บทความวิจัย

การสำรวจการใช้หัวเชื้อจุลินทรีย์ในการเพาะเลี้ยงกุ้งทะเล และประเมินประสิทธิภาพในการบำบัดน้ำทิ้งเบื้องต้น

A Field Survey of Microbial Products Used in Marine Shrimp Cultivation and Preliminary Evaluation of Their Wastewater Treatment Efficiency

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Key words : Microbial products, Shrimp farm effluent, Environment, Probiotic

Abstract

The aims of this study were documented the use of microbial products from shrimp farmers in Songkhla and Nakhonsithamarat province, to thier treatment efficiencies from shrimp aguaculture in from of Chemical Oxygen Demand (COD) and enumerate the composition of microorganisms in microbial products. Interviews were conducted with 66 shrimp farmers in the two major shrimp producing regions. It was found that farmer in the study used on average 59 different commercial microbial products. The results indicated that most shrimp farmers commonly treated shrimp pond water prior to and during the cultivation by using microbial products was 82% and not use was 18% of investigated farmers, respectively. It was found that the microbial products were used in three main purposes, namely for water treatment, feed additive as probiotic and for both purposes were 66 %, 24% and 10%, respectively. These product labels were indicated the composition of microorganisms comprising of *Bacillus* spp, lactic acid bacteria, nitrifying bacteria, denitrifying bacteria, yeast and fungi were following 54%, 17%, 10%, 4%, 8% and 3% of overall microbial products, respectively. *Bacillus* species were the most commonly bacteria found in microbial products, which consist of *Bacillus subtilis* 45%, *B. licheniformis* 16%, *B. megaterium* 11%, *B. polymyxa* 8% of overall microbial products and other few *Bacillus* spp. For the microorganism enumeration results was found microorganisms comprising lower than product label information. It was also found the amount of bacteria, yeast and actinomycetes in overall microbial products were 2.0×10^6 - 8.0×10^6, 10^3 - 6.0×10^5 and 10^3 - 9.0×10^3 cfu/g, respectively. Evaluation microbial product efficiencies were found that mostly commercial microbial products have shown low treatment efficiencies for shrimp ponds water improvement.

บทคัดย่อ

การศึกษาครั้งนี้ได้ทำการสำรวจเก็บรวบรวมข้อมูลการใช้หัวเชื้อจุลินทรีย์ในการเพาะเลี้ยงกุ้งทะเลในจังหวัดสงขลาและนครศรีธรรมราช โดยการสัมภาษณ์เกษตรกรจำนวน 66 คน ทดสอบประสิทธิภาพเบื้องต้นในการบำบัดน้ำทิ้งจากการเพาะเลี้ยงกุ้งทะเลโดยตรวจวัดจากค่า Chemical Oxygen Demand (COD) และศึกษาจำนวนและชนิดของเชื้อจุลินทรีย์ในหัวเชื้อจุลินทรีย์ทางการค้า หัวเชื้อจุลินทรีย์ที่เกษตรกรใช้มี 54 ชนิดที่แตกต่างกัน ผลการสำรวจการใช้หัวเชื้อจุลินทรีย์จากเกษตรกรจำนวน 66 คน พบว่ามีการใช้หัวเชื้อจุลินทรีย์ในการเพาะเลี้ยงกุ้งทะเล 82% และไม่ใช้หัวเชื้อจุลินทรีย์ 18% หัวเชื้อจุลินทรีย์ทางการค้าที่เกษตรใช้มีจำนวน 59 ชนิด พบว่าเป็นหัวเชื้อที่ใช้บำบัดน้ำ 66% สารเสริม

