

GROWTH AND MOTILITY OF THE DIATOM *CYLINDROTHECA CLOSTERIUM*: IMPLICATIONS FOR COMMERCIAL APPLICATIONS

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Abstract: A unialgal isolate of *Cylindrotheca closterium* (Ehremberg) Lewin and Reimann from the Newport River estuary was grown in f/2 seawater media under continuous saturating irradiance at 27°C. Growth rates and motility were measured in four replicate flasks under two experimental growth conditions: 1) unstirred batch cultures shaken once per day to suspend the cells and break down density gradients within the flasks, and 2) continuously stirred batch cultures on an orbital shaker (160 rpm). Cell densities were measured daily for six days by counting a minimum of 1,000 cells from each of four replicate culture flasks using a Neubauer hemacytometer. Daily measurements of motility were made by noting the percentage of cells moving within a 0.04 mm² area of the hemacytometer over a 10 sec interval. Both treatments showed similar logarithmic growth during the first three days but equilibrium cell density was twice as great in the unstirred treatment by day six. Growth rates on day one in both experimental conditions exceeded 2.3 divisions per day. Initial high motility rates (≥95%) were maintained until day three in the unstirred treatment but dropped in half by day two in the continuously stirred treatment. A significant negative correlation existed between culture age and motility in both treatments. These findings have implications for growing *C. closterium* in industrial applications including use as a settlement agent and feed in abalone hatcheries, bioassay for the detection of heavy metals in surface sediments, and remediation tool to remove nutrients from aquaculture effluents.

Key Words: *Cylindrotheca closterium*; diatoms; cell motility; biofilm.

INTRODUCTION

Cylindrotheca closterium (Ehremberg) Lewin and Reimann, a diatom commonly found in neritic waters, is best classified as tychoplagic or meroplanktonic since it exploits a half-planktonic, half-benthic existence more completely than other species (Round 1981). Meroplanktonic species are readily transported up into the water column where they survive and then return to the sediments under calm conditions. *C. closterium* attains high densities in the water column of the Newport River estuary in North Carolina following wind mixing events (Kenney, pers. comm.) and during wind-induced upwelling events off the central coast of Portugal (Moita et al. 2003). Under this selection regime, the ability to grow rapidly during the intermittent periods when *C. closterium* is transported upward into the well-lit water column would be adaptive. The growth rate of this species is much higher than that of many other diatom species (Tanaka 1984). When observed on benthic surfaces, *C. closterium* exhibits the typical gliding motility seen in other pennate diatoms (Edgar and Pickett-Heaps 1984) but is incapable of motility when suspended in the water column. A by-product of its movement across surfaces is the secretion of polymeric substances (deBrouwer and Stahl 2002; Apoya-Horton et al. 2006) which form the extracellular aggregative matrix of the biofilms that typically cover benthic surfaces within the euphotic zone. Given its high growth rate and meroplanktonic

existence, *C. closterium* has significant potential for a variety of industrial scale applications in unialgal biofilms or suspended in stirred bioreactors. Possible applications include use as a bioassay for heavy metal contamination of sediments (Moreno-Garrido et al. 2003), bioremediation agent for chloro-organic pollution (Keil et al. 1971) and shrimp farm effluents (Thompson et al. 2002), and mariculture feed in shrimp (Lebeau and Robert 2003) and shellfish hatcheries (Daume et al. 1999).

Biofilms composed of diatoms facilitate attachment and metamorphosis of abalone larvae and are widely used to induce larval settlement in abalone hatcheries (Kawamura and Kikuchi 1992; Gallardo and Buen 2003). Species composition of the diatom film not only influences the success of settlement (Roberts et al. 2007) but also the growth of post-larval abalone (Kawamura et al. 1995). Unlike many other diatom species studied, *C. closterium* supports rapid growth of post-larvae abalone because it is easily dislodged from the substratum (Kawamura et al. 1995) and its lightly-silicified frustule is easily broken by the radula of the abalone post-larvae (Takami and Kawamura 2003). Daume and coworkers (1999) reported a low settlement response on three day old diatom films composed of *C. closterium* which suggests that its value as food for abalone post-larvae exceeds its value as a settlement inducing agent for planktonic abalone larvae. Interestingly enough, biofilms of *C. closterium* grown for 19 days increased

settlement eight fold over three day old biofilms which suggests that older, non-growing cultures of *C. closterium* in stationary phase may induce settlement more than exponentially growing cultures. The control and management of diatom films represents a significant challenge in abalone mariculture (Seki 1997) that can result in low and variable larval settlement and survival (Searcy-Bernal et al. 1992).

