INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–353/DMA/2011/13–1–67–70
http://www.fspublishers.org

Full Length Article

Effects of Ultrasonic Irradiation on Degradation of Microcystin in Fish Ponds

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ABSTRACT

Surface cyanobacterial bloom causes deterioration of pond water and accumulation of toxins and musty odors (Geosmin & 2-methylisoborneol, MIB) in aquaculture activities. This study investigated the effectiveness of ultrasonic technology on *Microcystis* sp. surface scum settling, including microcystin (cyanotoxin) and musty odors degradation in pond water. Water from fish ponds, with 5.162±0.92 mg L^{-1} of initial chlorophyll 'a' was sonicated at 5 frequencies (29, 43, 108, 200 & 1000 kHz). Ultrasonic irradiation of 200 kHz had the greatest effect in settling *Microcystis* scum. Moreover, ultrasonication at 200 kHz effectively reduced microcystin and musty odors. Scanning electron microscopy (SEM) confirmed that sonification at a frequency of 200 kHz for 240 s did not disintegrate *Microcystis* sp. cells, but easily broke up the sticky mucus layer of the scum. Thus, ultrasonification at 200 kHz is promising technique to sink *Microcystis* surface scum, without cell disintegration, and could be used to reduce microcystin toxin and musty odor substances in aquaculture ponds. © 2011 Friends Science Publishers

Key Words: Ultrasonic irradiation; Cyanobacteria; Microcystin; Musty odors; Geosmin: MIB

INTRODUCTION

Although phytoplanktons are beneficial as natural food and maintenance of ecological balance in aquaculture ponds, some species can produce compounds that are extremely toxic to aquatic animals. Phytoplanktons may deteriorate water quality (Whangchai, 2001), with the accumulation of toxins and off-flavors in aquaculture products. Presently, freshwater fish and brackish water shrimp farms are facing an off-flavor contamination problem in fish/shrimp flesh caused by some cyanobacteria and actinomycetes (Tucker, 2000). Many cyanobacterial species are able to produce toxins, with microcystins. These toxins are usually found in drinking, fisheries and recreation (Carmichael, 1994).

Cyanobacterial toxins can affect public health and aquaculture such as fish, mussels, crayfish and shrimp, cause food safety issue, and adversely affect the quality of fish products (Shumway, 1990; Chen & Xie, 2005; Iblins & Chorus, 2007). Zimba *et al.* (2006) observed shrimp mortality due to cyanobacteria blooms and tissue damage. Sub-lethal levels of toxin reduces feed intake in shrimp and they become more liable to secondary infections (Smith, 1996). The cyanobacterial genera, which cause blooms in fresh and brackish water and release some toxins are, *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc* and *Anabaenopsis*. They produce cyclic peptide toxins, named microcystins (Chorus & Bartram, 1999). These toxins affect the liver by inhibiting protein phosphatase. Moreover, prawn pond water and sediment were also found to be contaminated with microcystin (Prommana *et al.*, 2006).

Nuisance odors cause problems in fisheries products such as tilapia reared in green water contaminated with geosmin (GSM) and 2-methylisoborneol (MIB) in fish flesh (Whangchai *et al.*, 2008). The causative compounds are produced by some actinomycetes (Wood *et al.*, 1983) and cyanobacteria (Izaguirre *et al.*, 1982; Persson, 1983). The most troublesome odors are GSM and MIB, which are difficult to neutralize in culture water.

Present strategies to prevent blooming of cyanobacteria include the application of chemicals (Ma & Liu, 2002), electrolytic treatments (Whangchai *et al*., 2003) and flushing with clean water (Welch, 2007). Chemicals may cause pollution problems and flushing with clean water may be difficult in closed-culture systems such as shrimp culture. Therefore, the effectiveness of ultrasonic devices on settling of *Microcystis* sp. scum was studied in aquaculture ponds. The effects of frequencies and sonication times on sedimentation of *Microcystis* scum, degradation of microcystin and eradication of musty odor substances,

To cite this paper: Srisuksomwong, P., N. Whangchai, Y. Yagita, K. Okada, Y. Peerapornpisal and N. Nomura, 2011. Effects of ultrasonic irradiation on degradation of microcystin in fish ponds. *Int. J. Agric. Biol*., 11: 67–70

iodine liberation and surface total solid were also investigated. Morphological changes of the cells were analyzed with scanning and transmission electron microscopy.

