

**Biofilms-Why good and bad bacteria can't be eliminated in your pond?**  
**By Mathew Vangel**

Let's take a shot at the fables handed down through the years to Koi Pond Enthusiasts around the world. **Number one-** It was told to me that using certain chemicals would devastate my filter and pond of "beneficial bacteria". **Number two-** It was told to me that washing out my filter media with anything but pond water would wash off the "beneficial bacteria". **Number three-** It was told to me that major water changes would eliminate most of the disease pathogens in my pond. Well..... DON'T BELIEVE IT!

**Why it isn't so.....**

Most of these beliefs are based on the idea that all pond microbes are planktonic (free-floating), and that simply is not true. Many of the microbes in your pond have the capability to adhere to surfaces, and actually demonstrate cell-communications to determine which medium to thrive on. Surface-designed Microbial Ponds have the capability to perform all of the functions for a successful Nitrogen Cycle without the use of a filter. Our verification test pond has enough surface area alone to maintain negligible nitrates, nitrites, and ammonia levels with the filter turned off for days. Although, filtration is usually required for fugitive particulate, and waste removal.

I will start explaining why this isn't the case by explaining what a Biofilm is, and that this formation is virtually indestructible. A Biofilm is a community of microorganisms attached to a solid surface. These microbes

(bacteria, fungi, algae, etc.) can form on a solid surface that comes in contact with water such as- pond liners, foam filters, rocks, hoses, piping, human skin, FISH (Slime Coat), plants, pots, etc. It is the leading cause of many nosocomial infections in hospitals growing inside of catheters, and implanted devices. The formation of a Biofilm starts with the adhesion of a microbe to the surface. Some microbes stay attached by proteinaceous appendages referred to as fimbriae (filament type legs). Once a number of fimbriae have glued the microbe to the surface, dislodging the microbe is highly unlikely. They form a spider-web formation on the surface (see below Figure 1-1 and Figure 1-2).

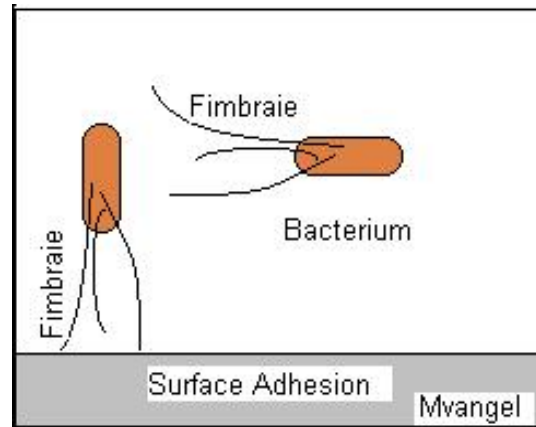


Figure 1-1

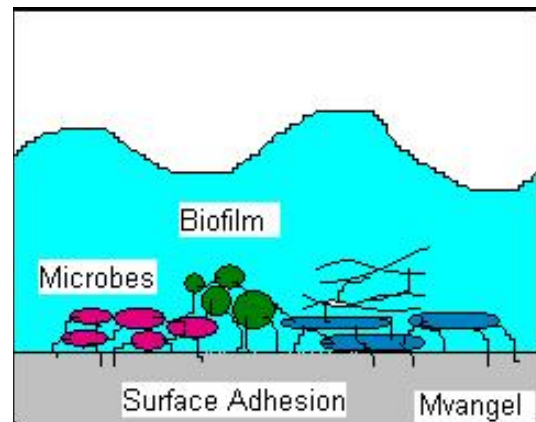


Figure 1-2

The next Biofilm process initiated is the creation of the extra cellular biopolymer consisting primary of polysaccharides and water. But between us lets call it plain old “slime”. This protective environment can exceed the mass of the microbe cell by over 100X, which makes it a very protective environment from the outside world. This is the same substance that may now be coating your teeth as you read this due to the bacteria metabolizing sugar into plaque. The industry has found that Biofilms are so resistant to destruction that in some cases water treatment plants have had to sand-blast the microorganisms from surfaces.

