Journal of Environmental Science and Management 17(1): 68-71 (June 2014)
ISSN 0119-1144

Adsorption and Biomass Concentration of Thraustochytrid *Schizochytrium aggregatum* (Goldstein and Belsky) in Bunker C Oil

Brian Gil S. Sarinas[*,1], Lorna D. Gellada[1], Ma. Lona T. Torrigue[1], Dolores N. Sibonga[1], Edmar S. Torrato[1], John G. Malagad[1], Joseph G. Feril[1], Lyle Arianne J. Bondoc[1], Juan Clemente A. Roncal[1] and Jilla A. Tornalejo[2]

Research Notes

ABSTRACT

Diverse array of microorganisms such as bacteria, fungi and protists are involved during oil spill. Each microorganism has its own specific function whether it has to degrade or adsorb hydrocarbons. One important microorganism is the Thraustochytrid that is a fungoid protist and are common in marine and estuarine habitats. Numerous studies existed on the biodegradation and adsorption of Thraustochytrids on various substances but not on Bunker C oil. Thus, this study aimed to determine the adsorption capacity and mean biomass of Thraustochytrids in Bunker C oil using different cell densities measured in grams. All of the three treatments or cell densities (1 x 10^5 cells ml^{-1}, 1 x 10^6 cells ml^{-1} and 1 x 10^7 cells ml^{-1}) were triplicated and average values were recorded. Oil dispersant was used as a control. It showed that Thraustochytrid with 1 x 10^7 cells ml^{-1} showed the highest adsorbed oil (.057 ḡ) among the three cell densities and showed significant difference at p = .01 but comparable to the control (.066 ḡ). In terms of biomass concentration, all cell densities showed no significant difference at p = .01. Thraustochytrid is a promising tool during oil spill because it has the capacity to adsorb oil.

Key words: *thraustochytrid, adsorption, biomass, Bunker C oil*

INTRODUCTION

Thraustochytrids are taxonomically classified as heterokont algae or two unequal flagella (C*avalier-Smith and Allsopp 1994; Lewis, Nichols and McMeekin 1999*). This organism belongs to phylum Heterokonta under the kingdom Chromista, and not fungi based on the latest taxonomy of the phylogenetic analysis through 18s rRNA sequence (*Cavalier-Smith and Allsopp 1994*).

Thraustochytrids are marine protist found in marine and estuarine habitats (*Fan et al. 2000*; *Fan, Vrijmoed and Jones 2002*; *Shene et al. 2010*), chemoorganotrophic, marine stramenipilan protist in the class Labyrinthulomycetes (*Bongiorni et al. 2005*). Yet, some are parasitic protists (*Scharer et al. 2007*). However, other researchers identified them as marine fungi (*Raikar et al. 2001*) and fungoid protist (*Bongiorni, Pignataro and Santangelo 2004; Naganuma et al. 2006*).

Six thraustochytrids such as *Schizochytrium* sp. KF-1, Schizochytrium mangrovei KF-2, KF-7, KF-12, *Thraustochytrium striatum* KF-9 and Ulkenia KF-13 from the fallen, senescent leaves of the mangrove species of *Kandelia candel* in Hong Kong were isolated, and identified (*Fan, Vrijmoed and Jones 2002*). The same study was found out by *Wong, Vrijmoed and Au* (*2005*) that thraustochytrids were also abundant on the fallen decaying leaves of *Kandelia candel* and in the sediments in Futian National Nature Reserve, China which speaks of their ubiquity in nature (*Bongiorni et al. 2005*). This supports the idea that thraustochytrids feed on decaying macroalgae and mangrove leaves that are rich in cellulose and probably act as food source for detritus and picoplankton feeders in mangrove ecosystem (*Naganuma, Takasugi and Kimura 1998*; *Wong, Vrijmoed and Au 2005*).

Thraustochytrids has the ability to recycle carbon in sandy shores and coastal waters (*Kimura, Fukuba and Naganuma 1999; Bongiorni, Pignataro and Santangelo 2004*). *Fan et al.* (*2000*) revealed that thraustochytrids has the ability to utilize food wastes as substrates in upgrading DHA production. This is for the fact that, the lipid content of thraustochytrids consist of a long chain of polyunsaturated fatty acids (PUFA) that has the ability to synthesize docosahexaenoic acid (DHA) (*Nakahara et al. 1996*) that is the major component of neural tissues, retina and responsible for brain development (*Lewis, Nichols and McMeekin 1999; Shene et al. 2010*).