MATERIALS AND METHODS

Ultrasonic devices set up: Ultrasonic devices with the same input power of 3 W and 5 varying frequencies; 29, 43, 108, 200 and 1000 kHz, were made by Honda Electronics Company (Toyohashi, Aichi, Japan). A polyethylene cylinder reactor, 10 cm in diameter, was equipped with a transducer at the lower part. Thirty mm of *Microcystis* suspension or KI solution in a beaker was sonicated in an ultrasonic reactor.

Surface scum collection and sonication: Scum was collected from the surface water of high bloom fish ponds. Species composition of surface scum by biovolume consisted of *M. aeruginosa* (80%), *Peridinium* sp. (9%), *Botryococcus braunii* (9%), *Pseudanabaena mucicola* (1%) and *Cylidrospermopsis raciborskii* (1%). The scum was filtrated through a 100 µm mesh screening net and kept as a suspension. Thirty mm of each sample with 5.162±0.92 mg L^{-1} of initial chlorophyll 'a' was treated with each sonication times (0, 30, 60, 120, 240 & 600 s) and different frequencies (29, 43, 108, 200 & 1000 kHz). Water samples were collected for chlorophyll a, microcystins and musty odors substances analyzes.

Measurement of chlorophyll a, iodine, microcystin and musty odor substances: The concentration of 2% potassium iodine solution, liberated during ultrasonic treatment with different irradiation times, was measured at 354 nm using a spectrophotometer (HACH, DR/4000U). The floating cells were pipetted and filtered by GC/F for chlorophyll 'a' analysis. Ten mL of 90% of methanol were added to each sample and incubated in a temperature controlled water bath (70ºC) for 20 min. The solution was centrifuged at 3,000 rpm for 10 min and measured at 630, 645, 665 and 750 nm using a spectrophotometer. The chlorophyll a concentrations in the extracts were calculated using the modified equations derived by Wintermans and de Mots (1965) and Saijo (1975). Microcystins were quantified by the enzyme-linked immunosorbant assay (ELISA) method. The QuantiPlate Kit (Envirologix Inc.,) was used for microcystin determination. Musty odor substances were analyzed by GC/MS and solid phase micro extraction (SPME) (Casey *et al.*, 2004). In 5 mL of water sample, 1.9 g of NaCl and 5 mL of methanol were added. The vial was sealed with a crimp cap and then fitted with a veton septum. The sample was then heated at 65ºC and exposed to the SPME fiber for 12 min absorption period with vigorous agitation. The autosampler was equipped with 1 cm long divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco). The fiber was withdrawn from the sample and desorbed at 270ºC for 5 min in the injection port of HP 6890 gas chromatograph, equipped with a 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). For qualitative analysis, the oven was heated up to 60ºC for 1 min, then the temperature was programmed at 15ºC min^{-1} to 220ºC and was maintained for 8 min.

Scanning electron microscopy observation: The samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) and with 1% OsO_4 in cacodylate buffer. They were dehydrated in ethanol series up to 80% and the residual water was removed, using the CO_2 critical point technique, sputter coated with gold and examined in a JEOL Scanning Electron Microscope JSM5910LV (JEOL Ltd., Japan).

Statistical analysis: Statistical comparison between groups was analyzed by one-way analysis of variance (ANOVA) and Post hoc Tukey's b test. Data in figures are given as mean ± standard deviation (SD) of three replicates [a], [b] and [c] are a statistical comparison between groups applied using Post hoc Tukey's b test ($P < 0.05$).

