Journal of Ecobiotechnology 2/2: 41-44, 2010
ISSN 2077-0464

Journal of Ecobiotechnology
http://journal-ecobiotechnology.com/

Efficacy of sugarcane bagasse to produce bacterial biofilm in water for fish culture

M.V. Radhakrishnan and E.Sugumaran
Department of Zoology, Annamalai University, Annamalainagar – 608 002, Tamilnadu, India

**Corresponding Author, Email: radkisnan@yahoo.co.in*

Keywords

Total Plate Count
Sugarcane Bagasse
Microbial Biofilm

Abstract

The present study has been conducted to understand the efficacy of sugarcane bagasse to produce bacterial biofilm in water. The study period was 90 days. Total Plate count (TPC) in water and substrate was estimated on nutrient agar at room temperature by spread plate method. The TPC in water was the highest (2.10 x 104 ml–1) after 45 days. The average TPC of bacteria on bagasse varied from 140.0 (15d) to 30.25 (90d) x104·g–1. The present study demonstrates that the sugarcane bagasse can produce more bacteria in water thereby the fish can effectively utilize biofilm grown on sugarcane bagasse and provision of a substrate reduces the need for artificial feed.

1. Introduction

Almost all submerged surfaces are covered with thin microbial biofilms. These biofilms are present in most marine waters including polar (Ford et al., 1989; Maki et al., 1990), temperate, (Berk et al., 1981) and tropical ecosystems (Hofmann et al., 1978) as well as freshwater environments (Lock, 1993). Biofilms form when bacteria attach to surfaces. Their subsequent multiplication and production of exopolymers forms a thin layer of organic matter that works to trap nutrients from the water column and provides protection for the microorganisms living within the biofilm (Van Loosdrecht et al., 1990). Biofilms can play an important role in mediating settlement and metamorphosis of invertebrate larvae (Kirchman and Mitchell, 1981; 1983; Kirchman et al., 1982a; 1982b; Maki and Mitchell, 1985; 1986; Maki et al., 1988; 1989; 1990; 1992; 1994; Mitchell, 1984; Mitchell and Kirchman, 1984; Mitchell and Maki, 1988; Rodriguez et al., 1995). Development of viable low-cost technologies and their application to current farming practices would help in enhancing aquaculture production. Substrate based aquaculture is one such technology that has generated a lot of interest in recent years (Wahab et al. 1999,Tidwell et al. 2000, Azim et al. 2001, Keshavanath et al. 2001). By providing organic matter and suitable substrates, heterotrophic food production can be increased several folds which in turn would support fish production. Substrates provide the site for epiphytic microbial production, consequently eaten by fish-food organisms and fish. Fish harvest microorganisms directly in significant quantities, either from microbial biofilm on detritus or from naturally occurring flocks in water column (Schroeder 1978). Provision of substrate would therefore, be useful for the growth of microbial biofilm. Apart from forming food for fish, biofilm improves water quality by lowering ammonia concentration (Langis et al. 1998, Ramesh et al. 1999), this implies the usefulness of substrates in improving water quality in culture systems, by lowering ammonia concentration. Hence, in the present study attempt has been made to assess the efficacy of sugarcane bagasse to produce biofilm in water.

2. Materials and Methods

The experiment was conducted over a period of 90 days in two 25 m^2 (5 x 5 x 1 m) cement tanks with 15cm soil base. The tanks were initially received 0.25 kg of quick lime and 2.5 kg of poultry manure. Water was filled to the tanks from a perennial well and a depth of 90 $\pm$ 2 cm was maintained throughout the experimental period. Subsequently, poultry manure was applied at 0.3 kg per tank every 15 days. Sugarcane bagasse, procured locally, was sun dried and bundles were made using nylon rope; they were introduced into the tanks randomly at the rate of 5 kg each, by suspending the bundles at regular distances from bamboo poles kept across the tanks. After 45 days, once again 1.25 kg of the substrate was supplemented to each of the designated tanks. Total Plate count (TPC) in water was estimated on nutrient agar at room temperature by spread plate method. TPC on substrate was enumerated according to Anwar et al. (1992). A known quantity of substrate was collected and rinsed three times to remove loosely adherent cells. Then it was resuspended in phosphate-buffered saline and vortexed for three minutes to dislodge the biofilm cells and TPC of the suspension estimated as number per gram of substrate.

