OPTIMIZATION OF FERMENTATION CONDITIONS FOR PROTEIN PRODUCTION FROM EFFECTIVE MICROORGANISMS-FERMENTED TIGER PRAWN WASTE

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ABSTRACT

Effective microorganisms or EM have been actively used for various reasons but mainly in the waste water treatment, as plant growth initiators and fertilizers. In this study, fermentations of local tiger prawn waste (*Panaeus monodon*) by EM were conducted. At the early screening stage, fermentations were carried out at various temperature conditions (25°C, 37°C and 60°C), using various starter inocula (EM/Lactic Acid Bacteria) and different sources of carbon (glucose/brown sugar (BS)). Their pH values, total titratable acidities and lactic acid bacterial growth were examined. The results showed that, all samples were successfully fermented after three days of fermentation process except for fermentation at 60°C. Temperature at 60°C had affected the fermentation considerably where the pH did not rapidly decrease. Various levels of carbon source and inoculum were also investigated during screening. The results showed that, acid production in the fermented sample were dependent on glucose concentration but less dependent on the inoculum level. Based on the screening results, fermentation of prawn waste was carried out using 10% EM inoculum, 10% BS and fermented at 37°C for three days and the produced protein liquor contained 46.67% (w/v), dry weight protein. In the optimization phase using central composite design (CCD), four factors were selected which are temperature (30°C – 50°C), incubation time (3 – 9 days), inoculum size (v/w) (1 - 3%) and carbohydrate level (w/w) (5 - 15%). The processing result was protein content. The results showed that, to obtain high protein content from fermented sample, the most significant factor was the interaction between temperature and % of sugar, and the least was the interaction between % of sugar and % inoculum. High protein content and lower pH value were obtained with the suggested conditions given by the software Design Expert® which are temperature of 30°C, 5% sugar, 1.05% inoculum and incubated for 3 days. After optimization studies, the liquor protein content was increased to 86.35%.

ABSTRAK

Mikroorganisma berkesan atau EM telah digunakan dalam pelbagai bidang terutamanya dalam rawatan air sisa, pertumbuhan pokok dan baja. Dalam kajian ini, fermentasi sisa tempatan udang harimau (*Panaeus monodon*) menggunakan EM telah dijalankan. Pada peringkat awal, fermentasi telah dijalankan pada pelbagai suhu (25°C, 37°C dan 60°C), menggunakan pelbagai mikroorganisma atau inokulum (EM / Bakteria Asid Laktik) dan pelbagai sumber karbon (glukosa / gula merah (BS)). Semasa proses fermentasi, nilai pH, jumlah keasidan dan pertumbuhan bakteria bakteria asid laktik telah dianalisa. Hasil kajian menunjukkan bahawa, semua sampel telah berjaya difermentasi selepas tiga hari kecuali sampel yang difermentasi pada suhu 60°C. Suhu 60°C memberi kesan yang ketara kepada proses fermentasi di mana pH tidak menurun dengan cepat. Pelbagai jumlah sumber karbon dan inokulum juga dikaji diperingkat awal ini. Hasil kajian menunjukkan, penghasilan asid sangat bergantung kepada jumlah sumber karbon yang dimasukkan dan kurang bergantung kepada jumlah inokulum yang digunakan. Berdasarkan hasil yang diperolehi di peringkat awal penyelidikan, proses fermentasi dilakukan menggunakan 10% EM sebagai inokulum, 10% BS sebagai sumber karbon dan difermentasi pada suhu 37°C selama tiga hari dan cecair protein yang mengandungi sebanyak 46.67% berat kering (b/i) telah berjaya diperolehi. Bagi fasa pengoptimuman menggunakan ‘central composite design’(CCD), empat faktor yang telah dipilih iaitu suhu (30°C - 50°C), masa pengeraman (3 – 9 hari), saiz inokulum (i/b) (1% - 3%) dan jumlah karbohidrat (b/b) (5% - 15%). Kandungan protein dianalisa dalam setiap sampel. Keputusan telah menunjukkan bahawa untuk mendapatkan nilai kandungan protein yang tinggi, faktor yang paling memberi kesan adalah interaksi antara suhu dan jumlah karbohidrat dan yang paling kurang memberi kesan ialah interaksi antara jumlah karbohidrat dan saiz inokulum. Kandungan protein yang tinggi dan nilai pH yang lebih rendah telah diperolehi berpandukan cadangan yang diberikan oleh perisian Design Expert ® iaitu melalui proses fermentasi selama tiga hari pada suhu 30°C, menggunakan 5% karbohidrat, dan 1.05% inokulum. Dengan melakukan proses pengoptimuman, kandungan protein meningkat kepada 86.35%.