Given its potential uses in a variety of biotechnological and commercial applications that would require industrial-scale production including abalone culture, I examined the effect of culture conditions on the growth rate and motility of *C. closterium*. It was hypothesized that continuous stirring would increase the growth rate of this meroplanktonic species under culture conditions since it attains high abundances in the water column following mixing events. Continuous stirring of cultures should act to ameliorate the effects of self-shading and local diffusion gradients that would limit production in a biofilm. This study also examined the general motility of *C. closterium* over time as this characteristic may influence its value in industrial-scale applications. A decrease in general motility over time as cultures attain stationary growth may explain the eight fold increase in abalone settlement that Daume et al. (1999) observed on 19-day old *C. closterium* biofilms. Recent research suggests that smothering by highly mobile diatoms interferes with abalone larvae settlement (Roberts et al. 2007). Consequently, a secondary hypothesis addressed by this study is that the motility of *C. closterium* will decrease with culture age.

METHODS

A non-axenic, uni-algal isolate of *C. closterium* was established from a sediment sample collected from a shallow-water sandflat of the Newport River just west of the Duke University Marine Laboratory on Pivers Island, Beaufort, North Carolina. The isolate was grown in 50 ml of Guillard's f/2 seawater media (Guillard and Ryther 1962) in 125 ml glass Erlenmeyer flasks stoppered with foam plugs at 27°C under continuous illumination between two banks of 36 W fluorescent lights (Grolux, Sylvania). Aged seawater used in the media was collected offshore several months earlier and filtered prior to storage (Millipore; HA type; 0.45 µm pore size). The final salinity of the media was 31 practical salinity units (PSU). The irradiance level was maintained at 240 µmol photons m⁻²·s⁻¹ in a culture chamber with a 24:0 L:D cycle. The initial isolate was grown under constant conditions and transferred to fresh media every two weeks for a three month period to ensure that any endogenous rhythms in growth or motility were free-running. These cultures were shaken once daily by hand to break down diffusion gradients.

Two days prior to the initiation of growth and motility experiments, the cells were again transferred to fresh media to produce exponentially growing cells.

Four 125 ml glass flasks containing 50 ml of Guillard's f/2 seawater media were inoculated with 6,250 two-day old, exponentially growing cells. These four flasks were grown under the same culture conditions described above for six days and shaken only once per day before performing cell counts and motility assays. The cell density in each flask was measured each day by counting the number of *C. closterium* cells in 15 to 30 samples (10⁻⁴ ml) until a minimum of 1,000 cells were counted. Cell counts were conducted using bright-field microscopy (100×) and an improved Neubauer hemacytometer. Daily measurements of cell motility were collected by noting the proportion of cells moving within a 0.04 mm² area within the hemacytometer over a 10 sec interval. The motility measurement for each day was based upon 4–8 replicate assays (50–100 cells per assay) performed on samples drawn from each flask totaling over 400 cells. The motility measures were converted to percentages by multiplying the proportion of cells moving during the 10 second interval by 100.

Following these incubations, another four 125 ml flasks with 50 ml of Guillard's f/2 seawater media were inoculated with 2,500 exponentially growing cells and grown for six days under the same conditions with one exception. These four flasks were continuously stirred on an orbital shaker (Rotomix Orbital, Thermo-Scientific) at 160 rpm within the culture chamber. Cell density and cell motility were measured as described above.