RESULTS

Effects of ultrasonic irradiation at different frequencies and sonication times on *Microcystis* cells and liberation of iodine: *Microcystis* surface scum collapsed and sank after sonication with 200 kHz frequency (Fig. 1). Ultrasonic treatment at 200 kHz had the highest performance (94.9%) within 30 s of sonication. Iodine liberation was direct measure of sonication effect. Iodine yield increased with increasing sonication frequencies and was highest at 200 kHz (0.0016 absorbance of OD354/s). Strong correlation was observed between iodine production and surface scum removal (Fig. 2).

Effect of ultrasonic irradiation at different frequencies and sonication times on the degradation of microcystin: Microcystin decreased significantly ($p<0.05$) in water sonicated at 108 and 200 kHz for 600 s by 72.3 and 80.8%, respectively (Fig. 3). A high correlation between iodine production and surface scum removal was evident (Fig. 4).

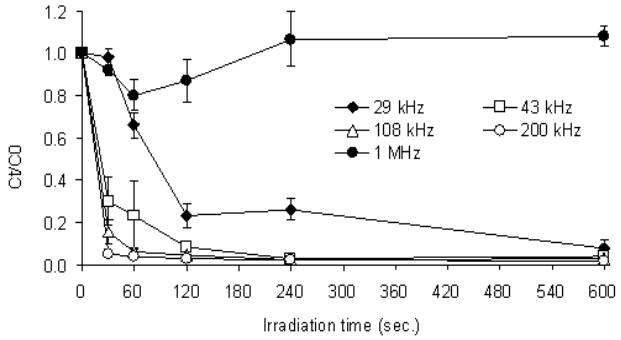
Effect of ultrasonic irradiation with different frequencies and irradiation times on degradation of musty odor substances: Ultrasonic irradiation at 200 kHz gave the highest performance for GSM and MIB removal (Fig. 5). GSM and MIB was removed from water by 27.1, 56.9, 66.7, 75.6, 23.8% and 17.4, 67.6, 67.9, 88.1 and 36.2%, respectively.

Surface scum observation using SEM: SEM pictures of *Microcystis aeroginosa* surface scum, before sonication (A) and after sonication (B) confirmed that 200 kHz ultrasonic irradiation (for 240 s) destroyed the sticky mucous layer, but did not disintegrate the cells (Fig. 6).

DISCUSSION

The present studies have confirmed that 200 kHz frequency destroyed sticky mucous layer before and after irradiation but did not disintegrate the cells.

Fig. 1: Chlorophyll a surface scum (Ct/C0) after sonication with 29, 43, 108, 200 and 1,000 kHz for 0, 30, 60,120, 240 and 600 sec



Surface scum removal rate (% of cell sink) after sonication 29, 43, 108, 200 and 1,000 kHz for 30 sec and Iodine liberation rate (absorbance of 354/sec) after sonication of 2 % potassium iodide with 29, 43, 108, 200 and 1,000 kHz