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LIST OF ABBREVIATIONS

AOAC	-	Association of Official Analytical Chemist
PBS	-	Palm Brown Sugar
cfu/mL	-	Colony- forming unit per mililitre
DNS	-	Dinitrosalicylic acid
EM	-	Effective microorganism
EM-A	-	Activated effective microorganism
EM-A7	-	Effective microorganism that has been activated for seven days
EM-M	-	EM Malaysia
EM-I	-	EM Indonesia
LAB	-	Lactic acid bacteria
MRS	-	De Man, Rogosa and Sharpe
PER	-	Protein Efficientcy Ratio
PMM	-	Porcine Meat Meal
PPTAP	-	Pusat Penyelidikan Ternakan Air Payau
SBM	-	Soybean Meal
P/E	-	Protein to Energy
TTA	-	Total Titratable Acidity
ANOVA	-	Analysis of variance
BG	-	Bacterial growth

Chapter 1

Introduction

1.1 Background Study

Aquaculture constitutes a vital and growing segment of agriculture worldwide. The increased demand for fish as a result of rapid population growth, and preference of fish over other animal proteins for personal, cultural or health reasons further accelerates the industry's growth. Economics, environmental issues and fish health are some of the important factors vital for the sustainability of the aquaculture industry. These factors increase the pressure on fish nutritionist to develop cost-effective, nutritionally balanced and low pollution diet as well as to improve feeding strategies (Briones *et al.*, 2004).

Asia is the world leader in aquaculture sector and Malaysia as one of the Asian countries had been requested to engage in a project on "Fish Supply and Demand in Asia". This project was supported by Asian Development bank and began in 2000 (Briones *et al*, 2004). In the aquaculture sector, the two most important things needed to be considered are nutrition and feeding strategies. At present, commercial diet containing imported fishmeal as the main protein source are the most commonly used diet for aquaculture purposes to maintain fish health and growth especially at the early growing stage. However, prices of fish feed pellet is increasing due to the escalating increase in fishmeal price around the world. The price of fishmeal from October 2006 to October 2011 is shown in Figure 1.1 (International Monetary Fund, 2011). Therefore,

finding alternative resources to replace fishmeal has become a very important and urgent issue as the cost for aquaculture activities will increase since 50-60% of the whole operation cost comes from feeds.

Figure 1.1: Prices of fishmeal from October 2006 to October 2011 (International Monetary Fund, 2011)

Description: Fishmeal, Peru fishmeal/pellet 65% protein, US$ per metric tonne
Unit: US Dollars per Metric Ton

One of the potential protein sources widely available in Malaysia is prawn waste from seafood processing industries where the waste generally contains 15-40% protein, and 14-30% chitin (Zakaria *et al.*, 1998). Protein from prawn waste has been proven to contain high quality amino acid content important both as i) feed attractant, and ii) easily digestible protein (Nwanna, 2003). The use of extracted protein as opposed to using whole waste is to avoid using a high fibre protein in the fish diet which will make the feed highly indigestible (Oduguwa *et al*., 2004). Fermentation involving lactic acid bacteria (LAB) for protein and chitin recovery has been proven successful (Zakaria *et*

al., 1998). A lactic acid bacterial fermentation using glucose as a substrate has been adopted in our laboratory with success and protein rich liquor is produced after 3 days. It has a protein content of 47.3 % (Nor *et al*. 2011) and has been preliminary tested for making fish feed. However, a complete study on digestibility was not done thus effectiveness of such fermented protein was not fully assessed. At present, a more stable EM technology which feeds on carbohydrate has been widely used and it is envisaged that the same technology can be applied to ferment prawn waste with expected similar success. Since EM is widely available with a reasonable price and also feeds on cheap carbohydrate, this is an added advantage and therefore adopted for this project. A full scale study on the best condition to obtain protein from fermented prawn waste was determined. The extracted protein can be added as an ingredient in fish or aquaculture diet formulation since prawn waste is generally reported to have good amino acid profile such as tryptophan and lysine which is important for growth of fish (Fanimo *et al*., 2004). The probiotic-rich fish pellet is also an added advantage in combating diseases and survivability.

