

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<sup>®</sup> 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 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 di peringkat 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%.

## TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	ABSTRACT	vi
	ABSTRAK	vii
	TABLE OF CONTENTS	viii
	LIST OF TABLES	xiii
	LIST OF FIGURES	xv
	LIST OF ABBREVIATIONS	xix
<b>1.0</b>	<b>INTRODUCTION</b>	1
	1.1 Background Study	1
	1.2 Statement of Problem	4
	1.3 Objectives of the Study	4
	1.4 Significant of the Study	4
	1.5 Research Scope	5
<b>2.0</b>	<b>LITERATURE REVIEW</b>	6
	2.1 Aquaculture in Malaysia	6
	2.2 Feed In Aquaculture	7
	2.3 Replacement of Fishmeal Diet	9
	2.4 Prawn Wastes as Animal Feed	12
	2.5 Essential Diet for Carnivorous Species	13
	2.5.1 Protein and Amino Acids	13
	2.5.2 Lipids and Fatty Acids	14
	2.5.3 Carbohydrate	14
	2.5.4 Vitamins and Minerals	15
	2.6 Effective Microorganism	16

2.6.1	Principle Microorganism in EM	17
2.6.1.1	Photosynthetic Bacteria	17
2.6.1.2	Lactic Acid Bacteria	17
2.6.1.3	Yeasts	18
2.6.1.4	Actinomycetes	18
2.6.1.5	Fermenting Fungi	19
2.6.2	Application of EM	19
2.6.2.1	Treating Food Waste	19
2.6.2.2	Fermenting Various Organic and Inorganic Materials	20
2.6.2.3	Treating Waste Water	20
2.7	Fermentation Process	21
2.7.1	General Introduction	21
2.7.2	Fermentation of Prawn Waste	22
2.7.3	Characterization of Protein from Fermented Prawn Waste	23
2.8	Design of Experiment	24
2.8.1	Factorial Designs	24
2.8.1.1	Two-level Fractional Factorial Design	24
2.8.2	Response Surface Methodology	25
2.8.2.1	Central Composite Design	25
2.8.3	Optimization Using Design of Experiment	25
<b>3.0</b>	<b>MATERIALS AND METHOD</b>	27
3.1	Materials	27
3.1.1	Bacteria	27
3.1.2	Prawn Waste	27
3.1.3	Media for Starter Inoculum	28
3.1.3.1	Lactic Acid Bacteria	28
3.1.3.2	Activation of Effective Microorganism	28
3.1.4	Carbohydrate Source	28
3.2	Experimental Methods	29

3.2.1 Screening Phase on Using EM for Fermentation of Prawn Waste	29
3.2.1.1 Preparation of Starter Cultures	29
a) LAB	30
b) EM	30
3.2.1.2 Fermentation of Prawn Waste	30
3.2.1.3 Effect of EM-Activation Period on the Fermentation of Prawn Waste	31
3.2.1.4 Effect of Starter Culture and Inoculum Level	31
3.2.1.5 Effect of Temperature	32
3.2.1.6 Effect of Carbon Source and level of Carbon Used	32
3.2.1.7 Effect of Prolong Storage on the Stability of Fermented Prawn Waste	33
3.2.1.8 Protein Recovery from Early Screening Stage	33
3.2.2 Optimization Study by Using Design of Experiment	34
3.2.2.1 Fractional Factorial Design	34
3.2.2.2 Central Composite Design	36
3.2.2.3 Model Adequacy Checking	37
3.2.2.4 F-distribution Test	37
3.2.2.5 Coefficient of Multiple Determinations ( $R^2$ )	37
3.2.2.6 Lack-of-fit Test	38
3.2.2.7 Pearson's Correlation Coefficient, r	38
3.2.2.8 Protein Production from Optimization Phase	39
3.2.3 Analysis of Fermented Products	39
3.2.3.1 Determination of pH and Total Titratable acidity (TTA)	39
3.2.3.2 Determination of Bacterial Growth	40
3.2.3.3 Proximate analysis	40