Division rates (div·d⁻¹) were calculated for 2, 3, and 6 day old cultures as: $\log_2(N_t/N_0) \times 1/\Delta t$, where N_t is the cell density at time t (cells·ml⁻¹), N_0 is the cell density (cells·ml⁻¹) at the time of inoculation, and Δt is the time interval between t and the time of inoculation. Student t -tests were used to compare the cell division rates and motility rates under the continuously stirred and unstirred treatments on days 2, 3 and 6 and to compare the cell densities in each treatment on day 6. When the motility rate data failed to meet the assumptions of the t -test, a Mann Whitney rank sum test was used to compare the continuously stirred and unstirred treatments. Spearman rank correlation analysis was used to evaluate the relationship between culture age and motility. All statistical analysis was conducted using SigmaStat statistical analysis software (Systat Software 2004).

RESULTS

The growth rate experiment showed similar logarithmic growth rates over the first three days of growth in both the stirred and unstirred treatments (Fig. 1).

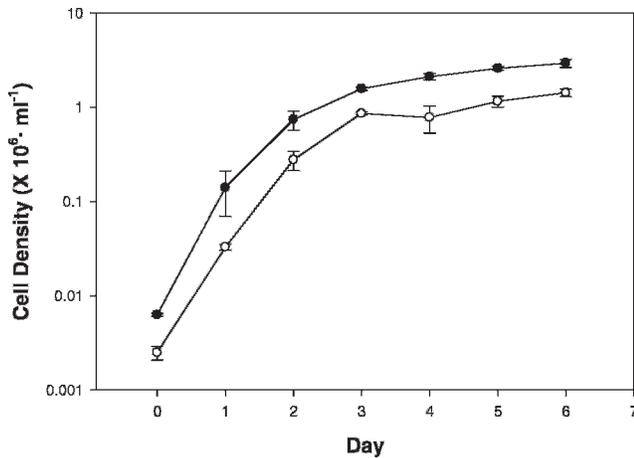


FIG. 1. Growth of *Cylindrotheca closterium* over a 6 day period as measured by cell density ($\times 10^6$ cells·ml⁻¹) in continuously stirred and unstirred batch cultures. The filled circles (●) are the means ($n = 4$) of the unstirred cultures and the open circles (○) are the means ($n = 4$) of the continuously stirred cultures. The error bars illustrate the standard errors of the means.

Average division rates on day 2 were 2.34 div·d⁻¹ in the unstirred treatment and 2.51 div·d⁻¹ in the stirred treatment. Average division rates had dropped to 1.84 div·d⁻¹ and 1.96 div·d⁻¹, respectively, by day 3 when the cultures were closer to entering stationary growth. Student *t*-tests revealed that the observed differences in the growth rates between the two treatments ($n = 4$) were not significantly different on day 2 ($p = 0.227$) or day 3 ($p = 0.08$). Once stationary phase growth was attained by day 6, the average growth rate of the unstirred treatment (1.08 div·d⁻¹) was significantly greater (*t*-test; $p = 0.02$) than the stirred treatment (1.02 div·d⁻¹). A Student *t*-test indicated that the average cell density during stationary growth was significantly higher ($p = 0.008$) in the unstirred treatment (2.94×10^6 cells·ml⁻¹) than in the stirred treatment (1.56×10^6 cells·ml⁻¹).

The motility experiments revealed that average cell motility under both treatment conditions exceeded 95% on the day following inoculation (Fig. 2); however, a Mann-Whitney rank sum test revealed that the average motility in the unstirred treatment (50%) was significantly lower ($p = 0.029$) than the average motility in the stirred treatment (95%) by day 2. Average motility in both treatments had fallen to 47.5% by day 3. Student *t* tests revealed that the average motilities in the unstirred treatment (~47%) on days 4 and 5 were significantly higher ($p = 0.002$ and $p = 0.025$) than in the continuously stirred cultures (~25%). Once the cultures had attained stationary growth on day 6, average motility in the unstirred treatment (39.5%) was not statistically different ($p = 0.709$, *t*-test) than the continuously stirred treatment (37.3%). Spearman rank correlation analysis revealed a significant ($p < 0.001$)