ชีวภาพ (Probiotic) 24% และใช้บำบัดน้ำและสารเสริมชีวภาพ 10% ตามลำดับ ข้อมูลระบุในฉลากหัวเชื้อจุลินทรีย์ที่เกษตรกรใช้ประกอบด้วยจุลินทรีย์ชนิดสายพันธุ์เดี่ยวและชนิดสายพันธุ์ผสม *Bacillus* spp. 54%, Lactic acid bacteria 17%, Nitrifying bacteria 10%, Yeast 8%, Denitrifying bacteria 4%, และ Fungi 3% ตามลำดับ สำหรับสายพันธุ์ของแบคทีเรีย *Bacillus* spp พบมากที่สุดในหัวเชื้อจุลินทรีย์ทางการค้าประกอบด้วย *Bacillus subtilis* 45%, *B. licheniformis* 16%, *B. megaterium* 11%, *B. polymyxa* 8% และ *Bacillus* สายพันธุ์อื่นพบอีกเล็กน้อย การนับจำนวนเชื้อจุลินทรีย์ในผลิตภัณฑ์จุลินทรีย์พบว่าผลิตภัณฑ์จุลินทรีย์ส่วนใหญ่มีจำนวนเชื้อจุลินทรีย์น้อยกว่าที่ระบุไว้ในฉลาก โดยนับเชื้อแบคทีเรีย ยีสต์ และแอคติโนมัยซิสได้มากที่สุด 2.0×10^6 - 8.0×10^6, 10^3 - 6.0×10^5 และ 10^3 - 9.0×10^3 cfu/g ตามลำดับ การทดสอบเปรียบเทียบประสิทธิภาพในการปรับปรุงคุณภาพน้ำในบ่อเพาะเลี้ยงกุ้ง โดยวัดจากค่าการบำบัดสารอินทรีย์ในน้ำทิ้งจากฟาร์มเพาะเลี้ยงกุ้ง พบว่าหัวเชื้อจุลินทรีย์ทางการค้าส่วนใหญ่มีประสิทธิภาพต่ำในการปรับปรุงคุณภาพน้ำจากการเพาะเลี้ยงกุ้งทะเล

Introduction

The shrimp farming industry is an important economic sector in many Asian countries, including Thailand, which is the world's top producer. However shrimp has generated huge volume of wastewater farms (Tavarutmaneekul and Tookwinas, 1995). Currently, intensive shrimp culture in Thailand has rapidly expanded especially along the coast during the last decade. Intensive shrimp farms need large amounts of feed to support high densities of shrimp, and flush correspondingly high loads of wastes into coastal waters, lead to eutrophication (Sansanayuth *et al.*, 1996). A serious problem confronting the shrimp farming industry in Thailand is that of attaining equilibrium between levels of shrimp production and water quality that will assure adequate profits and protect water supplies. The two significant components of pond environment are water and sediment that interact continuously to influence the culture environment. Accumulated sediment was known to be undesirable. Problems associated with accumulated sediment occur when excessive organic material builds up causing the release of ammonia (Wang and Fast, 1992; Avnimelech, 1996). Shrimp farm discharges contain considerable quantities of organic carbon, nitrogen and phosphorus in receiving waterways, which can lead to eutrophication (Sansanayuth *et al.*, 1996). There is a growing interest in water recycle system throughout the shrimp farming industry. Water recycle, combined with effective water treatment, is essential for improving water management for most shrimp farms.

Generally, shrimp farmers improve water quality in shrimp ponds by enhancing the mineralization processes and preventing the accumulation of organic matter. Bioremediation or bioaugmentation is a concept of reducing organic wastes to environmentally safe levels through the use of microbial products. Examples of common bacteria involving to mineralization of organic wastes are in genera of *Bacillus, Pseudomonas, Acinetobacter, Cellulomonas, Nitrosomonas* and *Nitrobacter* (Thomas *et al.*, 1992). Currently, there are several kinds of microbial products available in the market for aquacultural activities. Live bacterial cells, enzyme preparations, plant and yeast extracts are also used in aquaculture ponds to help in mineralization processes of organic wastes such as Effective Microorganisms or EM (Boyd and Gross, 1998). However, the efficiency of commercial microbial products is being questioned on the limited environments such as the only active in freshwater condition but less is effective in brackish or saline water (Shariff *et al*., 2001). To date there has been no convincing demonstration of commercial microbial products efficacy.