### How does this apply to your pond?

**First-** medications that are commonly used in Koi Ponds to eradicate parasites will not fully penetrate these Biofilms in your filter systems to kill all of the beneficial, or bad bacteria. This includes all of the colonizing bacteria adhering to every hard surface in your pond. The free-floating cells (planktonic) have a much higher kill rate with non-intrusive medication methods than non-suspended cells (Biofilm). The introduced microbial agent has many defenses to overcome in Biofilms. It usually is stopped in the beginning when it becomes depleted to inefficient levels coming into contact with the Biofilm. A certain amount of growing Biofilm layers may be penetrated, or sloughed off during this attempt to kill the microbes that lay beneath. The newly exposed underneath layers begin to grow that previously laid dormant due to the starvation from nutrients. The Biofilm now starts to rebuild its lost layers. Antimicrobial failure of penetration can be seen in Figure 1-3.

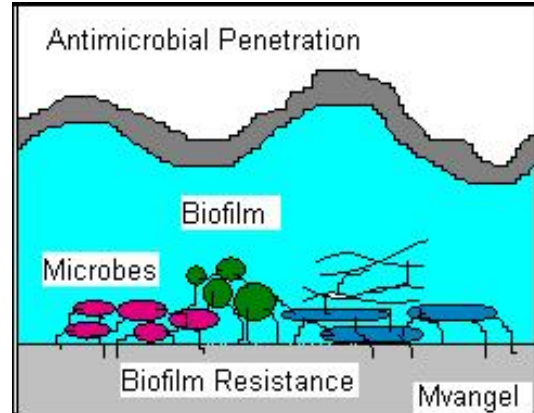


Figure 1-3

The microbial agent may find it impossible to be successfully transported any further into the cellular walls. The most common result is a partial microbe kill. That means you will never positively kill everything you want, or do not want to eradicate. The best you can expect are small Biofilm log kill rates, and large planktonic kill rates. That result most often times is favorable for the hobbyist.

### No Wonder !

Ever contemplate why your pond recovers so fast after water, and disease treatments? The microbes in the Biofilm as we said are almost bulletproof in this sphere of slime. You can punch a few holes into the slime, but almost never fully remove their existence from the face of the EPDM, or in certain fortunate areas of the country “cement ponds”.

Biofilms can stay active for days and even weeks when dry....I mean fully dried out (see below in Figure 1-3).



Figure 1-3 Are Microbes Dead or Alive?

The filter media shown above was dried out for 3-days, and the replaced with no noticeable difference to water quality. If this leads you to believe that dehydration does not kill everything, then you have understood this concept very well. **For example-** Do you know how long Hepatitis B can remain viable in a smear of dried blood? **Answer-** Up to one week where it can be rehydrated back to it's active pathogenic state. Where it is fully capable to infect you through an open sore, as one transport method.

### Summary

Microbes have many methods of survival. Now you know why ponds recover so well after water changes, and why diseases are never fully eradicated due to Biofilm existence in your pond. Biofilms will always be a friend and foe in your aquatic environment. Balancing microbe colony levels are the key to managing a successful ecosystem. My follow-up article will describe the "Best Practices of Disinfection" that you should follow when handling, and cleaning surfaces relating to your hobby.

**Author's Background-** Mathew Vangel is an Advanced Technology Research Engineer, and licensed Medical Researcher in Massachusetts studying

Infectious Disease Control, cross-contamination from aquatic to human environments, blood pathogens, Biofilm Structures, and Endocrinology. For more information and additional article references write to- [info@pondanalysis.com](mailto:info@pondanalysis.com) or [www.pondanalysis.com](http://www.pondanalysis.com)

### References

1. Heukelekian H, Heller A. Relation between food concentration and surface for bacterial growth. *J Bacteriol* 1940;40:547-58.
2. Zobell CE. The effect of solid surfaces on bacterial activity. *J Bacteriol* 1943;46:39-56.
3. Jones HC, Roth IL, Saunders WM III. [Electron microscopic study of a slime layer.](#) *J Bacteriol* 1969;99:316-25.
4. Characklis WG. Attached microbial growths-II. Frictional resistance due to microbial slimes. *Water Res* 1973;7:1249-58.
5. Costerton JW, Geesey GG, Cheng K-J. [How bacteria stick.](#) *Sci Am* 1978;238:86-95.
6. Characklis WG, McFeters GA, Marshall KC. Physiological ecology in biofilm systems. In: Characklis WG, Marshall KC, editors. *Biofilms*. New York: John Wiley & Sons; 1990. p. 341-94.
7. Fletcher M, Loeb GI. Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. *Appl Environ Microbiol* 1979;37:67-72.