Since prawn waste protein is cheaper to obtain, it is expected to be economically viable to be used in diets for carnivorous fish such as Siakap (*Lates calcarifer*) which requires about 45% protein-based diet (Mohammed Suhaimee, 2005). To date, no work has been reported on fermentation of prawn waste using EM to obtain protein for the use in aquaculture diet to replace the more expensive fishmeal as the main protein source. This study was therefore designed to provide an optimum condition for the production of high quality protein through EM-fermented prawn waste which has the potential to be used as an ingredient in aquaculture feed. Optimization was done using design of experiment (DOE) which are fractional factorial design and central composite design (CCD).

At present, aquaculture industry uses commercial diet in the form of pellets for feeding especially for the first two months. Commercial diets contain among others animal protein such as fishmeal which is usually imported for its high quality grade. Cost of fishmeal has escalated and many local farmers are highly dependent upon such

diets for fear of high fatality rate if they use a lower quality product especially in the first two months. Malaysia has been blessed with marine products and frozen food industries involving processing of prawn species such as tiger prawn and in return the industry is also producing about 50% of waste materials (Cira *et al.*, 2002). This waste has been reported to contain valuable amounts of protein (15-40%) and chitin (Zakaria *et al.*, 1998) and if protein can be extracted in good form, it can be used to replace the costly fishmeal.

1.2 Statement of Problem

Previous study (Nor *et al*, 2011) had shown that prawn waste was fermented by LAB in order to obtain protein liquor for aquaculture purpose. However, since LAB is a single culture, it's resistance to current environment is limited, which may reduce the performance of LAB during fermentation processes. In order to improve this problem, EM technology was applied since it contains a consortium of organisms that can work together to enhance the population of beneficial organisms. Furthermore, EM is simple, inexpensive, versatile and easy to use (Higa *et al.*, 1994).

1.3 Objectives of the Study

This study embarks on two main objectives:

(i) To investigate the effectiveness of EM to ferment prawn waste to obtain high quality, probiotic rich-protein liquor.

(ii) To optimize the fermentation for production of protein liquor.

1.4 Significance of the Study

The result of this project is hoped to create awareness amongst farmers to use updated technologies and at the same time saving production cost. By providing basic understanding of the working of these microorganisms proposed by this project, it will promote further useful applications especially in the agricultural and environmental sectors. The results of this work can be used in the larger laboratory scale fermentations involving suitable bioreactors.

1.5 Research Scopes

In order to achieve the research objectives, the following scopes of study have been drawn:

1. Fermentation study on tiger prawn waste using effective microorganisms and lactic acid bacteria. Characterization of fermentation products namely protein liquor.
2. Optimization study on the fermentation conditions using Response Surface Methodology which is factorial design and central composite design.

REFERENCES

Khaliq, A., Abbasi, K. M., and Hussain, T., (2006). Effect of Integrated Use of Organic and Inorganic Nutrient Sources of Effective Microorganisms (EM) on Seed Cotton Yield in Pakistan. *Bioresource Technology*. **97**, 967-972.

Adler-Nissen, J. (1986). *Enzymic Hydrolysis of Food Protein*. London: Elsevier.

Ai Q., Mai K., Li H., Zhang C., Zhang L., Duan Q., Tan B., Xu W., Ma H., Zhang W., and Liufu Z. (2004). Effect of Dietary Protein to Energy Ratios on Growth and Body Composition of Juvenile Japanese Seabass, *Lateolabrax japonicas Aquaculture.* **230**, 507-516.

Anderson, L., V., and McLean, A., R., (1974). Design of Experiments A realistic Approach. Marcel Dekker, Inc. 326-386

Anderson, T., M. (2009). Encyclopedia of Microbiology. 3rd Ed. 349-361.