The aims of this work are to survey the available commercial microbial products, commercial microbial inoculum products composition, used of microbial products from shrimp farmers and potential their treatment efficiency used for water quality improvement in shrimp ponds.

Materials and Methods

Microorganisms

Seven commercial microbial products obtained directly from shrimp framers were compared the wastewater treatment efficiencies. These products were *Bacillus subtilis* 1070® (Asian Aquaculture Cp., Ltd., Thailand), *All baczyme*® (Allvet Co., Ltd., Thailand), *Bactapur-N300*® (International Ecological technological Inc., Canada), *Biobaczyme*® (Planet Aquatic Chemicals Co., Ltd., Thailand), *Treat sludge*® (Topvet Co., Ltd., Thailand), *Naturebac*® (Nature vision Co., Ltd., Thailand) *and Bactocel*® (Thai Technology Agriculture Co., Ltd., Thailand).

Synthetic shrimp farming wastewater

The synthetic wastewater composition mimicking to shrimp farming effluent was prepared. The wastewater compositions was 2,400 ± 650 mg/l total COD, 1,200 ± 210 mg/l soluble COD, 160 ± 25 mg/l total Kjedahl nitrogen, 5.9 ± 0.7 mg/l ammonia-nitrogen, 10 ± 1.2 mg/l phosphate, salinity approx. 25 ppt. (as 2.5% NaCl) and pH 7.5. Details of its composition are shown in Table 1.

Table 1 Compositions of synthetic shrimp farming wastewater for reactor experiments

Component	Constituent	Concentrations (g/l)
Shrimp feed (Grobest®-2)	Chemical Oxygen Demand	1.5 (as 1,200 mgO_2/l)
Artificial sea water	$MgSO_4 7H_2O$	0.25
	KCl	0.5
	$CaCl_2 2H_2O$	0.25
	$FeSO_4 7H_2O$	0.001
	Sea salt	20
Inorganic carbon	$NaHCO_3$	0.5
Ammonium sulfate	Total ammonium nitrogen	0.024 (as 5.9 mg NH_4^+ -N/l)
Phosphate	K_2HPO_4	0.044 (as 10 mg PO_4^{3-}-P/l)

Source: Modified from Shan and Obbard, 2001

Survey of microbial product usages from shrimp farmers in Songkhla and Nakhonsithamarat provinces, Thailand

Information of commercial microbial products utilization was surveyed by interviewing and sending questionnaires in order to know how many these products were used from 66 shrimp farmers in Songkhla and Nakhonsithamarat provinces, Thailand. In addition, the methods of product preparation from commercial were investigated. The method preparing commercial microbial products and conventional microbial products before used such as fermented in molass, vegetables, fish and starch. The composition of microorganisms presences in 59 products of commercial microbial products were counted depends on details available from product labels.

Enumeration of microorganisms in commercial microbial products by viable plate counts technique

The microbial densities of seven representatives of commercial microbial products were enumerated for microorganisms per gram of products by using culture-dependent method with conventional media. Commercial microbial products were appropriately diluted in 0.1% peptone water before being cultured. Estimation of total plate counts on Trypticase soy agar (TSA), Yeast extract malt (YEM) and Actinomyces Agar (AA) being added 2.0% NaCl were carried out according to the pour plate techniques (Clark, 1965)

Evaluation of microbial products treatment efficiency of shrimp farm effluent

Seven commercial microbial products obtained directly from shrimp farmers were compared the wastewater treatment efficiencies. These products were *Bacillus subtilis* 1070®, *All baczyme®*, *Bactapur-N300®*, *Biobaczyme®*, *Treat sludge®*, *Naturebac®* *and Bactocel®*. Ten ml of collected samples were cultivation with 100 ml of sterile synthetic shrimp farming wastewater. Primary cultivation was performed in a 250 ml flask and applied the agitation with rotary shaker at 200 rpms at room temperature (28-30°C) for 6 days. The effluent samples were collected when were operated for 6 days and analyzed for the COD content by using Merck Spectroquant COD test kit (Merck Ltd., Germany). Two products were selected according to their COD removal efficiency performance for evaluating effect of bioaugmentation to shrimp farming wastewater treatment in 1 L reactor under room temperature condition. Mixing and aeration was achieved by bubbling air through the reactor. It must be noted that this experiment was focused only the COD removal efficiency. The effluent samples were collected at 0, 1, 3, 5 and 7 of operational days and analyzed the COD content.

