place, the microbes will also assimilate nitrogen, phosphorus, potassium and sulfur which are bound in the cell protoplasm. The main objective of handling waste biologically is to degrade by oxidizing organic waste, so that complex compounds can be broken down into simpler and more soluble compounds, besides that they can also be used as nutrients by indigenous bacteria [16].

The efficiency of the rate of reducing lipid content is influenced by the ability of bacteria to degrade lipid both individually and in a mixture (consortium). Mixed culture (consortium) was effective in reducing lipid for 12 days of incubation [17]. It can be seen from Figures 5 and 6 that the proteolytic bacteria consortium in the crab canning and surimi processing wastewater has the ability to degrade the lipid content more than the consortium of each isolate, namely the consortium of proteolytic bacteria or the consortium of lipolytic bacteria.

![Figure 6. Degradation of lipid content in surimi wastewater](image)

The effectiveness of biodegradation organic matter with the addition of a bacterial inoculum is higher when compared to the decomposition of organic matter without the addition of an inoculum. Microorganisms can consume organic pollutants and convert these organic pollutants into carbon dioxide, water and energy for growth and reproduction. The addition of a mixed culture inoculum of bacteria will stimulate the decomposition process to occur faster than those without adding the bacterial inoculum, because the time needed to decompose is longer than to which the inoculum is added [16]. The highest percentage of degradation in dissolved protein was 46.73% in surimi wastewater using a lipolytic bacteria consortium while in crab canning wastewater was 12.48% with the same consortium of bacteria. The highest reduction rate of lipid content was 84% in crab canning wastewater using proteo-lipolytic consortium, while in surimi wastewater was 62.28% using the same consortium.

The efficiency of reducing lipid content has a difference between each bacteria and the combination (consortium) due to the reaction system. The presence of lipase is not only a catalyst for the hydrolysis reaction but also catalyzes the interesterification reaction depending on the source of the lipase and the reaction conditions. The use of bacterial consortium is able to reduce the lipid content of surimi wastewater on laboratory scale [17]. Biodegradation will maximize the process of decomposing organic substances, resulting in a decrease in BOD levels, because lipid degradation continues, so that
the lipid content will decrease. By decreasing the lipid content, the amount of oxygen consumed by the microbes in the process of decomposition will decrease [16].

4. Conclusion

The decrease in dissolved protein content in surimi wastewater was higher than in crab canning wastewater. The dissolved protein content of surimi wastewater decreased from 11.38 to 3.28 mg/L using a proteo-lipolytic consortium, while there was a decrease of 3.19 to 2.64 mg/L in crab canning wastewater with the same consortium. The lipid content of crab canning wastewater decreased from 0.0623 to 0.0100 mg/L using a proteo-lipolytic consortium while there was a decrease of 0.0318 to 0.0120 mg/L in surimi wastewater with the same consortium. The highest percentage of degradation in dissolved protein was 46.73% in surimi wastewater using a lipolytic bacteria consortium while in crab canning wastewater was 12.48% with the same consortium of bacteria. The highest reduction rate of lipid content was 84% in crab canning wastewater using proteo-lipolytic consortium, while in surimi wastewater was 62.28% using te same consortium. Consortium bacteria in surimi wastewater tended to degrade protein higher than bacteria in crab canning wastewater.

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