Andrew Jackson, (2010). Fishmeal, Fish Oil Prime Feed Ingredients Not Limiting Factors For Responsible Aquaculture, *Global Aquaculture Advocate*, January/February 2010, UK.

Axelsson, L. T. (1993). Lactic Acid Bacteria: Classification and Physiology. In: Salminen, S. and Wright, S.V. Eds. *Lactic Acid Bacteria*. New York: Marcel Dekker Inc, 1-65.

Bhaskar, N., Suresh, P. V., Sakhare, P. Z., and Sachindra, N. M. (2007). Shrimp Biowaste Fermentation with *Pediococcus acidolactici* CFR2182: Optimization of Fermentation Conditions by Response Surface Methodology and Effect of Optimized Conditions on Deproteination/Demineralization and Carotenoid Recovery. *Enzyme and Microbial Technology*. **40**, 1427-1434.

Boonyaratpalin. M., Suraneiranat. P., Tunpibal. T. (1998). Replacement of Fish Meal With Various Types of Soybean Products in Diets for the Asian Seabass, *Lates calcarifer*. *Aquaculture.* **161**, 67-78.

Briones M., Dey M.M. and Ahmed M. (2004). The Future for Fish in the Food and Livelihoods of the Poor in Asia. *NAGA, worldfish center quarterly* **27**, 3.

Bueno-Solano, C., Lopez-Cervantes, J., Campas-Baypoli, O. N., Lauterio-Garda, R., Adan-Bante, N. P., and Sanchez-Machado, D. I. (2009). Chemical and Biological Characteristics of Protein Hydrolysates from Fermented Shrimp by-Products. *Food Chemistry*. **112**, 671-675.

Caprice, E. and Fitzgerald, G.F. (1999). Food fermentations: Role of microorganisms in food production and preservation. International Journal of Food microbiology. **50**, 131-149.

Catacutan, M. R. and Coloso, R. M. (1997). Effect of Dietary Protein to Energy Ratios on Growth, Survival, and Body Composition of Juvenile Asian Seabass, *Lates Calcarifer,* Fed Varying Carbohydrates and Lipids Levels. *Aquaculture*. **149**, 137-144.

Chapman, F., and Miles, R. (2006). Interpreting a Fish Food Package Label. University of Florida. IFAS Extension. 1-6.

Choorit, W., Patthanamanee, W., Manurakchinakorn, S. (2008). Use of response Surface Method for the Determination of Demineralization Efficiency in Fermented Shrimp Shells. *Bioresource Technology* **99**, 6168-6173.

Christopher, L., D., Dulgado, L., Wada, N., Meijer, S., Rosegrand, W.., M., Ahmad, M. (2003). Fish to 2020 Supply and Demand in changing Global Markets. Worldfish Centre, Penang Malaysia. International Food Policy Research Institute. Washington D.C. 1-137.

Cira, L. A., Huerta, S., Hall, G. M., and Shirai, K. (2002). Pilot Scale Lactic Acid Fermentation of Shrimp Wastes for Chitin Recovery. *Process Biochem*. **37**, 1359-1366.

Cornell, J. A. (1990). *How to apply Response Surface Methodology (RSM)*. 2nd Ed. Wisconsin, US: ASQC. 1-20.

Cowey, C. B. (1998). The Nutrition of Fish: The Developing Scene. *Nutrition Research Reviews* **1**, 255-280.

Devore, J. L. and Farhum, N. R. (1999). *Applied statistics for engineers and scientists*. Pacific Grove: Duxbury Press.

Elizabeth, C., and Fitzgerald, F., G. (1999). Food Fermentations: Role of Microorganisms in Food Production and Preservation. *International Journal of Food Microbiology* **50**, 131-149.

Espe, M., Haaland, H., Njaa, L. R. (1992). Autolysed fish silage a feed ingredient for Atlantic Salmon (Salmo salar). *Composition Biochemistry Physiology* **103**, 369-372.

Fagbenro, O., Jauncey, K., and Haylor, G. (1994). Nutritive Value of Diet Containing Dried Lactic Acid Fermented Fish Silage and Soybean Meal for Juvenile. *Oreochromis niloticus and Clanas gariepinus. Aquat. Living Resour.* **7**, 79-85.