3. Results and Discussion

Microbial communities play key roles in the Earth's biogeochemical cycles. A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material. Non-cellular materials such as mineral crystals, corrosion particles, clay or silt particles depending on the environment in which the biofilm has developed, may also be found in the biofilm matrix. The solid-liquid interface between a surface and an aqueous medium (e.g., water) provides an ideal environment for the attachment and growth of microorganisms. The solid surface may have several characteristics that are important in the attachment process. Characklis et al. (1990) noted that the extent of microbial colonization appears to increase as the surface roughness increases. Other characteristics of the aqueous medium, such as pH, nutrient levels, ionic strength, and temperature, may play a role in the rate of microbial attachment to a substratum. Several studies have shown a seasonal effect on bacterial attachment and biofilm formation in different aqueous systems (Mitchell, 1984; Mitchell and Maki, 1988). This effect may be due to water temperature or to other unmeasured, seasonally affected parameters. Fletcher (1988, 1988a) found that an increase in the concentration of several cations (sodium, calcium, lanthanum, ferric iron) affected the attachment of *Pseudomonas fluorescens* to glass surfaces. Cowan et al. (1991) showed in a laboratory study that an increase in nutrient concentration correlated with an increase in the number of attached bacterial cells. A material surface exposed in an aqueous medium will inevitably and almost immediately become conditioned or coated by polymers from that medium, and the resulting chemical modification will affect the rate and extent of microbial attachment. Loeb and Neihof (1975) were the first to report the formation of these conditioning films on surfaces exposed in water. These researchers found that films were organic in nature, formed within minutes of exposure, and continued to grow for several hours. Moreover, properties of the cell surface, specifically the presence of fimbriae, flagella, and surface-associated polysaccharides or proteins, also are important and may possibly provide a competitive advantage for one organism where a mixed community is involved.

Table1. Total plate count of bacteria in water ($x10^4 \cdot ml^{-1}$) and on substrate ($x10^4 \cdot g^{-1}$) (n = 3; $X \pm$ SE)

Medium	Days						
	0	15	30	45	60	75	90
Water	0.42 ± 0.21	0.98 ± 0.10	1.84 ± 0.16	2.10 ± 0.22	1.90 ± 0.21	1.98 ± 0.20	1.90 ± 0.44
Substrate	-----	140 ± 1.35	171.00 ± 25.00	162.40 ± 3.75	72.00 ± 4.12	34.18 ±1.20	30.25 ± 0.75

In the present study, the TPC in water showed a gradual increase from 15days (d) to 90d and was highest (2.10 x 10^4 ml^{-1}) after 45 days. The TPC of bacteria on bagasse showed a decreasing trend from 15d to 90d (140.0 to 30.25 $x10^4 \cdot g^{-1}$). More details are given in Table1. Nutrient enrichment of water is known to increase both the thickness and cellular density of bacterial biofilm (Radhakrishnan and Sugumaran, 2010). The higher bacterial density in water from bagasse based tanks is due to the organic matter contributed by manure as well as the nutrients present in bagasse (Radhakrishnan and Sugumaran, 2010). In addition, bacteria in the biofilm could have added free cells to the water. This might be the reason for the continuous increase of bacteria in water and decrease in the colony in the sugarcane bagasse for a long time. Moreover, the nutrients present in the bagasse cause an initial increase. The present result demonstrates that the sugarcane bagasse can produce more bacteria in water thereby the fish can effectively utilize biofilm grown on sugarcane bagasse and provision of a substrate reduces the need for artificial feed. Since biodegradable wastes have high C: N ratio and harbour higher periphytic biomass, they are better suited as substrates (Azim et al., 2001). Use of cheaper substrates like sugarcane bagasse can greatly improve economic viability of aquaculture.

Acknowledgements

The authors are thankful to University Grants Commission, New Delhi, India for financial assistance in the form of Major Research Project

[No. F 37-47/2009(SR)] and Dr. M. Sabesan, Professor and Head for the encouragement.