	a) Determination of Protein Content (Kjeldahl Method)	40
	b) Determination of Crude Fat	41
	c) Determination of Crude Fibre	42
	d) Determination of Moisture Content	43
	e) Determination of Ash Content	44
<b>4.0</b>	<b>RESULTS AND DISCUSSIONS</b>	<b>45</b>
4.1	Early Screening Stage on EM as Starter Culture for Fermentation of Prawn Waste	45
4.1.1	EM-Activation	45
4.1.2	General Observation on Fermented Prawn Waste	47
4.1.3	Effect of EM-Activation Period on the Fermentation of Prawn Waste	48
4.1.4	Effect of Starter Culture and Inoculum Level	50
4.1.5	Effect of Temperature	54
4.1.6	Effect of Sugar Source and Level of Sugar Used	57
4.1.7	Effect of Prolonged Storage on the Stability of Fermented Prawn Waste	60-
4.1.8	Protein Recovery from Fermented Prawn Waste using Early Screening Stage Conditions	61
4.1.9	Summary of the Early Screening Experiment	63
4.2	Optimization Study by using Design of Experiment	64
4.2.1	Factorial Design	64
4.2.2	Effect of Processing Parameters on the pH Value of Fermented Sample	65
4.2.3	Effect of Processing Parameters on the Protein	70



	Production From Fermented Sample	
	4.2.4 Summary of Preliminary Optimization Phase (Factorial Design)	76
	4.3 Optimization Using Central Composite Design	77
	4.3.1 Effect of Processing Parameters on the Protein Production from Fermented waste	78
	4.3.2 ANOVA Analysis and Fitting of Quadratic Model	79
	4.3.3 Effect of Parameters on Protein Production	84
	4.3.3.1 Effect of Temperature and Percentage of Sugar	84
	4.3.3.2 Effect of Incubation Time and Percentage of Sugar	86
	4.3.3.3 Effect of Incubation Time and % Inoculum on Protein production	87
	4.3.3.4 Effect of Temperature and Incubation Time on Protein production	88
	4.3.3.5 Effect of Temperature and Percentage of Inoculum on Protein production	89
	4.3.3.6 Effect of Sugar and Inoculum Level on Protein Production	90
	4.3.4 Optimization of Models	91
	4.3.4.1 Summary of Optimization by CCD	92
	4.3.4.2 Comparison of Protein Production at Different Fermentation Condition	93
	4.3.5 Verification of Optimization Model	93
<b>5.0</b>	<b>CONCLUSIONS AND SUGGESTIONS</b>	<b>95</b>
	5.1 Conclusions	95
	5.2 Suggestion for Future Work	96
	<b>REFERENCES</b>	<b>97</b>
	<b>APPENDIXES</b>	<b>108</b>

## LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Research on partial or total replacement of fishmeal	11
2.2	The dietary requirements for various vitamins with their respective signs of deficiency in sea bass	16
3.1	Experimental matrix for pH and protein analysis: fractional factorial design ( $2^3$ )	35
3.2	Experimental matrix for protein analysis: central composite design ( $2^4$ )	36
4.1	Weight of the material involve in screening study	62
4.2	Proximate analysis on the initial waste (unfermented) and the liquor (protein fraction) after 72 hours of fermentation	63
4.3	Mass balance for protein after fermentation process	63
4.4	Design summary of the factorial design	65
4.5	Fractional factorial design for four variables together with the observed response of pH value	66
4.6	ANOVA response surface linear model (responses: pH value)	67
4.7	Fractional factorial design for four variables together with the observed response of protein production	71
4.8	ANOVA response surface linear model (responses: % protein)	71
4.9	Selection criteria of the processing parameter	76