Fanimo A.O, Oduguwa B.O., Oduguwa O.O., Ajasa O.Y. and Jegede O. (2004). Feeding Value of Shrimp Meal for Growing Pigs. *Arch. Zootec* **53**, 77-85.

Food and Agriculture Organization of the United Nations, FAO (2010). The State of Food Insecurity in the World Addressing Food Insecurity in Protacted Crises. Rome, Italy. Policy and Support Branch.

Hall, G. M., and Silva, S. D. (1992). Lactic Acid Fermentation of Scampi (*Penaeus monodon*) Waste for Chitin Recovery. In Brine, C. J., Sandford, P. A., and Zikakis, J. P. (Eds) *Advance in Chitin and Chitosan*. London: Elsevier Applied Science.663-668.

Hall, G. M., Silva, S. D. (1994). Shrimp Waste Ensilation. *INFOFISH Int*. 2(94), 27-30.

Hamizatul Zakiah Binti Daud (2000). Pemencilan Bakteria Asid Laktik Daripada Fermentasi Sisa Udang. BSc. Thesis. Universiti Teknologi Malaysia, Malaysia.

Hassan, T. E, Health, J. L. (1986). Biological Fermentation of Fish Waste for Potential Use in Animal and Poultry Feeds. *Agricultural Wastes* **15**, 1-5.

Hernandez. C., Miguel. A., Novoa. O., Aguilar-Vejar K., Gonzalez-Rodriguez. B., and De La Parra. I. A. (2008). "Partial Replacement of Fish Meal by Porcine Meat in Practical Diets for Pacific White Shrimp (*Litopenaeus vannamei*)". *Aquaculture* **277**, 244-250.

Heu M-S, Kim J-S, Shahidi, F. (2003). Components and Nutritional Quality of Shrimp Processing by-Products *Food Chemistry* **82**, 235-242.

Higa, T., and Parr, F., J. (1994). Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment. International Nature Farming Research Centre Atami, Japan.

Higa,T., James, P. (1994). Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment. International Nature Farming Research Center. 7.

Horwitz, W. (1980). *Official Methods of Analysis*. 13th Ed. Washington, D.C: Association of Official Analytical Chemists.

International Monetary Fund (IMF), 2011. Fishmeal Monthly Prices-Commodity Prices. www.indexmundi.com/commodity = fishmeal.

Jonathan, W. (2011). Fish Farming is Answer to Increasing Global Meat Demands, Says Report. 14 June 2011, 16.45 BST.

Kaushik, S. J., Coves, D., Dutto, G., and Blanc, D. (2003). Almost Total Replacement of Fish Meal by Plant Protein Sources in the Diet of a Marine Teleost, the European Seabass *Dicentrarchus labrax*. *Aquaculture* **230**, 391-404.

Kuehl, R. (2000). *Design of Experiments: Statistical principles of research design and analysis*. 2nd Ed. Duxbury Press. Pacific Grove.

Lopez A. R., Guerrero, I. L., Huerta, S. (2009). Astaxanthin Extraction from Shrimp Waste by Lactic Acid Fermentation and Enzymatic Hydrolysis of the Carotenoprotein Complex. *Journal of Chromatograph Science*. **67**, 1002-1006.

Madigan, M. T., Martinko, J. M., and Paker, J. B. (1997). *Biology of Microorganism*. 8th Ed. Englewood Cliffs: Prentice Hall.

Mahat, M., K., Illias, M., R.,Rahman., A., R., Rashid., A., A., N., Mahmood, N., N., A., Hassan, O., Aziz, A., S., and Kamaruddin, K. (2004). Production of Cyclodextrin Glucanotransferase (CGTase) from Alkalophilic *Bacillus* sp. TS1-1: Media Optimization Using Experimental Design. *Enzyme and Microbial Technology* **35**, 467-473.

Malaysian Fisheries Department (2004). Annual Fisheries Statistic 2004. Malaysia: Malaysian Fisheries Department.

Mohammed Suhaimee A. M. (2005). The Use of Protein from Palm Kernel Meal in the Diets of Seabass (*Lates calcarifer*). Universiti Teknologi Malaysia: MSc. Thesis.