References

Anwar H., Janice L.S. and Costerton J.W., 1992. Susceptibility of biofilm cells of *Pseudomonas aeruginosa* to bacterial action of whole blood serum. FEMS Microbiol. Lett., 92: 235–242.

Azim M.E., Wahab M.A., van Dam A.A., Beveridge M.C.M. and Verdegem M.C.J., 2001. The potential of periphyton-based culture of two Indian major carps, rohu *Labeo rohita* (Hamilton) and gonia *Labeo gonius* (Linnaeus). Aquacult. Res., 32: 209–216.

Berk, S.R., Mitchell, R.J., Bobbie, J.S., Nickels and White. D.C., 1981. Microfouling on metal surfaces exposed to seawater. *International Biodeterioration Bulletin*. 17: 29-37.

Characklis W.G., Mc Feters G.A. and Marshall K.C., 1990. Physiological ecology in biofilm systems. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons; p. 341–94.

Cowan M.M., Warren T.M. and Fletcher M., 1991. Mixed species colonization of solid surfaces in laboratory biofilms. Biofouling. 3:23–34.

Donlan R.M., Pipes W.O. and Yohe T.L., 1994. Biofilm formation on cast iron substrata in water distribution systems. Water Res. 28:1497–1503.

Fera P., Siebel M.A., Characklis W.G. and Prieur D., 1989. Seasonal variations in bacterial colonization of stainless steel, aluminum, and polycarbonate surfaces in a seawater flow system. Biofouling.1: 251–61.

Fletcher M.,1988. Attachment of *Pseudomonas fluorescens* to glass and influence of electrolytes on bacterium-substratum separation distance. J Bacteriol.170:2027–30.

Fletcher M., 1988. The applications of interference reflection microscopy to the study of bacterial adhesion to solid surfaces. In: Houghton DR, Smith RN, Eggins HOW, editors. Biodeterioration 7. London: Elsevier Applied Science; p. 31–5.

Ford, T.E., Walch M.R., Mitchell M.J., Kaufman J.R., Vestal S.A. and Ditner Lock M.A., 1989. Microbial film formation on metals in an enriched Arctic river. Biofouling. 1: 301-311.

Hofmann, D.K., Neumann R. and Henne K., 1978. Strobilation, budding, and initiation of scyphistoma morphogenesis in the rhizostome *Cassiopea andromeda* (Cnidaria: Scynhozoa). Marine Biology. 47:161-176.

Keshavanath P., Gangadhar B., Ramesh T.J., Van Rooij J.M., Beveridge M.C.M., Baird D.J., Verdegem M.C.J. and Van Dam A.A., 2001. Use of artificial substrates to enhance production of freshwater herbivorous fish in pond culture. Aquacult. Res., 32: 189–197.

Kirchman, D. and Mitchell, R., 1981. A biochemical mechanism for marine biofouling. Ocean. September, pp 537-541.

Kirchman, D. and Mitchell, R.,1983. Biochemical interactions between microorganisms and marine fouling invertebrates. In: *Biodeterioration 5*. Oxley, T.A. and Barry, S., Eds., John Wiley & Sons Itd, New York, NY, pp 281-290.

Kirchman, D., Graham, S., Reish, D., and Mitchell. R. 1982a. Bacteria induce settlement and metamorphosis of *Janua (Dexiospira) brasiliensis* Grube (Polychaeta: Spirorbidae). Journal of Experimental Biology and Ecology. 56: 153-163.

Kirchman, D., S. Graham, D. Reish, and R. Mitchell. 1982b. Lectins may mediate in the settlement and metamorphosis of *Janua (Dexiospira) brasiliensis* Grube (Polychaeta: Spirorbidae). Marine Biology Letters. 3: 131-142.

Langis, R., Proulex D., de la Noue J., and Couture P., 1998. Effect of bacterial biofilms on intensive *Daphnia* culture. Aquacult. Engg., **7**: 21–38.

Lock, M.A. 1993. Attached microbial communities in rivers. In: *Aquatic Microbiology: An Ecological Approach*. Ford,T.E., Ed., Blackwell Scientific Publications, Boston, MA. Pp 113-138.

Loeb, G.I. and Neihof, R.A., 1975. Marine conditioning films. Advances in Chemistry; 145: 319–35.