Another important feature of thraustochytrid where this study is anchored is the ability of this organism to degrade hydrocarbons present in oil during oil spill event (*Raikar et al. 2001; Battlung, Metillo and Oclarit 2011*).

[1] John B. Lacson Foundation Maritime University-Arevalo, Sto. Niño Sur, Arevalo, Iloilo City, Philippines. E-mail: bg_sarinas@yahoo.com (*corresponding author)
[2] Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC-AQD)Tigbauan, Iloilo, Philippines

Battlung, Metillo and Oclarit (*2011*) utilized the thraustochytrid Schizochytrium strain to degrade hydrocarbon present in used diesel oil with different enrichment culture and suggested that *Schizochytrium* sp. has the ability to degrade oil because it exhibits adsorption of hydrocarbons. The same study was also found out by *Raikar et al.* (*2001*) that thraustochytrids adhere itself to an oil droplet using crude oil. They further added that thraustochytrids grow more in tarballs after seven days which showed that thraustochytrids play an important role in the biodegradation of tarballs in sediments during oil spill.

This study aimed to determine the adsorption capacity and biomass concentration of thraustochytrid *Schizochytrium aggregatum* (Goldstein and Belsky) exposed in Bunker C oil after seven days. Since there is no study yet on the adsorption and biomass concentration of thraustochytrid in Bunker C oil, thus this study is conducted.

MATERIALS AND METHODS

Materials

The strain of thraustochytrid *Schizochytrium aggregatum* (Goldstein and Belsky) was taken at the Phycology laboratory of Southeast Asian Fisheries Development Center-Aquaculture Department (SEAFDEC-AQD), Tigbauan, Iloilo. There were four treatments namely, A: $1x10^5$ cells ml^{-1}, Treatment B: 1×10^6 cells ml^{-1}, Treatment C: 1×10^7 cells ml^{-1} and D: control group (containing oil and oil dispersant, Termamyl SC).

Data Collection

Preparing of media and culture of thraustochytrid isolates. Growth media is composed of yeast extract, agar, glucose, MSG, one drop of lactic acid and with seawater (1000 ml). It was distributed in the nine 250 ml flasks and was covered with cotton plug and aluminum foil. These were autoclaved for two hours at 121°C. At the same time, one 500 ml flask was taken in the media for thraustochytrid culture that was observed for two days.

Setting-up of treatments.There were three replicates for every treatment. Treatment A was composed of 1×10^5 cells ml^{-1} treatment B: 1×10^6 cells ml^{-1} and treatment C: 1×10^7 cells ml^{-1}. The formula d (cells ml^{-1}) = (total count/no. of blocks) x 10^4 was used to determine the desired cells ml^{-1} for each treatment following the work of *Guillard* (*1973*). The control set-up (D) was the oil dispersant (Termamyl SC) containing 75 ml for the three replicates. All set-ups contained 100 µl of Bunker C oil and were observed for seven days.

Reading of adsorbed Bunker C oil in g after seven days. After seven days, the set-up treatments were centrifuged to separate the oil component and these were measured in grams. Average values were recorded for each treatment.

Reading of biomass of thraustochytrids in g after seven days. The set-up treatments were centrifuged to separate the cell component from Bunker C oil and these were measured in grams by the use of electronic weighing scale.

Use of the haemacytometer for checking the microscopic adsorbed oil by thraustochytrids. This was to check for the adsorption of Bunker C oil by thraustochytrid isolates for each treatment using the compound microscope.

Data Analysis

In addition, one-way analysis of variance (ANOVA) set at 0.01 level of significance using SPSS was employed to determine if no significant differences existed in the amount of oil adsorbed in grams among treatments and if there was or no significant differences in the biomass of thraustochytrids in grams for each treatment after seven days.