8. Pringle JH, Fletcher M. Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. *Appl Environ Microbiol* 1983;45:811–17.
9. Bendinger B, Rijnaarts HHM, Altendorf K, Zehnder AJB. Physicochemical cell surface and adhesive properties of coryneform bacteria related to the presence and chain length of mycolic acids. *Appl Environ Microbiol* 1993;59:3973–77.
10. Loeb GI, Neihof RA. Marine conditioning films. *Advances in Chemistry* 1975;145:319–35.
11. Marsh PD. Dental plaque. In: Lappin-Scott HM, Costerton JW, editors. *Microbial biofilms*. Cambridge: Cambridge University Press; 1995. p. 282–300.
12. Mittelman MW. Adhesion to biomaterials. In: Fletcher M, editor. *Bacterial adhesion: molecular and ecological diversity*. New York: Wiley-Liss, Inc.; 1996. p. 89–127.
13. Ofek I, Doyle RJ. Bacterial adhesion to cells and tissues. In: Ofek I, Doyle RJ, editors. *New York: Chapman & Hall*; 1994.
14. Characklis WG. Microbial fouling. In: Characklis WG, Marshall KC, editors. *Biofilms*. New York: John Wiley & Sons; 1990. p. 523–84.
15. Rijnaarts HH, Norde W, Boucher EJ, Lyklema J, Zehnder. Bacterial adhesion under static and dynamic conditions. *Appl Environ Microbiol* 1993;59:3255–65.
16. Zheng D, Taylor GA, Gyananath G. Influence of laminar flow velocity and nutrient concentration on attachment of marine bacterioplankton. *Biofouling* 1994;8:107–20.
17. Donlan RM, Pipes WO, Yohe TL. Biofilm formation on cast iron substrata in water distribution systems. *Water Res* 1994;28:1497–1503.
18. Fera P, Siebel MA, Characklis WG, Prieur D. Seasonal variations in bacterial colonization of stainless steel, aluminum, and polycarbonate surfaces in a seawater flow system. *Biofouling* 1989;1:251–61.
19. Fletcher M. [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; 1988. p. 31–5.
20. Fletcher M. Attachment of *Pseudomonas fluorescens* to glass and influence of electrolytes on bacterium-substratum separation distance. *J Bacteriol* 1988;170:2027–30.
21. Cowan MM, Warren TM, Fletcher M. Mixed species colonization of solid surfaces in laboratory biofilms. *Biofouling* 1991;3:23–34.
22. Rosenberg M, Kjelleberg S. Hydrophobic interactions in bacterial adhesion. *Advances in Microbial Ecology* 1986;9:353–93.
23. Corpe WA. Microbial surface components involved in adsorption of microorganisms onto surfaces. In: Bitton G, Marshall KC, editors. *Adsorption*