4.10	Solution of the best condition for fermentation process in obtaining high protein content from preliminary optimization	76
4.11	Design summary of optimization study	78
4.12	Central Composite Design (CCD) for four variables together with the observed response	79
4.13	ANOVA Table (partial sum squares) for response surface quadratic model	80
4.14	Central composite design's solution of the best condition for fermentation process in obtaining high protein content	91
4.15	Comparison of protein production at different condition of fermentation process	93
4.16	The verification phase results based on the most appropriate processing parameters	94

## LIST OF FIGURES

<b>FIGURES NO.</b>	<b>TITLE</b>	<b>PAGE</b>
1.1	Prices of Fishmeal from October 2006 to October 2011	2
2.1	Global growth of aquaculture and it's used of fish meal and fish oil	9
4.1	The pH profile during activation period of EM	46
4.2	Lactic acid bacterial growth during activation period of EM	47
4.3	Effect of types of activated EM (EM-A) on pH value of EM fermented waste (liquor) after three days of fermentation process	49
4.4	Effect of types of activated EM (EM-A) on percentage protein of EM fermented waste (liquor) after three days of fermentation process	50
4.5	The pH profile of fermented waste using various starter inoculum at 37°C	52
4.6	TTA of prawn waste fermented using various starter inoculum at 37°C	52
4.7	Bacterial growth of prawn waste fermented using various starter inoculum at 37°C	53
4.8	pH profile of fermented prawn waste using different level of EM-M inoculum	54

4.9	pH profile of EM-M fermented prawn waste by using brown sugar at various temperatures	55
4.10	TTA of EM-M fermented prawn waste by using brown sugar at different temperature	55
4.11	Bacterial growth of EM-M fermented prawn waste by using brown sugar at various temperatures	55
4.12	pH profile of EM-M fermented using different sugar sources at 37°C	58
4.13	TTA of EM-M fermented prawn waste using different carbon sources at 37°C	58
4.14	Bacterial growth of EM-M fermented prawn waste using different sugar sources at 37°C	59
4.15	pH value of fermented sample using different level of sugar	60
4.16	Effect of prolonged storage on the stability of the EM-M fermented prawn waste	61
4.17	Normal probability plots of residuals (pH drop)	69
4.18	The residual versus the predicted response (pH drop)	69
4.19	Interaction graph between temperature and incubation time	70
4.20	Normal probability plot of residuals (protein production)	73
4.21	The residual versus the predicted response (protein production)	74
4.22	Interaction graph between temperature and incubation time on protein production	74
4.23	Interaction graph between temperature and % sugar on protein production	75

4.24	Interaction graph between temperature and % of inoculum on protein production	75
4.25	Ramp diagram of fermentation condition for maximum protein production	77
4.26	Normal plot of residuals for protein production	82
4.27	Residuals vs predicted for protein production	82
4.28	Predicted vs actual for protein production	83
4.29	Outlier T for protein production	83
4.30	Contour plot (a) and 3D plot (b) showing effect of temperature and percentage of sugar on protein production at fixed incubation time (6 day) and % inoculum (2%)	85
4.31	Contour plot (a) and 3D plot (b) showing effect of incubation time and percentage of sugar on protein production at fixed temperature (40°C) and % inoculum (2%)	86
4.32	Contour plot (a) and 3D plot (b) showing effect of incubation time and percentage of inoculum on protein production at fixed temperature (40°C) and % sugar (10 %)	87
4.33	Contour plot (a) and 3D plot (b) showing effect of temperature and incubation time on protein production at fixed sugar level (10 %) and inoculum level (2%)	88
4.34	Contour plot (a) and 3D plot (b) showing effect of temperature and inoculum level on protein production at fixed sugar level (10 %) and incubation time (6 day)	89
4.35	Contour plot (a) and 3D Plot (b) showing effect of sugar and inoculum level on protein	90

	Production at fixed temperature (40°C) and incubation time (6 day)	
4.36	Ramp diagram of CCD for optimum condition for protein production	92
4.37	3D plot of CCD for the optimum condition for protein production	92

## 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 Efficiency 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.

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