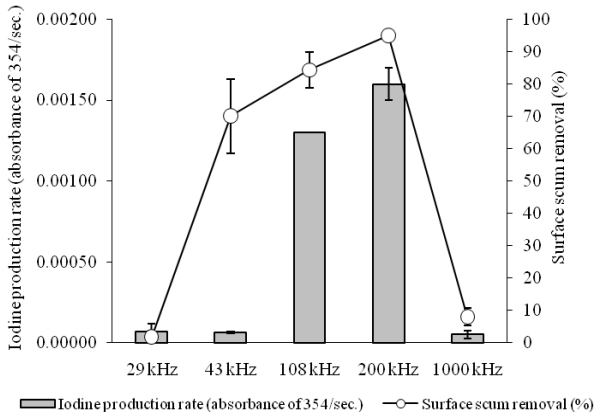
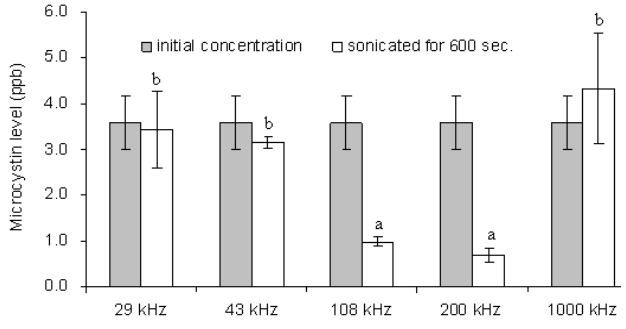
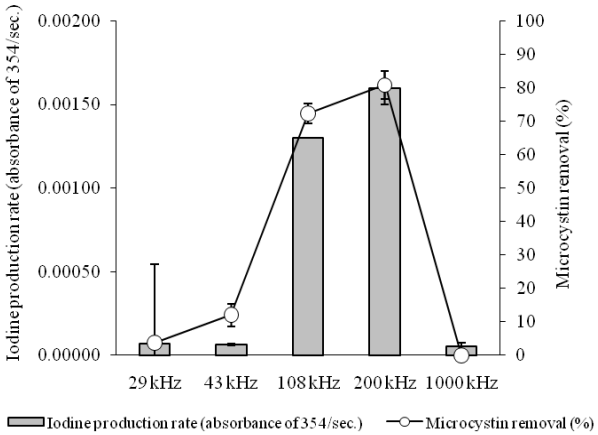


Fig. 3: Microcystin change by ultrasonic irradiation after sonication of *Microcystis* surface scum with 29, 43, 108, 200 and 1,000 kHz for 600 s



Ultrasonification, however, is known to have harmful effects on the structural and functional state of organisms too (Rott, 1981). When applied to water, power ultrasound causes acoustic cavitation, in which millions of small bubbles collapse rapidly to reach temperature as high as

Fig. 4: Correlation between iodine production rates (absorbance of OD 354/sec) after sonication 2% KI solution and microcystin reduction (%) during ultrasonic irradiation with 108 and 200 kHz of surface scum



5000 K and pressure as high as 100 MPa (Petrier *et al.*, 1998). Ultrasonic irradiation generates highly active hydroxyl free radicals, which react with potassium iodide resulting in iodine liberation (Hart & Henglein, 1985; Petrier *et al.*, 1998). Sonication frequency affected reaction kinetics. In this study, it was found that the higher frequency was effective than lower frequency to settle the *Microcystis* scum and reduced both microcystin and musty odor compounds. The main *Microcystis* sinking mechanism seems ultrasonic cavitation. The efficiency of ultrasonic to reduce cell sedimentation and toxin/musty odor compound correlated with the liberation of iodine after irradiation. Koda *et al.* (2003) found that 200 kHz was the optimum frequency to KI Irradiation. Ma *et al.* (2005) reported that 150 kHz is the optimum frequency for microcystin degradation. Ultrasonic irradiation promotes the growth and collapse of gas bubbles (cavitation), leading to extreme conditions, which produces ▪H and ▪OH, which attack the benzene ring and diene of the ADDA peptile residue and cleave the Mdha-Ala peptile bond (Song *et al.*, 2005; Song *et al.*, 2006).

Natural synthesis or collapse of gas vacuoles inside cells controls vertical migration of natural *Microcystis* and the migration is governed by 2 factors; light and nitrogen (Walsby, 1969). Transmission electron microscope of ultrasonic treated cells revealed the disrupted vacuoles (Lee *et al.*, 2001). In this study, SEM observation showed that ultrasonic irradiation destroyed the sticky layer, surrounding the cells but it did not disintegrate the scum size to single cells. Treated scum/cells settled readily to the pond bottom. These conditions may promote cell destruction by algal lysing microorganisms (Shilo, 1970; Gunnison & Alexander, 1975; Moriarty, 1997). Furthermore, SEM observations showed that low power ultrasonic irradiation (200 kHz) did not disintegrate cells. Consequently,

Fig. 5: Percent removal of GSM and MIB after ultrasonic irradiation with different frequencies for 600 s