Sampling and analysis

Collected samples were analyzed COD concentrations using commercial test kits from Spectroquant (Merck Ltd., Germany).

Results and Discussions

Shrimp framing industry have given negative image in countries that import shrimp, among environmentalist within the producing countries. There is an increasing interest in an environmentally friendly shrimp culture within the shrimp farming industry and efforts to reach a sustainable production are being made. Thus shrimp farmers used microbial products instead of chemicals (Grashlund and Bengtsson, 2001). Application of commercial microbial products in aquaculture ponds is rapidly increasing as probiotics, disease management strategy and improving water quality are referred to as bioremediation products (Gatesoupe, 1999) There are several commercial microbial products marketed for aquaculture use to clean up the pond bottom, maintain good water quality and improve shrimp health particularly for intensive shrimp aquaculture practices. However, the beneficial effect of using such microbial products in aquaculture is still debatable, as their efficacy is yet unclear. In Songkhla province, Thailand, several imported microbial products are being used in shrimp farms but no attempt has been made to verify the performance of these products. These investigations were shown the high proportion of commercial microbial products usage in Songkhla. It was also found that the microbial products were used in three main purposes, namely for water treatment, for feed additive as probiotics and for both purposes. From their labels, they mostly comprised of *Bacillus* spp. The lower number of microorganisms enumerated on suitable medium than the information claim in the products labels was found. The efficiency of commercial microbial products was limited for only the freshwater environments and may be less effective in brackish or saline environments. A few microbial commercial products can removal COD in synthetic shrimp farming wastewater.

Survey of microbial product usages from shrimp farmers in Songkhla and Nakhonsithammarat province, Thailand

A wide variety of commercial microbial products were commonly used to treat water and sediment of ponds in shrimp farming as well as in shrimp hatcheries. These studies aimed to survey the use of microbial inoculum from shrimp farmers in Songkhla province, Thailand and its potential of water quality improvement in shrimp ponds. From 66 shrimp farmers was found that mostly shrimp farmers treat water in shrimp ponds prior to and during the cultivation by using commercial microbial products and other methods, which were found at 73% and 17%, respectively (Figure 1).

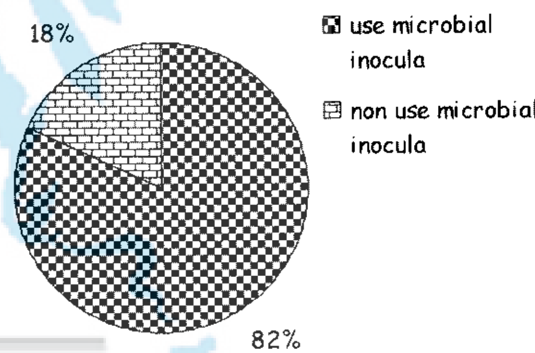

Figure 1 Survey results of the proportion the microbial product usage in Songkhla and Nakhonsithammarat province.

