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IMPROVING FEED QUALITY WITH LIQUID SUBSTRATE FERMENTATION

Yuli Andriani and Rusky I. Pratama

[1)] *Staff at Fisheries Department, Faculty of Fisheries and Marine Sciences, University of Padjadjaran*
[*)] *E-mail: yuliyusep@yahoo.com*

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ABSTRACT

Feed is one of the factors that play an important role in fish farming activities. The feed given must have nutritional content that can meet the needs of fish. Fermentation using liquid substrate is a solution that can be done to increase the nutritional value of feed. The fermentation time for liquid substrates is faster because microorganisms can come into contact with the media and during fermentation, oxygen, pH and nutrients are thoroughly distributed. Types of microbes that can be used in liquid substrate fermentation are Trichoderma viride, Bacillus subtilis and Bacillus licheniformis. Fermentation of feed ingredients using Bacillus licheniformis on ketapang fruit is known to increase crude protein from 4.89% to 8.85% and reduce crude fiber from 14.95% to 11.88%. Fermentation of feed ingredients using Bacillus subtilis on chicken feathers can increase the crude protein content from 8.56% to 10.40% and reduce crude fiber from 5.30% to 0.90%. Liquid substrate fermentation has been shown to increase crude protein content, reduce crude fiber content and increase feed digestibility.

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1. INTRODUCTION

The success of fish farming is determined by several factors, one of which is the provision of quality feed. In the aquaculture industry, feed is the main supporting component as well as an important factor in the success of an aquaculture activity. In addition, feed also contributes greatly to production costs, which range between 40% -80%. The feed given must have sufficient nutritional value to meet the needs of fish growth. At present, fish cultivators face the problem of the price of expensive components for the preparation of feed ingredients and some of these materials must be imported to meet the needs of aquaculture activities. It is hoped that this can be overcome by utilizing local raw materials which are abundantly available but have not been optimally utilized so that it is hoped that the use of these local materials can reduce the use of ingredients in the manufacture of fish feed.

The home-scale food industry in its processing produces by-products in the form of liquid waste. In Indonesia, liquid waste is waste that has not been paid attention to by processors and industry or it can be said that liquid waste is simply thrown away so that it can be bad for the environment. For example, in the tofu processing business, a lot of tofu dregs are not utilized even though if the tofu dregs are processed further, they can be used as a substitute for soy flour. Several obstacles were encountered in processing tofu dregs into feed, including the high crude fiber of 23.5%, and the presence of anti-nutritional substances (phytic acid) which can inhibit the digestion and absorption of nutrients.

The solution that can be done to increase the low nutrient content in liquid waste is one of them with fermentation. According to [1], fermentation is a technique in improving the quality of feed ingredients. In general, all of these fermented products have compounds that are simpler and more quickly digested than the original ingredients. Fulfilling the need for protein sources for feed production, local feed ingredients whose nutritional value has been enhanced by fermentation technology can be used as substitutes. The large amount of water content in industrial waste makes fermentation using liquid substrates suitable for the utilization of industrial liquid waste which is still not widely managed. By optimizing the use of alternative feed ingredients, feed problems which are important in aquaculture activities can be overcome. This is in accordance with research that has been carried out regarding solutions to increase the nutritional content of alternative feeds using the liquid fermentation method.

2. LIQUID SUBSTRATE FERMENTATION

2.1 Definition and Purpose

Fermentation is the process of chemical changes in an organic substrate through enzyme activity produced by microorganisms, whereas according to another study fermentation is the use of microbial metabolism to make raw materials into products that have a higher value, for example cell protein. monopolymers, biopolymers, organic acids, and antibiotics. Additional nutrients can affect the speed of fermentation and also the growth of microorganisms. Apart from requiring carbohydrates, the fermentation process also requires sufficient nitrogen and minerals so that microorganisms can grow and produce optimally. Apart from using mushrooms or yeast, fermentation can also be carried out using bacteria or a mixture of different microorganisms, for example you can use EM4 (Effective Microorganism 4).

Fermentation can remove unwanted odors, improve digestion, remove toxins from raw materials, and create desired colors. Microbes that are commonly used as fermentation inoculums are fungi, bacteria and yeast. Fermentation is divided into two, namely liquid substrate fermentation (submerged fermentation) and solid substrate fermentation (solid state fermentation). Liquid substrate fermentation is a fermentation in which microorganisms are grown on a substrate that is dissolved in the liquid phase. The liquid substrate fermentation process can be carried out in 3 ways, namely closed fermentation (batch culture), continuous fermentation and fed-batch fermentation.