RESULTS AND DISCUSSION

For treatment A (1×10^5 cells ml^{-1}), the mean adsorbed oil was .005 ḡ (sd=.006), for treatment B (1×10^6 cells ml^{-1}) it was .042 ḡ (sd=.018), for treatment C (1×10^7 cells ml^{-1}) it was .051 ḡ (sd=.024) and for control (treatment D), it was .066 ḡ (sd=.012) (**Table 1**). This study supports the idea of *Raikar et al.* (*2001*) and *Battlung, Metillo and Oclarit* (*2011*) that thraustochytrid has the capacity to adsorbed oil.

Table 1. Mean Values of Adsorbed Oil (g) of Thraustochytrids after Seven Days.

Treatment	cells ml^{-1}	ḡ	sd
A	$1x10^5$	.005	.006
B	$1x10^6$	.042	.018
C	$1x10^7$	.051	.024
D	Control	.066	.012

For treatment A (1×10^5 cells ml^{-1}), the mean biomass concentration of thraustochytrid was 1.123 ḡ (sd=.618), for treatment B (1×10^6 cells ml^{-1}) it was 1.177 ḡ (sd=.127) and for treatment C (1×10^7 cells ml^{-1}) it was 1.883 ḡ (sd=.425) (**Table 2**). This simply shows that thraustochytrids can grow even with the presence of hydrocarbons (*Raikar et al. 2001*).

Table 2. Mean Values of Thraustochytrid Biomass Concentration after Seven Days.

Treatment	cells ml^{-1}	ḡ	sd
A	$1x10^5$	1.123	.618
B	$1x10^6$	1.177	.127
C	$1x10^7$	1.883	.425

After seven days, adsorbed oil of thraustochytrids show a significant difference among treatments, $F_{(3,8)}$= 8.300, p = .008 (**Table 3**).

Table 3. One-Way ANOVA for Mean Values of Adsorbed Oil of Thraustochytrid after Seven Days.

Sources of Variation	SS	df	Mean Square	F	Sig.
Treatment	.007	3	.002	8.300*	.008
Error	.002	8	.000		
Total	.009	11			

* - significant at 1% level of probability

Treatment A and B have no significant differences while treatment B, C, and D were also not significant (**Table 4**). However, treatment A was significant with treatments C and D, respectively.

Table 4. Scheffe Test for Mean Values of Adsorbed Oil of Thraustochytrids after Seven Days.

Treatment	Mean weight (g̃)
A (1x10^5)	. 005^a
B (1x10^6)	. 042^{ab}
C (1x10^7)	. 051^b
D (Control)	. 066^b
Level of Sig.	.008

The biomass concentration of thraustochytrids after seven days showed no significant difference among treatments, $F_{(2,6)}$ = 2.799, p = .138 (**Table 5**)

Table 5. One-Way ANOVA for Significant Difference in the Mean Values of Biomass of Thraustochytrids after Seven Days.

Grouping	SS	df	Mean Square	F	Sig.
Between Groups	1.080	2	.540	2.799 ^{ns}	.138
Within Groups	1.157	6	.193		
Total	2.237	8			

ns – not significant at 1% level of probability

CONCLUSION

Thraustochytrid *Schizochytrium aggregatum* (Goldstein and Belsky) is an alternative tool in the adsorption of hydrocarbons. It was shown that treatment C (1x10^7 cells ml^{-1}) had the highest average value of adsorbed oil than the other treatments but is comparable to the control although there is a significant difference. On the other hand, the biomass concentration of thraustochytrid showed no significant difference among treatments. It is recommended that other type of oil will be used for further information about the adsorption capacity of the thraustochytrids.

REFERENCES

Bongiorni, L., Pignataro, L. and G. Santangelo. 2004. "Thraustochytrids (Fungoid-Protists): An Unexplored Component of Marine Sediment Microbiota." *Scientia Marina* 68(1): 43-48.

Bongiorni, L., Jain, R., Raghukumar, S. and R.K. Aggarwal. 2005. "Thraustochytrium sp. nov.: A New Thraustochytrid Stramenopilan Protist From Mangroves of Goa, India." *Protist* 156: 303-315.