From the survey results, it was found 59 types of microbial products were available commercially on the market. It was also found that the microbial inoculum were used in three main purposes, namely, for water treatment at 66%, for feed additive as probiotic at 24% and for both purposes at 10%, respectively (Figure 2). The treatment concept relies on the microbial species composition in aquaculture ponds can be changed

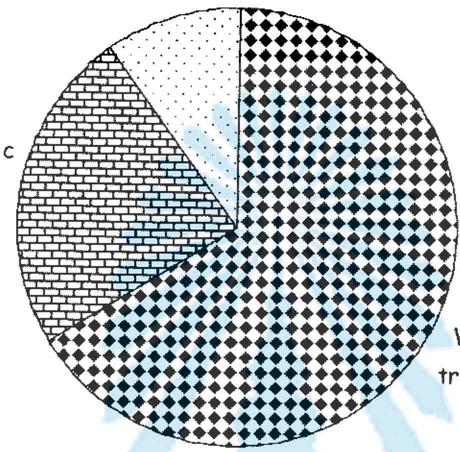

Figure 2 Survey results regard to the purpose of the microbial products usage from shrimp farmers in Songkhla and Nakhonsithammarat provinces, Thailand.

by adding selected species to displace deleterious common bacteria. Success depends upon defining the ecological process or processes to be changed, the type of deleterious species that are dominate and the desirable alternative species or strains of bacteria that could be added. Competitive exclusion is one of the ecological processes that allow manipulation of the bacterial species composition in the water and sediment (Moriarty, 2000). These products' label were indicated the composition of microorganisms comprising of *Bacillus* spp, lactic acid bacteria, nitrifying bacteria, denitrifying bacteria, yeast and fungi were 54%, 17%, 10%, 4%, 8%, and 3%, respectively (Figure 3). *Bacillus* species are commonly found in marine sediments and therefore are naturally ingested by aquatic animals such as shrimps that feed on the sediment. An advantage of using *Bacillus* species is due to they are indigenous microorganisms, some species can produce proteolytic enzyme, capable of producing endospores (Moriarty, 2000; Devaraja *et al.*, 2002).

It was also found that there were many *Bacillus* species containing in the products, those were *Bacillus subtilis* 46%, *B. licheniformis* 17%, B. megaterium 11%, *B. polymyxa* 8% and other few species as show in figure 4. Panpornmin (2002) found that *Bacillus subtilis* and *Bacillus licheniformis* dominated in shrimp framing sediment using culture-depend method. Several farmers pointed out that they regarded microbial products to be a more environmentally friendly alternative to chemicals. In spite of the size and importance of the shrimp framing industry,

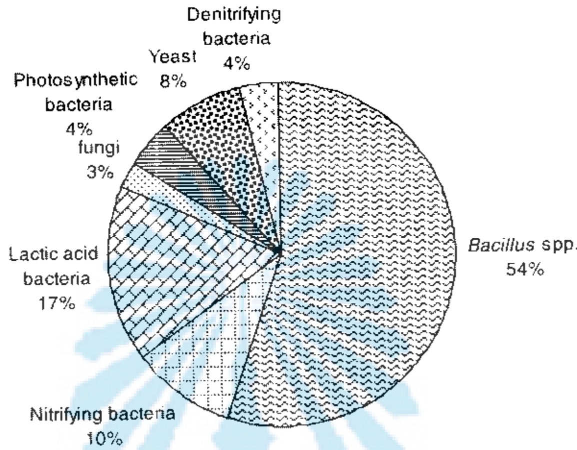

Figure 3 Percentage of major type of microorganism from commercial microbial products used by shrimp farmers in Songkhla and Nakonsrithamarat provinces (information was obtained from product labels).