2.2 Types of Liquid Substrate Materials

One of the fermentation techniques that is widely used in fermentation technology is liquid substrate fermentation using microorganisms which are worked on the substrate dissolved in the liquid phase. The water content used in the liquid fermentation process is very large and has a high humidity threshold so that the liquid fermentation technique is more effective when used in a fermentation process that uses organisms with aw requirements (water activity). Bacteria, soluble sugar, molasses, fruit or vegetable juice, wastewater, and agricultural waste are examples of commonly used liquid substrates in fermentation. Several local feed raw materials that have potential as alternative feed raw materials that can use liquid substrates come from agricultural, livestock and plantation industry wastes such as tofu dregs, palm oil cake, rubber seed cake, coconut cake, copra, hemp leaves, *kiambang* flour, banana plant waste, and rumen contents..

2.3 Potential and Constraints in Utilizing Liquid Substrate Fermentation

Liquid substrate fermentation has the potential to produce a more diverse product than solid substrate fermentation products. Liquid substrate fermentation can also produce feed directly if the fermentation activity takes place according to the amount of biomass being used. The application of liquid substrate fermentation is more popular in developed countries. This is marked by the development of the liquid substrate fermentation industry accompanied by advanced advances in fermentation technology in producing various liquid substrate fermentation products. Indonesia actually has the capacity of abundant Natural Resources, various types of microbes that have the potential for the enzyme industry and can be processed through a liquid substrate fermentation process, but until now Indonesia does not yet have an industry that produces liquid substrate fermentation. This is also motivated by the expensive operational costs of fermenting liquid substrates. Even though it is clear that Indonesia has the potential to produce a more diverse range of fermented products, such as for example the production of amylase, especially amyloglucosidase. In addition, Indonesia also has many starchy plants that grow and contain various microbes.

There are several types of liquid substrate fermentation methods, one of which is Submerged Fermentation or better known as SmF. The addition or replacement of nutrients in Submerged Fermentation (SmF) media is continuous. Bacteria that use high amounts of water are suitable for application using this fermentation technique. This was clarified in another study which stated that liquid substrate fermentation is more suitable for use in fermentation processes where the bacteria used require a high-water content. This is because in the fermentation process, bacteria require a large enough water content and have a high humidity level. In terms of time, the fermentation of liquid substrates is faster in terms of increasing the number of microorganisms in contact with the media and during the fermentation the oxygen is spread throughout, as well as the pH and nutrients.

The use of SmF fermentation has several advantages such as being easy to engineer by scientists because of easy supervision in terms of reaction and sterilization. In addition, enzyme production can be stimulated and can yield various forms of product depending on the stain and culture media used [2]. Liquid media fermentation is more profitable because the conditions can be adjusted as desired but it also has disadvantages, namely operational costs and the tools are very expensive.

3. LIQUID SUBSTRATE FERMENTATION PROCESS

Submerged fermentation (SmF) is the most widely used technique for cellulase production. Meanwhile, the aerobic fungus Trichoderma reesei is one of the most widely used strains. The following is a flow diagram of the submerged fermentation process (SmF).

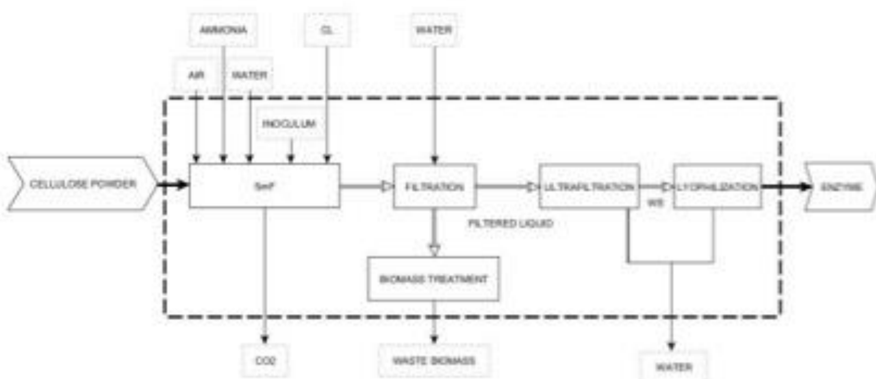

Figure 1. Submerged fermentation process flow chart (SmF)

The main stages of the fermentation process are shown below:

1) Inoculum: the sequence of the inoculum phase is seed growth (3 bioreactors) to provide the required amount of inoculum (5% of the process volume) and preparation of bioreactor media. Special media for inoculums contain different components such as ammonium sulfate, potassium phosphate or calcium dichloride (Heinzle et al 2006). Three growth bioreactors produce inoculum for the production vessel (100 L). This reactor is designed to provide 5% inoculum for each submerged bioreactor to the next scale. The fermentation time for each of the three growth bioreactors is estimated to be 40 hours [3].