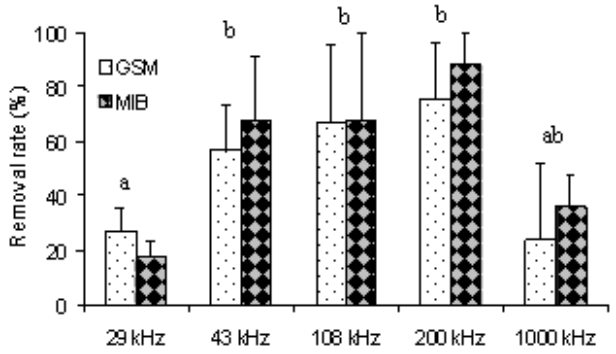
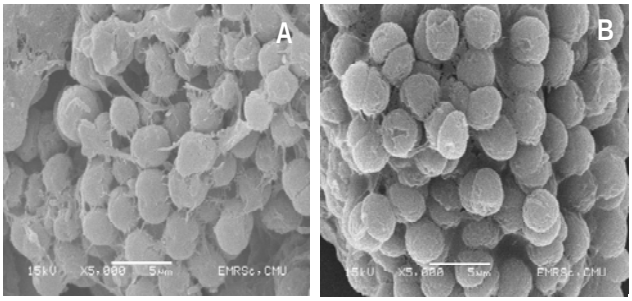


Fig. 6: SEM of surface scum (without ultrasonic irradiation, A) and with ultrasonic irradiation (200 kHz, for 240, B)



microcystin in the *Microcystis* cell was not released into water body during ultrasonic irradiation.

It is concluded that ultrasonic irradiation of 200 kHz is the optimum treatment to sink surface scum from fish ponds water, without cell disintegration and could degrade microcystin and musty odors effectively as well. This technology would be an effective method for cyanobacterial bloom control. The effect of this technology on phytoplankton profile and fish is our plan for future study.

Acknowledgement: This work was financially supported by The Thailand Research Fund (TRF) No. DIG5180001.

REFERENCES

Carmichael, W.W., 1994. The toxins of cyanobacteria. *Sci. American*, 270: 78–86

Casey, C.G., W.L. Steven and V.Z. Paul, 2004. Instrumental versus sensory detection of off-flavors in farm-raised channel catfish. *Aquaculture*, 236: 309–319

Chen, J. and P. Xie, 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large shallow, eutrophic lake of the subtropical China. *Toxicon*, 45: 615–625

Chorus, I. and J. Bartram, 1999. *Toxic Cyanobacteria in Water*. E and FN Spon. An imprint of Routledge, London

Gunnison, D. and M. Alexander, 1975. Resistance and susceptibility of algae to decomposition by natural microbial communities. *Limnol. Oceanogr*., 20: 64–70

Hart, E.J. and A. Henglein, 1985. Free radical and free atom reactions in the sonolysis of aqueous iodide and formate solutions. *J. Phys. Chem*., 89: 4342–4347

Ibelings, B.W. and I. Chorus, 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review. *Environ. Pollut.*, 150: 177–192

Izaguirre, G., C.J. Hwang, S.W. Krasner and M.J. McGuire, 1982. Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. *Appl. Environ. Microbiol*., 43: 708–714

Koda, S., T. Kimura, T. Kondo and H. Mitome, 2003. A standard method to calibrate sonochemical efficiency of an individual reaction system. *Ultrason. Sonochem*., 10: 149–156

Lee, T.J., K. Nakano and M. Matsumura, 2001. Ultrasonic irradiation for blue-green algae bloom control. *Environ. Technol*., 22: 383–390

Ma, J. and W. Liu, 2002. Effectiveness and mechanism of potassium ferrate (VI) preoxidation for algae removal by coagulation. *Water Res*., 36: 871–878