Myers, H., R., and Montgomery, C., D., (2002). Response Surface Methodology Process and Product Optimization Using Designed Experiments. 2nd Ed. A Wiley-Interscience Publication. 1-156.

NACA/FAO (2008). Reducing the Dependence on the Utilization of Trash Fish/ Low Value Fish as Feed for Aquaculture of Marine Finfish in the Asian Region. Golden Beach Resort, Krabi, Tahiland, 8th to 10th Sepetember 2008. 1-61.

NAGA (2004). World Fish Center Quaterly **27**, 3-4.

Nor, N. M. , Zakaria, Z. , Manaf, M. S. A. and Salleh, M. M (2011). The Effect of Partial Replacement of Dietary Fishmeal with Fermented Prawn Waste Liquor on Juvenile Sea Bass Growth. *Journal of Applied Aquaculture* **23**, 51-57.

Nwanna, L. C. (2003). Nutritional Value and Digestibility of Fermented Shrimp Head Waste Meal by African Catfish *Clarias gariepinus*. *Pakistan Journal of Nutrition* **2**(6), 339-345.

Oduguwa, O.O., Fanimo A.O., Olayemi V.O. and Oteri N. (2004). The Feeding Value of Sun-dried Shrimp Waste-Meal Based Diets for Starter and Finishing Broilers. *Arch. Zootec* **53**, 87-90.

Olive –Teles, A., and Goncalves, P. (2001). Partial Replacement of Fshmeal by Brewers Yeast *(Saccaromyes cerevisae)* in Diets for Seabass *(Dicentrarchus labrax)* Juveniles. *Aquaculture* **202**, 269-278.

Oseni, M. M. (2001). Replacement of Fishmeal by Animal by-Product Meals in a Practical Diet for Grow-Out Culture of Grouper *Epinephelus coioides*. *Aquaculture.* **204**, 75-84.

Oyedapo, A., Fagbenro & Oluayo, A., and Bello-Olusoji (1996). Preparation, Nutrient Composition and Digestibility of Fermented Shrimp Head Silage. *Food chemistry*. **60**, 489-493.

Parker, R. (2002). Aquaculture Science. 2nd Ed. Englewood Cliffs: Prentice Hall.

Rahman, A. R. (2004). Response Surface Methods (RSM). *A two days course using Design Expert Software version 6.0* (Stat-Ease, 2002). 6-7 February, 2004. Chemical Engineering Pilot Plant (CEPP), UTM Skudai, Johor Darul Takzim.

Ramyadevi, D., Subathira, A., and Saravanan, S., (2012). Potential Recovery of Protein from Shrimp Waste in Aqueous two Phase System. *Research Journal of Chemical Science*. **7**, 47-52

Rao, M. S., Munoz, J., and Stevenns, W. F. (2002). Critical Factors in Chitin Production by Fermentation of Shrimp Biowaste. *Appl Microbial Biotechnol*. **54**, 808-813.

Rao, M., S., and Stevens, W., F. (2006). Fermentation of Shrimp Biowaste under Different Salt Concentrations with Amylolytic and Non-Amylolytic *Lactobacillus* Strains for Chitin Production. *Food Technol. Biotechnol.* **44**, 83-87.

Rashid, M. T., and West, J., (2007). Dairy Wastewater Treatment with Effective Microorganisms and Duckweed for Pollutants and Pathogen Control. Zaidi, M. K., Wastewater Reuse-risk Assessment, Decision-making and Environmental Security, Springer. 93-102.

Regost, C., Arzel, J., Kaushik, S. J. (1999). Partial or Total Replacement of Fishmeal by Corn Gluten Meal in Diet for Turbot (*Psetta maxima*). *Aquaculture* **180**, 99-117.

Ronald W. H. (1996). Alternative Protein Sources for Salmon and Trout Diets. *Animal Feed Science Technology*. **59**, 71-80.

Sachindra, M., N., and Bhaskar, N. (2008). In Vitro Antioxidant Activity of Liquor from Fermented Shrimp Biowaste. *Bioresource Technology* **99**, 9013-9016.