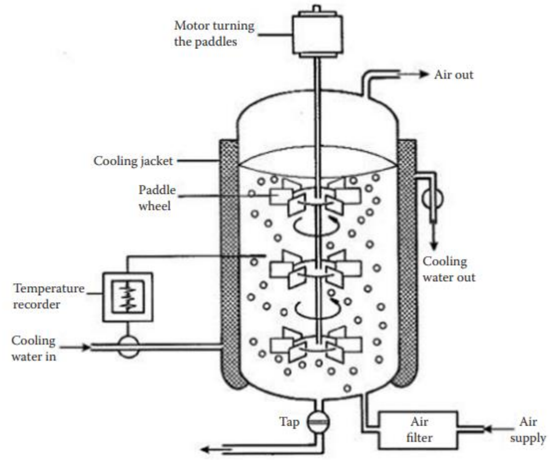

Figure 2. Structure of a modern fermenter used for submerged fermentation

2) SmF: The working volume of the bioreactor used is 80% of the total volume including the compressor and air filter which function for sterilization and provide oxygen needed during fermentation under optimal conditions. This bioreactor converts raw materials into the desired product, namely cellulase enzymes. Ammonium is used to control pH and provide additional nitrogen for fermenting microorganisms. Corn liquid and other nutrients are also added to the bioreactor as a carbon source. The bioreactor is aerated by compressed air with the aim of providing oxygen. Additionally, a system of chilled water flowing through the internal coils is used to control the temperature. The output of the fermentation is CO_2 which is released during cellulase production. Microorganisms are given nutrients to form biomass, cellulase enzymes (metabolism products), and waste water. The flow rate is estimated from the number of raw materials and waste.
3) Filtration: The first sub-phase is filtration, which consists of a tubular centrifugation and a 0.22 m membrane filter in which approximately 99.9% of the total waste biomass is removed.
4) Ultrafiltration: The supernatant is ultrafiltrated via tangential filtration to obtain a concentrated liquid without microorganisms. Ultrafiltration (10 kDa) was repeated until the concentration factor was 10.
5) Lyophilization: in the lyophilization stage, all the remaining water is removed, and a dry, solid enzyme is obtained as the final product.

4. QUALITY IMPROVEMENT OF FEED MATERIALS THROUGH LIQUID FERMENTATION

According to [2] the application of submerged fermentation (SmF) has been going on for more than a century, longer than solid fermentation. Many studies on liquid fermentation have been carried out and have shown results in improving the nutritional quality of feed ingredients. This can be seen in Table 1.

Table 1. Results of Liquid Substrate Fermentation Research Studies in Feed

Methods	Treatment	Result	Reference
Fermentation using liquid microorganism mix.	There were 3 treatments with different lengths of fermentation of tofu dregs, namely 3 days (Treatment A), 6 days (Treatment B) and 9 days of fermentation (Treatment C).	The best fermentation time, namely fermentation for 6 days (treatment B) showed a dry matter digestibility value of 55.65% and an organic matter digestibility of 54.23%, making it suitable for use as raw material for fish feed.	[4]
Liquid fermentation using *Bacillus licheniformis*	There were 3 treatments with different doses of inoculum and fermentation time, namely 1% 24 hours (Treatment 1), 2% 48 hours (Treatment 2) and 3% 72 hours	Crude protein increased from 4.89% to 8.85% and decreased crude fiber from 14.95% to 11.88%.	[5]

	(Treatment 3).		
Liquid fermentation	Five treatments with different amounts of time, namely for two days (D-1), four days (D-2), six days (D-3), eight days (D-4), and ten days (D-5) with inoculum dose of 2%	Fermentation that lasts more than two days decreases the crude fiber content (5.7%), reduces the oil palm cake fat (4.37%) and increases the protein content (15.37%)	[6]

Conclusion

Based on the review above, it can be concluded that liquid fermentation can improve the quality of feed ingredients in terms of increasing protein content, reducing crude fiber and anti-nutritional substances. The range of increase in crude protein value was in the range of 1.32% -12.1%, meanwhile, the value of crude fiber decreased by 3.07% -44%. Increasing the nutritional value of feed ingredients is carried out by fermenting liquid substrates through the stages of inoculum preparation, Smf, filtration, ultrafiltration and lyophilization.

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