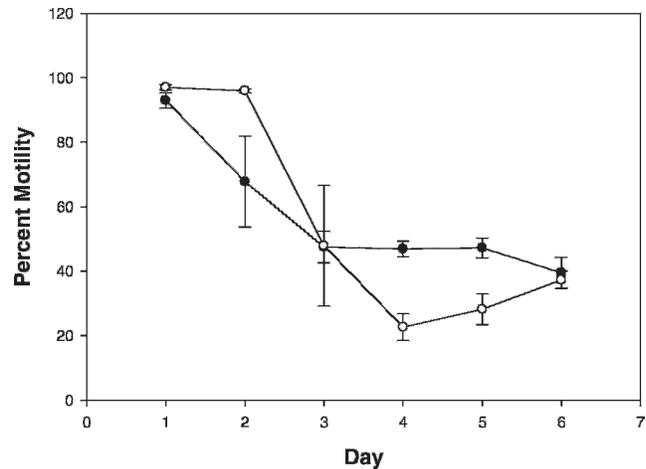


FIG. 2. Percent motility of *Cylindrotheca closterium* over the 6 day growth period. The filled circles (●) are the means ($n = 4$) of the unstirred cultures and the open circles (○) are the means ($n = 4$) of the continuously stirred cultures. The error bars illustrate the standard errors of the means.

inverse relationship between culture age and motility with a correlation coefficient of -0.7 for both unstirred and continuously stirred treatments.

DISCUSSION

Growth rates for *C. closterium* reported here are higher than most of those reported in the published literature but there was no statistically significant difference between the growth rates of continuously stirred and unstirred cultures. During the steepest portion of the growth curve (day 2), the average growth rate exceeded 2.5 div·d⁻¹ in the continuously stirred treatment and it remained above 1.9 div·d⁻¹ on day 3. Rijstenbil (2003) reported slower exponential growth of *C. closterium* in batch cultures of 0.48 div·d⁻¹ but these experiments were conducted with a 12:12 L:D cycle at 15°C. Exponential growth of *C. closterium* growing on cleaned sand after 3 days was reported as 1.23 div·d⁻¹ but these cultures were grown in *f*/2 media without EDTA (Moreno-Garrido et al. 2003). A maximal cell division rate of 1.60 div·d⁻¹ for *C. closterium* in continuous culture on a 10:14 L:D cycle at 19°C was measured by Tanaka (1984). Williams (1964) reported a division rate of 2.0 div·d⁻¹ for *C. closterium* at 30–31 PSU in batch culture which is very similar to the results reported here for cells grown at 31 PSU but he reported even higher growth rates of *C. closterium* (3.2 div·d⁻¹) at 18 PSU. The high growth rates originally reported by Williams (1964) with a single culture and confirmed by replicate cultures in this study indicate that this species can be rapidly grown for commercial applications. The hypothesis that continuous stirring would increase exponential growth rates in this meroplanktonic species must be rejected suggesting that the motility of this

species on the glass surfaces of the unstirred culture flasks was sufficient to overcome the negative effects of self-shading and micro-scale diffusion gradients.

One puzzling finding of the current study was the difference in the 6-day biomass of the continuously stirred and unstirred cultures with the unstirred treatment having almost twice the cell density of the stirred treatment. The initial inocula of the unstirred cultures was approximately three times as dense as the stirred cultures but this should not have produced this dramatic difference in final biomass. One possible explanation might involve a cellular-level response to turbulence. It is possible that the constant agitation induced the incorporation of more silica in each cell's frustules which resulted in silica limitation in the continuously stirred treatment flasks. It is generally held that the extent of silicification and the thickness of diatom frustules are inversely correlated with growth rate (Claquin et al. 2002). Although growth is often controlled by environmental factors such as light, temperature, and nutrients, few studies have addressed how these and other environmental factors might affect frustules thickness independent of their influence on growth. Previous work in laboratory-scale batch cultures has documented that vigorous mixing of some benthic diatom species can result in hydrodynamic stress (Camacho et al. 2001) and cell damage (Contreras et al. 1998). Given its meroplanktonic lifestyle, *C. closterium* may have evolved a dynamic response to cope with vigorous mixing, e.g., laying down thicker or more reinforced frustules, that is not elicited in the absence of turbulence. Such a response could be mediated through the differential expression of genes involved in the synthesis, regulation, and localization of silicic acid transporters (Martin-Jézéquel et al. 2000).