Salasiah Binti Mohamad (2009). Optimization of Enzymatic Degradation of Pretreated Shrimp Waste Using Crude Chitinase Produced by Bacillus licheniformis Using Central Composite Design (CCD). Report of Degree of Bachelor of Science (Industrial Biology) Faculty of Bioscience and Bioengineering, Universiti Teknologi Malaysia.

Sekeran, V., Balaji, C., Bagavathi Phuspa, T. (2005). Evaluation of Effective Microorganism in Solid Waste Management, *Electronic Green Journal* **21**, 1.

Shahidi, F., and Synowiecki, J. (1991). Isolation and Characterization of Nutrients Value-Added Products Fron Snow Crab (*Chinoecetes ipoloi*) abd Shrimp (*Pandalus borealis*) Processing Discards. *J. Agric. Food. Chem.* **39**, 1527-1532.

Shirai, K., Gurrero, I., Huetta, S., Saucedo, G., Castillo, A., Gonzalez, O., and Hall, G.M. (2001). Effect of Initial Glucose Concentration and Inoculation Level of Lactic Acid Bacteria in Shrimp Waste Ensilation. *Enzyme and Microbial Technology*. **28**, 446-452.

Shirai, K.(1999), Utilisation pf Shrimp Wastes for Chitin, Protein and Pigment Recovery by Microbial Route (Ph.D. Thesis). Mexico City, Mexico. Universidad Autonoma Metropolitana.

Simpson, B. K. and Gagne, N. and Simpson, M. V. (1994). Bioprocessing of Chitin and Chitosan In: A. M. Martin. Fisheries processing: Biotechnological Applications. London:Chapman and Hall.162-170.

Speck, M. L. (1972). Control of Food Borne Pathogens by Starter Cultures. *J.Dairy Sci*. **55**, 1019

Stat-Ease (2002). *Design-Expert 6.0 user's guide*. Version 6.0 Design-Expert® software.

Sung, C. K., Young-Chae, S., and In-Soo, K. (2000). Treatment of Food Waste Material by Effective Microorganisms and Its Use in Crop Production, Department of Environmental Engineering, Korea Maritime University, Pusan Korea.

Synder, C. (2011). Trout Growers Feel Pinch of High Feed Price. Times News. 11 October 2011.

Szymanski, N., and Patterson, R. A., (2003). Effective Microorganisms (EM) and Wastewater Systems. *Future Directions for On-Site Systems: Best Management Practice Proceedings of On-Site '03 Conference*. 30th September to 2nd October 2003. University of New England, 347-354.

The Global Aquaculture Alliance 2010, November 6-9, Santiago Chile.

Thongchai, P., and Pranee, P. (2006). How Effective are Effective Microorganisms (EM). EEAT yearbook. 43-44.

Uyan, O., Koshio, S., Teshima, S., Ishikawa, M., Thu, M., Alam, S., M., Michael, R., F., (2006). Growth and phosphorus loading by partially replacing fishmeal with tuna muscle by-product powder in the diet of juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture* **257**, 437-455

World Fish Centre, 2011. Reducing Poverty and Hunger by Improving Fisheries and Aquaculture. World Fish Centre Report 2010/2011 Including Financial Report for 2010.

Wassef, E., A., (2001). Alternative Protein Source for fish Feeds in Egypt. *Cahiers Options Mediterranian* 63, 127-141.

Webster C. D. and Lim C. E. (2002). *Nutrient Requirement and Feeding Of Finfish For Aquaculture*. New York: CABI publishing.

Yusuf, C. (1999). Department of Chemical Enginering University of Alneria, Spain. Copyrighy Acad Press.

Zakaria, Z., Hall G.M. and Shama G. (1998). Lactic Acid Fermentation of Scampi Waste in a Rotating Horizontal Bioreactor for Chitin Recovery. *Process Biochemistry* **33**, 1-6.

Zhiong Li, Lee, J., and Cho, M. H., (2010). Antioxidant, Antibacterial, Tyrosinase Inhibitory, and Biofilm Inhibitory Activities in Fermented Rice Bran Broth with Effective Microorganisms. *Biotechnology and Bioresource Engineering*. **16**, 139-144.