Battlung, M.J.V., Metillo, E.B. and J.M. Oclarit. 2011. "Increased Biomass and Adsorption of Used Diesel Oil by Thraustochytrids." Paper presented at the 5th International Conference on Bioinformatics and Biomedical Engineering (iCBBE). Wuhan, China. May 10-12, 2011.

Cavalier-Smith, T., Allsopp, M.T.E.P. and E.E. Chao. 1994. "Thraustochytrids are Chromists, not Fungi: 18s rRNA Signatures of Heterokonta." *Philosophical Transactions: Biological Sciences* 346(1318): 387-397.

Fan, K.W., Chen, F., Jones, E.B.G. and L.L.P. Vrijmoed. 2000. "Utilization of Food Processing Waste by Thraustochytrids." In: Aquatic Mycology across the Millennium: (eds K. D. Hyde, W. H. Ho and S. B. Pointing). *Fungal Diversity.* 5:185-194.

Fan, K.W., Vrijmoed, L.L.P. and E.B.G. Jones. 2002. "Physiological studies of subtropical mangrove thraustochytrids." *Botanica Marina* 45: 50-57.

Guillard, R.L. 1973. "Division Rates." In: Phycological methods (ed. J. R. Stein). Cambridge University Press: *Cambridge.* pp. 289-311.

Kimura, H., Fukuba, T. and T. Naganuma. 1999. "Biomass of Thraustochytrid Protoctists in Coastal Water. *Marine Ecology Progress Series* 189: 27-33.

Lewis, T.E., Nichols, P.D. and T.A. McMeekin. 1999. "The Biotechnological Potential of Thraustochytrids." *Marine Biotechnology* 1: 580-587.

Naganuma, T., Takasugi, H., and H. Kimura. 1998. "Abundance of Thraustochytrids in Coastal Plankton." *Marine Ecology Progress Series* 162: 105-110.

Naganuma, T., Kimura, H., Karimoto, R. and N.V. Pimenov. 2006. "Abundance of Planktonic Thraustochytrids and Bacteria and the Concentration of Particulate ATP in the Greenland and Norwegian Seas." *Polar Bioscience* 20:37-45.

Nakahara, T., Yokochi, T., Higashihara, T., Tanaka, S., Yaguchi, T. and D. Honda. 1996. "Production of Docosahexaenoic and Docosapentaenoic Acid by Schizochytrium sp. Isolated from Yap Islands." *Journal of the American Oil Chemists' Society* 73: 1421-1426.

Raikar, M., Raghukumar, S., Vani, V., David, J.J. and D. Chandramohan. 2001. "Thraustochytrid Protists Degrade Hydrocarbons." *Indian Journal of Marine Science* 30: 139-145.

Scharer, L., Knoflach, D., Vizoso, D.B., Rieger, G. and U. Peintner. 2007. "Thraustochytrids as Novel Parasitic Protists of Marine Free-Living Flatworms: *Thraustochytrium caudivorum* sp. nov. Parasitizes Macrostomum liglano." *Marine Biology* Springer-Verlag. doi: 10.1007/s00227-007-0755-4.

Shene, C., Leyton, A., Esparza, Y., Flores, L., Quilodran, B., Hinzpeter, I. and M. Rubilar. 2010. "Microbial Oils and Fatty Acids: Effect of Carbon Source on Docosahexaenoic Acid (C22:6 N-3, DHA) Production by Thraustochytrid Strains." *Journal of Soil Science and Plant Nutrition* 10(3): 207-216.

Wong, M.K.M., Vrijmoed, L.L.P. and D.W.T. Au. 2005. "Abundance of Thraustochytrids on Fallen Decaying Leaves of Kandelia candel and Mangrove Sediments in Futian National Nature Reserve, China. *Botanica Marina* 48(12): 374-37.

ACKNOWLEDGEMENT

We would like to thank Dr. Mary Lou Lacson Arcelo, President Emeritus and Chairman of the Board of Trustees of John B. Lacson Foundation Maritime University and Dr. Ronald Raymond Lacson Sebastian, Chief Executive Officer and Vice-Chairman of John B. Lacson Foundation Maritime University for the full support extended in this study and the Southeast Asian Fisheries Development Center Aquaculture Department (SEAFDEC-AQD) for the laboratory equipment.