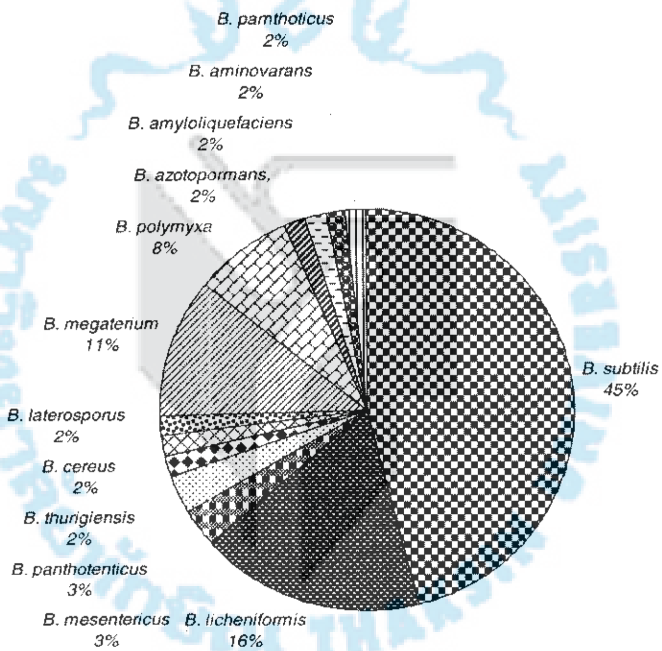

Figure 4 Percentage of 12 Bacillus species commonly found in the commercial microbial products used from Shrimp farmers (data were obtained from product labels).

documentation of the quality and quantity of commercial microbial products used during farming is scarce.

Enumeration of microorganisms in commercial microbial products by viable plate count technique

To obtain the number of microbial cells claimed to present in the commercial microbial products, seven commercial microbial products obtained directly from shrimp farmers were enumerated on suitable media. These products were *Bacillus subtilis* 1070®, *All baczyme*®, *Bactapur-N300*+®, *Biobaczyme*®, *Treat sludge*®, *Naturebac*® *and Bactocel*®. It was found that the total plate counts of *Treat Sludge*® and *Bacillus subtilis 1070*® showed higher number of bacterial enumerated on Trypticase Soy Agar with 2.0% NaCl addition, namely 8.0×10^6 cfu/g in both products. Total plate count of commercial microbial products were found in range of $<10^3$ to 8.0×10^6 cfu/g from each commercial microbial products. The highest number of yeast enumerated on yeast extract malt agar that being added with 2.0% NaCl were also found in *Biobaczyme*® and *Bactocel* ®, among of both were 6.0×10^5 cfu/g. The highest number of actinomycetes enumerated on actinomycetes agar was found in *Bactapur-N300+*® was 9.0×10^3 cfu/g (Table 2). Generally, it was found that there were lower number of microorganisms enumerated on suitable media than were claimed from the products labels. This may be due to the efficiency of commercial microbial products was limited for only the freshwater environments and may be less effective in brackish or saline environments, shelf life and storage conditions.

Evaluation of microbial products treatment efficiency of synthetic shrimp farm effluent

The efficacies of commercial microbial products were tested in treating synthetic shrimp

Table 2 The enumeration of microorganisms presence in 7 commercial microbial products by standard plate count technique on 3 cultural media.

Commercial microbial products	Trypticase Soy Agar 2.0% NaCl (cfu/g)	Yeast Extract Malt Agar 2.0% NaCl (cfu/g)	Actinomycetes Agar 2.0% NaCl (cfu/g)
Bacillus subtilis 1070®	8.0×10^6	$<10^3$	$<10^3$
All baczyme®	2.0×10^6	5.0×10^4	2.0×10^4
Bactapur-N300+®	nd	1.0×10^4	9.0×10^3
Biobaczyme®	7.0×10^6	6.0×10^5	$<10^3$
Treat Sludge®	8.0×10^6	7.0×10^4	$<10^3$
Naturebac®	2.0×10^6	2.0×10^4	$<10^3$
Bactocel®	6.0×10^6	6.0×10^5	7.0×10^3

nd: not determined due to contamination

farming wastewater. This experiment was focused only for COD removal efficiency. The effluent samples were collected at 0, 1, 3, 5 and 7 of operation days. Seven commercial microbial products obtained directly from shrimp framers were compared their wastewater treatment efficiencies. These products were *Bacillus subtilis* 1070®, *All baczyme*®, *Bactapur-N300*+®, *Biobaczyme*® and *Treatsludge*®. It was found that *Bacillus subtilis* 1070®, *All baczyme*®, *Bactapur-N300*+®, *Biobaczyme*®, *Treat sludge*®, natural and control (sterile synthetic shrimp farming wastewater) gave COD removal efficiencies at 35%, 50%, 47%, 61%, 82%, 27% and 0%, respectively (Figure 5). The overall results showed that the *Biobaczyme*® and *Treat sludge*® gave higher COD removal efficiencies than other products. Subsequently, two products were selected according to their performance of COD removal for evaluating effect of bioaugmentation experiment in 1 L glass reactor. The synthetic wastewater composition was prepared to mimicking the shrimp farm wastewater. It was found that *Biobaczyme*® and *Treat sludge*® can remove COD from synthetic shrimp farming wastewater up to 62% and 72%, respectively (Figure 6).