Although similar trends in decreased motility with increased culture age during the first three days were noted in both unstirred and continuously stirred treatments, the transition between high motility ($\geq 95\%$) and low motility ($\leq 50\%$) began a day earlier in the unstirred treatment flasks. Following this transition, the motility in the unstirred treatment was significantly higher than in the continuously stirred treatment until day 6 when both treatments averaged approximately 40% motility. One explanation for higher motility observed on day 2 in the unstirred treatment as exponential growth was slowing is that motility may play a role in increasing nutrient uptake and breaking down diffusion gradients when nutrients begin to become limiting. Once population growth has stopped and the cells have become nutrient limited, motility stabilizes at 40%.

The motility findings reported here yield insights into the use of *C. closterium* in commercial applications and also support the idea that increased settlement of

abalone larvae on older biofilms of *C. closterium* may be related to lower motility in older biofilms. Highly mobile *Nitzschia longissima* smother newly settled abalone larvae (Roberts et al. 2007) so it is possible that the high motility of exponentially growing *C. closterium* explains the inhibitive effect of young *C. closterium* biofilms on abalone settlement reported by Daume et al. (1999). The lower motility of stationary phase cultures observed in this study is consistent with this interpretation. For applications which involve its use as a bioassay or bioremediation agent, the use of exponentially growing cultures of *C. closterium* may be preferred since their high motility may result in enhanced exposure and absorption of environmental compounds and their higher metabolic rates would facilitate faster chemical transformations. Non-growing, stationary phase cultures may be preferable in applications using *C. closterium* as an agent to induce the settlement of shellfish larvae.

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LITERATURE CITED

- APOYA-HORTON, M. B., L. YIN, G. J. C. UNDERWOOD, AND M. R. GRETZ. 2006. Movement modalities of the mudflat diatom *Cylindrotheca closterium* (Bacillariophyceae). *J. Phycol.* 42:379–390.
- CAMACHO, F. G., E. M. GRIMA, A. S. MIRÓN, V. G. PASCUAL, AND Y. CHISTI. 2001. Carboxymethyl cellulose protects algal cells against hydrodynamic stress. *Enzyme Microb. Technol.* 29:602–610.
- CLAQUIN, P., V. MARTIN-JÉZÉQUEL, J. C. KROMKAMP, M. J. W. VELDHUIS, AND G. W. KRAAY. 2002. Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the cell cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen and phosphorus control. *J. Phycol.* 38:922–930.
- CONTRERAS, A., G. GARCÍA, E. MOLINA, AND J. C. MERCHUK. 1998. Interaction between CO₂-mass transfer, light availability, and hydrodynamic stress in the growth of *Phaeodactylum tricorutum* in a concentric tube airlift photobioreactor. *Biotechnol. Bioeng.* 60:317–325.
- DAUME, S., S. BRAND-GARDNER, AND W. J. WOELKERING. 1999. Preferential settlement of abalone larvae: diatom films vs. non-geniculate coralline red algae. *Aquaculture* 74:243–254.
- De BROUER, J. F. C., AND L. STAHL. 2002. Daily fluctuations of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia* sp. (Bacillariophyceae). *J. Phycol.* 38:464–472.
- EDGAR, L. A., AND J. D. PICKETT-HEAPS. 1984. Diatom locomotion, an explanation. *Prog. Phycol. Res.* 3:47–88.
- GALLARDO, W. G., AND S. M. A. BUEN. 2003. Evaluation of mucus, *Navicula*, and mixed diatoms as larval settlement