Ma, B., Y. Chen, H. Hao, M. Wu, B. Wang, H. Lu and G. Zhang, 2005. Influence of ultrasonic field on microcystins produced by bloom-forming algae. *Colloids and Surfaces B: Biointerfaces*, 41: 197–201

Moriarty, D.J.W., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture*, 151: 333–349

Persson, P.E., 1983. Off-flavors in aquatic ecosystem-an introduction. *Water Sci. Technol*., 15: 1–11

Petrier, C., Y. Jiang and M. Lamy, 1998. Ultrasound and environment: sonochemical destruction of chloromatic derivatives. *Environ. Sci. Technol*., 32: 1316–1318

Prommana, R., Y. Peerapornpisal, N. Whangchai, L.F. Morrison, J.S. Metcalf, W. Ruangyuttikarn, A. Towprom and G.A., Codd, 2006. Microcystins in cyanobacterial blooms from two freshwater prawn (*Macrobrachium rosenbergii*) ponds in northern Thailand. *Science Asia*, 32: 365–370

Rott, E., 1981. Some results from phytoplankton counting intercalibrations. *Schweiz. Z. Hydrol*., 43: 34–62

Shilo, M., 1970. Lysis of blue-green algae by myxobacter. *J. Bacteriol.*, 104: 453–461

Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. *J. World Aquacul. Soc*., 21: 65–104

Saijo, Y., 1975. A method for determination of chlorophyll. *Japanese J. Limnol*., 36: 103–109

Smith, T.P., 1996. Toxic effects of blooms of marine species of oscillatoriales on farmed prawns (*Penaeus monodon*, *Penaeus japonicus*) and brine shrimp (*Artemia salina*). *Toxicon*, 34: 857–869

Song, W., T. Teshiba, K. Rein and K.E., O'Shea, 2005. Ultrasonically induced degradation and detoxification of microcystin-LR (cyanobacterial toxin). *Environ. Sci. Technol*., 39: 6300–6305

Song, W., A. De la Cruz, K. Rein and K.E. O'Shea, 2006. Ultrasonically induced degradation of microcystin-LR and -RR: identification of products, effect of pH, formation and destruction of peroxides. *Environ. Sci. Technol*., 40: 3941–3946

Tucker, C.S., 2000. Off-flavor problems in aquaculture. *Rev. Fisheries Sci.*, 8: 45–88

Walsby, A.E., 1969. The permeability of blue green algal gas-vacuole membrane to gas. *Proc. Royal Soc. B*., 173: 235–255

Welch, E.B., 2007. The dilution/flushing technique in lake restoration. *J. American Water Res. Assoc*., 17: 558–564

Whangchai, N., 2001. Development of ozonation for water quality improvement in intensive shrimp cultivation. *Ph.D. Thesis*, University of Tsukuba, Japan

Whangchai, N., N. Nomura and M. Matsumura, 2003. Phytoplankton control in artificial seawater using electrolytic treatments. *Thai J. Agric. Sci*., 36: 297–304

Whangchai, N., K. Kannika, S. Deejing, T. Itayama, N. Iwami, T. Kuwabara and Y. Peerapornpisal, 2008. Growth performance and accumulation of off flavor in red tilapia, *Oreochromis niloticus* x *Oreochromis mosambicus*, cultured by green water system using chicken manure. *Asian Environ. Res.*, 1: 8–16

Wintermans, J.F.G.M. and A. de Mots, 1965. Spectrophotometric characteristics of chlorophyll a and b and their pheophytins in ethanol. *Biochim. Biophys. Acta*., 109: 448–453

Wood, S., S.T. William and W. R. White, 1983. Microbes as a source of earthy flavors in potable water. A review. *Int. Biodeterior. Bull*., 19: 83–97

Zimba, P.V., A. Camus, E.H. Allen and J.M. Burkholder, 2006. Co-occurrence of white shrimp, *Litopenaeus vannamei*, mortalities and microcystin toxin in a southeastern USA shrimp facility. *Aquaculture*, 261: 1048–1055

(Received 07 July 2010; Accepted 12 September 2010)