Conclusions

It was found that shrimp farmers at 82% of the studied population commonly used commercial microbial products. These products were either used to treat the water or sediment, or apply directly to the shrimp feed as a probiotic. Some products used to improved water quality also were contained enzyme and bacteria. Mostly farmers used commercial microbial products containing *Bacillus* spp. The products administered in the feed were used to enhance shrimp's digestion, while the purpose of water and sediment treatment was mainly to increase decomposition of organic matter and to out-competed pathogenic bacteria.

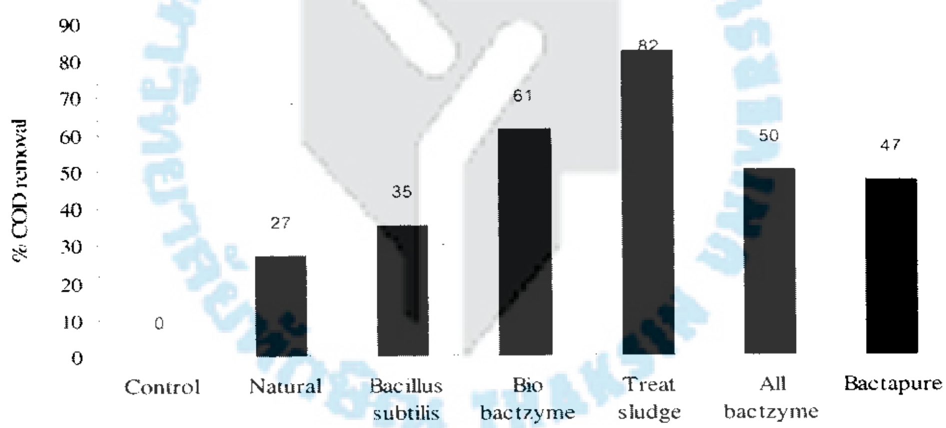

Figure 5 Chemical oxygen demand (COD) treatment efficiencies of synthetic shrimp farming wastewater by 7 types of commercial microbial products.

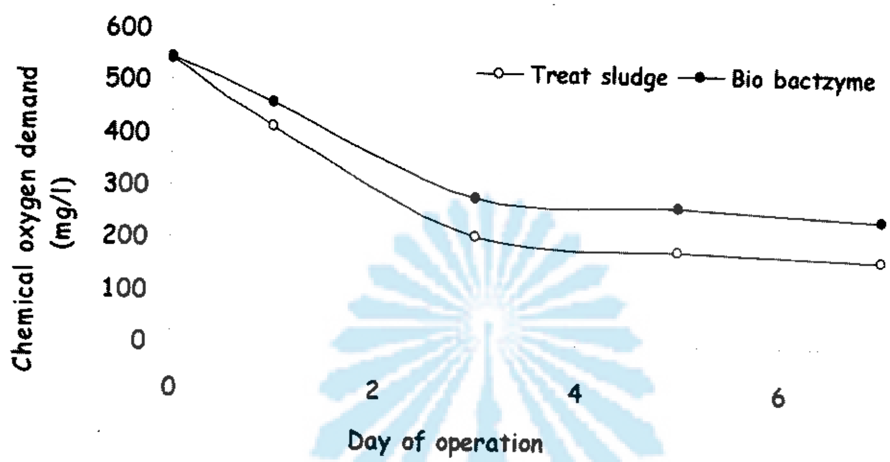

Figure 6 Profiles of Chemical oxygen demand (COD) concentrations from synthetic shrimp farming wastewater when compared the use of two selected commercial microbial products.

