BIOLOGICAL TREATMENT OF WASTEWATER FROM FISH PROCESSING PLANT USING *BACILLUS SPHAERICUS*

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Introduction

Biological treatment of wastewater from food processing plant to reduce the COD/BOD is one potential method that can be adopted to alleviate the pollution problem faced by this industry. In order to reduce the cost of treatment, the biological treatment process should be coupled with the production of valuable products. Production of biomass, which could be used as a valuable source of protein for animal feed, is one of the methods that is widely used in reducing the costs of wastewater treatment (Karim and Kamil, 1989). The spores of bacteria, *Bacillus sphaericus* and *Bacillus thuringenesis*, are commonly used as biological control agent to control the population of mosquito i.e., it is highly toxic towards mosquito larvae. These bacteria are unable to grow on glucose or sucrose (Yousteen et al. 1984). This paper deals with work that was undertaken to treat the wastewater from fish processing plant by biological means using *Bacillus sphaericus* aimed at reducing the waste strength and recovering the bacterial spores to be used as biological control agent. This strain is highly toxic towards *Culex*, has moderate toxicity towards *Anopheles*, and is relatively ineffective towards *Aedes*. i.e., it is highly toxic to mosquito larvae.

Materials and Methods

The fresh wastewater samples were obtained from fish processing plant, Perikanan LKPP Fort Sdn Bhd., at Kuantan, Pahang, Malaysia. The bacterium, *Bacillus sphaericus* strain 2362, was used throughout this study. A 100 ml wastewater or 0.5 g/l yeast extract was filled in 500 ml shake flask and the minerals [$MnSO_4.H_2O$; 8.0 g/l; $MgCl_2.6H_2O$; 0.23 g/l; and $CaCl_2.H_2O$; 0.103 g/l] were added according to the requirement of each experiment. After sterilisation for 15 min at 121°C, 15 psi, the flasks were inoculated with 10 ml of inoculum and then incubated in an orbital shaker at 200 rpm and 30°C. At time intervals, 10 ml of sample was taken for analysis (total cell, number of spored, COD and total nitrogen). The spores pellet was used for larvicidal activity determination against *Culex aquinqefasciatus*. Six larvae were added to 100 ml deionised water. The sample of *B. spaherícus* spore plus a standard (Sp88-Pasteur Institute, Paris, France) was added over a range of six dilutions (plus one control), each dilution was performed in duplicate. The mortality was read at 24 h and 48 h incubation and the response was converted to an LD_{50} by probit analysis.

Results and Discussion

Fish processing wastewater contained high amount of total nitrogen and amino acids, which are essential elements for growth of the microorganisms. Reducing sugars were only present in trace amounts. Growth of B. sphaericus in fish wastewater was very fast and reached a stationary growth phase after about 12 h. Spores formation was only started after 24 h of cultivation and increased almost linearly with time. The maximum concentration of spores (0.89 x 10^9 spores/ml) was obtained at 120 h of cultivation. The similar patterns of growth and sporulation were also observed for cultivation in yeast extract. However, the rate of sporulation was slightly faster in cultivation using yeast extract than using wastewater. In addition, the maximum total cell and spore obtained in cultivation using yeast extract were about 30% higher than cultivation using wastewater. A drastic reduction in COD, from 6,500 mg/l to 2,500 mg/l, was observed during active growth of the bacterium in waste water i.e., 0-20 h. During the sporulation phase, a slight reduction in COD (from 2,500 mg/l to 985 mg/l) was observed. Biological treatment of fish processing wastewater using *B. sphaericus* had a COD reduction of about 85%. The larvicidal activity of *B. sphaericus* spores was about 75 times higher after 48 h of incubation as compared to 24 h. The degree of sporulation and the level of toxin activity in *B. sphaericus* are not necessarily related (Karim et al. 1993). The larvicidal activity is greatly affected by the medium composition and cultural conditions. In this study, it was found that the larvicidal activity [LD_{50}] for spores of *B. sphaericus* grown in wastewater was almost the same to those spores grown in yeast extract. However, the larvicidal activity of *B. sphaericus* spores was lower than a standard.

Conclusions

The treatment of wastewater from fish washes using *B. sphaericus* coupled with the recovering its spores for subsequence use as biological control agent can be an alternative pretreatment technique in reducing the polluting srength of the effluent. Using this treatment method, about 85% reduction in COD of the wastewater from fish washes could be achieved after 120 h with an overall spores production of 0.92 x 10^7 spores/ml.h. The larvicidal activity of *B. sphaericus* spores grown in wastewater was comparable to spores obtained by growing in yeast extract.

References

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