- inducers for the tropical abalone *Haliotis asinina*. *Aquaculture* 236:221–239.
- GUILLARD, R. R. L., AND J. H. RYTHER. 1962. Studies on marine planktonic diatoms. I. *Cyclotella nanna* Hustedt and *Detonula confervaceae* (Cleve) Gran. *Can. J. Microbiol.* 8:229–239.
- KAWAMURA, T., AND H. KIKUCHI. 1992. Effects of benthic diatoms on settlement and metamorphosis of abalone larvae. *Suisanzoshoku* 40:403–409.
- KAWAMURA, T., T. SAIDO, H. TAKAMI, AND Y. YAMASHITA. 1995. Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. *J. Exper. Mar. Biol. Ecol.* 194:189–199.
- KEIL, J. E., L. E. PRIESTER, AND S. H. SANDIFER. 1971. Polychlorinated biphenyl (Aroclor 1242®): Effects of uptake on growth, nucleic acids, and chlorophyll of a marine diatom. *Bull. of Env. Contam. Tox.* 6(2):156–159.
- LEBEAU, T., AND J.-M. ROBERT. 2003. Diatom cultivation and biotechnologically relevant products. Part I: Cultivation at various scales. *Appl. Microbiol. Biotechnol.* 60:612–623.
- MARTIN-JÉZÉQUEL, V., M. HILDEBRAND, AND M. A. BRZEZINSKI. 2000. Silicon metabolism in diatoms: Implications for Growth. *J. Phycol.* 36:821–840.
- MOITA, M. T., P. B. OLIVEIRA, J. C. MENDES, AND A. S. PALMA. 2003. Distribution of chlorophyll a and *Gymnodinium catenatum* associated with coastal upwelling plumes off central Portugal. *Acta Oecologia* 24:S125–S132.
- MORENO-GARRIDO, I., M. HAMPEL, L. M. LUBIÁN, AND J. BLASCO. 2003. Sediment toxicity tests using benthic marine microalgae *Cylindrotheca closterium* (Ehremberg) Lewin and Reimann (Bacillariophyceae). *Ecotox. & Env. Safety* 54(3):290–295.
- RIJSTENBIL, J. W. 2003. Effects of UVB radiation and salt stress on growth, pigments, and antioxidative defence of the marine diatom *Cylindrotheca closterium*. *Mar. Ecol. Prog. Ser.* 254:37–48.
- ROBERTS, R. D., T. KAWAMURA, AND C. M. HANDLEY. 2007. Factors affecting settlement of abalone (*Haliotis iris*) larvae on benthic diatom films. *J. Shellish Res.* 26(2):323–334.
- ROUND, F. E. 1981. *The ecology of algae*. Cambridge University Press, Cambridge, UK. 653 pp.
- SEARCY-BERNAL, R., A. SALAS-GARZA, R. FLORES-AGUILAR, AND P. HINOJOSA-RIVERA. 1992. Simultaneous comparison of methods for settlement and metamorphosis induction in the red abalone (*Haliotis rufescens*). *Aquaculture* 105:241–250.
- SEKI, T. 1997. Biological studies on the seed production of the northern Japanese abalone. *Bull. Tohoku Nat. Fish. Res. Inst.* 59:1–71.
- SYSTAT SOFTWARE INC. 2004. *SigmaStat Software for Windows*, V 3.1, Point Richmond, CA, USA.
- TAKAMI, H., AND T. KAWAMURA. 2003. Dietary changes in the abalone, *Haliotis discus hannai*, and relationship with the development of the digestive organ. *Jarq.* 37(2):89–98.
- TANAKA, N. 1984. The cell division rates of 10 species of attaching diatoms in natural sea water. *Nippon Suisan Gakkaishi.* 50(6):969–972.
- THOMPSON, F. L., P. C. ABREU, AND W. WASIELESKY. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* 203:263–278.
- WILLIAMS, R. B. 1964. Division rates of salt marsh diatoms in relation to salinity and cell size. *Ecol.* 45(4):877–880.

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