Several farmers understood the benefit of the microbial products and regarded as environmentally friendly alternative to chemicals. Commercial microbial products were indicated the composition of microorganisms. From their labels, they comprises of *Bacillus* spp, lactic acid bacteria, nitrifying bacteria, denitrifying bacteria, yeast and fungi at 54%, 17%, 10%, 4%, 8%, and 3%, respectively. *Bacillus* species were commonly found in marine sediments and therefore are naturally ingested by aquatic such as shrimps that feed on the sediment. It was found that the number of microorganisms obtained from culturing method were lower than those indicated on products' label. The efficiency of commercial microbial products is limited for only the freshwater environments and may be less effective in brackish or saline environments. A few microbial commercial products can removal COD in synthetic shrimp farming wastewater.

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References

Avnimelech, Y. 1999. Carbon / nitrogen ratio as a control element in aquaculture systems. Aquaculture. 176: 227-235

Boyd, C. E. and Gross, A. 1998. Use of probiotics for improving soil and water quality in aquaculture ponds. *In:* Advances in Shrimp Biotechnology (ed. By T. W. Flegel) pp. 101-105. National center for Genetics Engineering and Biotechnology, Thailand.

Clark, F. E. 1965. Method of soil analysis, in Black, C. A. Editor, Agar-plate method for total microbial count: Madison, Wisconsin, USA, American society of agronomy.Inc., Publisher.

Devaraja, T. N., Yusoff, F. M. and Shariff, M. 2002. Changes in bacterial population and shrimp production in ponds treated with commercial microbial products. Aquaculture. 206: 245-256.

Gatesoupe, F. J. 1999. The use of probiotic in aquaculture. Aquaculture. 180: 147-165.

Graslund, S. and Bengtsson, B. 2001. Chemicals and biological products used in south east Asian shrimp farming and their potential impact on the environment a: review. Sci. Total. Environ. 280: 93-131

Moriarty, D.J.W. 2000. Disease control in shrimp culture with probiotic bacteria. *In:* Bell, C.R., Brylinsky, M. and P. Johnson-Green (eds.), Microbial Interactions in Aquaculture. Proceedings of the 8th International Symposium on Microbial Ecology, Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 2000. pp. 237-243.

Panprommin, K. 2002. Biodegradation of organic matter contaminated sediment from shrimp farming by *Bacillus subtilis* and *Bacillus lichaniformis.* M. Sc. Thesis. Mahidol University. Thailand.

Sansanayuth, P., Phadungchep, A., Ngammontha, S., Ngdngam, S., Sukasem, P., Hoshino, H. and Ttabucanon, M.S. 1996. Shrimp pond effluent: Pollution problems and treatment by constructed Wetlands. Wat. Sci. Tech. 34: 93-98.

Shan, H. and Obbard, J. P. 2001. Ammonia removal from prawn aquaculture water using immobilized nitrifying bacteria. Appl. Microbiol. Biotechnol. 57: 791-798.

Shariff, M., Yusoff, F. M. and Sinivasa Rao, P. S. 2001. The effectiveness of a commercial microbial products in poorly prepared tiger shrimp, *Penaeus monodon* (fabricius) ponds. Aqua. Res. 32: 181-187.

Tavarutmaneekul, P. and Tookvinas, S. 1995. Aquaculture sustainability and the environment. Thailand study report. NACA/ ADB

Thomas, G. M., Ward, C. H., Raymond, R. L., Wilson J. T. and Loehr, R. C. 1992. Bioremediation. *In:* Encyclopedia of Microbiology (ed. By J. Leperberg), Academic Press, London, pp. 369-385.

Wang, J. K., and Fast, A. W. 1992. Shrimp pond engineering considerations. *In* "Marine shrimp culture: Principles and practices" (A. W. Fast and L. J. Lester, Eds.), Elsevior, Amsterdam, pp. 415-429.