Maki, J.S. and Mitchell, R.1985. Involvement of lectins in the settlement and metamorphosis of marine invertebrate larvae. Bulletin of Marine Science. 37: 675-683.

Maki, J.S. and Mitchell, R.,1986. The function of lectins in interactions among marine bacteria, invertebrates, and algae. In: *Microbial Lectins and Agglutinins: Properties and Biological Activity*. Mirelman, D., Ed., John Wiley & Sons, Inc., New York, NY, pp 409-425.

Maki, J.S., Yule, A.B., Rittschof, D., and Mitchell, R., 1994. The effect of bacterial films on the temporary adhesion and permanent fixation of cypris larvae, *Balanus amphitrite* Darwin. Biofouling. 8:121-131.

Maki, J.S., Little, B.J., Wagner, P., and Mitchell, R., 1990. Biofilm formation on metal surfaces in antarctic waters. Biofouling. 2: 27-38.

Maki, J.S., Rittschof, D., Schmidt, A.R., Snyder, A.G., and Mitchell, R., 1989. Factors controlling attachment of bryozoan larvae: A comparison of bacterial films and unfilmed surfaces. Biological Bulletin. 177:295-302.

Maki, J.S., Rittschof, D., and Mitchell, R., 1992. Inhibition of larval barnacle attachment to bacterial films: An investigation of physical properties. Microbial Ecology. 23: 97-106.

Maki, J.S., Rittschof, D., Costlow, J.D., and Mitchell, R., 1988. Inhibition of attachment of larval barnacles, *Balanus amphitrite*, by bacterial surface films. Marine Biology. 97: 199-206.

Maki, J.S., Rittschof, D., Samuelsson, M. O., Szewzyk, U., Yule, A.B., Kjelleberg, S., Costlow, J.D., and Mitchell, R., 1990. Effect of marine bacteria and their exopolymers on the attachment of barnacle cypris larvae. Bulletin of Marine Science. 46: 499-511.

Mitchell, R., 1984.Colonization by higher organisms. In: *Microbial Adhesion and Aggregation*. Marshall, K.C., Ed., Springer-Verlag, Berlin, Germany, pp 189-200.

Mitchell, R., and Kirchman, D., 1984. The microbial ecology of marine surfaces. In: *Marine Biodeterioration: An Interdisciplinary Study*, Costlow, J.D. and Tipper, R.C., Eds., Naval Institute Press, Annapolis, MD, pp 49-56.

Mitchell, R., and Maki, J.S., 1988. Microbial surface films and their influence on larval settlement and metamorphosis in the marine environment. In: *Marine Biodeterioration: Advanced Techniques Applicable to the Indian Ocean*. Thompson, M., Sarojini, R., and Nagabhushanam, R., Eds., Oxford & IBH Publishing Co, New Delhi, India, pp 489-497.

Radhakrishnan M.V., and Sugumaran E., 2010. Utilization of Sugarcane Bagasse as a Substrate for Plankton Productivity. Recent Research in Science and Technology, 2(3): 19-22.

Ramesh M.R., Shankar K.M., Mohan C.V., and Varghese T.J., 1999. Comparison of three plant substrates for enhancing growth through bacterial biofilm. Aquacult. Engg., 19: 119–131.

Rodriguez, S.R., Riquelme, C., Campos, E.O., Chavez, P., Brandan, E., and Inestrosa, N.C., 1995. Behavioral responses of *Concholepas concholepas* (Bruquière, 1789) larvae to natural and artificial settlement cues and microbial films. *Biological Bulletin*. 189:272-279.

Schroeder G.L., 1978. Autotrophic and heterotrophic production of microorganisms in intensely manured fishponds and related fish yields. Aquaculture, 14: 303–325.

Tidwell J.H., Coyle S.D., van Arnum A., and Weibel C., 2000. Production response of freshwater prawn, *Macrobrachium rosenbergii* to increasing amounts of artificial substrate in ponds. J. World Aquacult. Soc., 31: 452–458.

Wahab M.A., Azim M.E., Ali M.H., Beveridge M.C.M. and Khan S., 1999. The potential of periphyton-based culture of the native major carp calbaush, *Labeo calbasu* (Hamilton). Aquacult. Res., 30: 409–419.