- of microorganisms to surfaces. New York: John Wiley & Sons; 1980. p. 105–44.
24. Rosenberg M, Bayer EA, Delarea J, Rosenberg E. Role of thin fimbriae in adherence and growth of *Acinetobacter calcoaceticus* RAG-1 on hexadecane. *Appl Environ Microbiol* 1982;44:929–37.
  25. Bullitt R, Makowski L. [Structural polymorphism of bacterial adhesion pili](#). *Nature* 1995;373:164–7.
  26. Bashan Y, Levanony H. Active attachment of *Azospirillum brasilense* Cd to quartz sand and to a light-textured soil by protein bridging. *J Gen Microbiol* 1988;134:2269–79.
  27. Danielsson A, Norkrans B, Bjornsson A. On bacterial adhesion - the effect of certain enzymes on adhered cells in a marine *Pseudomonas* sp. *Bot Marina* 1977;20:13–7.
  28. Williams V, Fletcher M. *Pseudomonas fluorescens* adhesion and transport through porous media are affected by lipopolysaccharide composition. *Appl Environ Microbiol* 1996;62:1004.
  29. Marshall KC, Stout R, Mitchell R. Mechanisms of the initial events in the sorption of marine bacteria to surfaces. *J Gen Microbiol* 1971;68:337–48.
  30. Fletcher M, Lessman JM, Loeb GI. Bacterial surface adhesives and biofilm matrix polymers of marine and freshwater bacteria. *Biofouling* 1991;4:129–40.
  31. Beech IB, Gaylarde CC. Adhesion of *Desulfovibrio desulfuricans* and *Pseudomonas fluorescens* to mild steel surfaces. *J Appl Bacteriol* 1989;67:2017.
  32. Zottola EA. Characterization of the attachment matrix of *Pseudomonas fragi* attached to non-porous surfaces. *Biofouling* 1991;5:37–55.
  33. Korber DR, Lawrence JR, Sutton B, Caldwell DE. Effect of laminar flow velocity on the kinetics of surface recolonization by Mot<sup>+</sup> and Mot<sup>-</sup> *Pseudomonas fluorescens*. *Microb Ecol* 1989;18:1–19.
  34. Davies DG, Geesey GG. [Regulation of the alginate biosynthesis gene algC in \*Pseudomonas aeruginosa\* during biofilm development in continuous culture](#). *Appl Environ Microbiol* 1995;61:860–7.
  35. Prigent-Combaret C, Vidal O, Dorel C, Lejeune P. [Abiotic surface sensing and biofilm-dependent regulation of gene expression in \*Escherichia coli\*](#). *J Bacteriol* 1999;181:5993–6002.
  36. Becker P, Hufnagle W, Peters G, Herrmann M. [Detection of different gene expression in biofilm-forming versus planktonic populations of \*Staphylococcus aureus\* using micro-representational-difference analysis](#). *Appl Environ Microbiol* 2001;67:2958–65.
  37. Pulcini E. The effects of initial adhesion events on the physiology of *Pseudomonas aeruginosa* [Ph.D. dissertation]. Bozeman (MT): Montana State University; 2001.
  38. Flemming H-C, Wingender J, Griegbe, Mayer C. Physico-chemical properties of biofilms.

- In: Evans LV, editor. Biofilms: recent advances in their study and control. Amsterdam: Harwood Academic Publishers; 2000. p. 19–34.
39. Sutherland IW. [Biofilm exopolysaccharides: a strong and sticky framework](#). Microbiology 2001;147:3–9.
  40. Hussain M, Wilcox MH, White PJ. The slime of coagulase-negative staphylococci: biochemistry and relation to adherence. FEMS Microbiol Rev 1993;104:191–208.
  41. Leriche V, Sibille P, Carpentier B. [Use of an enzyme-linked lectinsorbent assay to monitor the shift in polysaccharide composition in bacterial biofilms](#). Appl Environ Microbiol 2000;66:1851–6.
  42. Donlan RM. [Role of biofilms in antimicrobial resistance](#). ASAIO J 2000;46:S47–S52.
  43. Tolker-Nielsen T, Molin S. [Spatial organization of microbial biofilm communities](#). Microb Ecol 2000;40:75–84.
  44. Lewandowski Z. Structure and function of biofilms. In: Evans LV, editor. Biofilms: recent advances in their study and control. Amsterdam: Harwood Academic Publishers; 2000. p. 1–17.
  45. Stoodley P, Boyle JD, Dodds I, Lappin-Scott HM. Consensus model of biofilm structure. In: Wimpenny JWT, Gilbert PS, Lappin-Scott HM, Jones M, editors. Biofilms: community interactions and control. Cardiff, UK: Bioline; 1997. p. 1–9.
  46. James GA, Beaudette L, Costerton JW. Interspecies bacterial interactions in biofilms. Journal of Industrial Microbiology 1995;15:257–62.
  47. Tolker-Nielsen T, Brinch UC, Ragas PC, Andersen JB, Jacobsen CS, Molin S. [Development and dynamics of \*Pseudomonas\* sp. biofilms](#). J Bacteriol 2000;182:6482–9.
  48. Durack DT. [Experimental bacterial endocarditis. IV Structure and evolution of very early lesions](#). J Pathol 1975;115:81–9.
  49. Tunney MM, Jones DS, Gorman SP. Biofilm and biofilm-related encrustations of urinary tract devices. In: Doyle RJ, editor. Methods in enzymology, vol. 310. Biofilms. San Diego: Academic Press; 1999. p. 558–66.
  50. Donlan RM. Biofilm control in industrial water systems: approaching an old problem in new ways. In: Evans LV, editor. Biofilms: recent advances in their study and control. Amsterdam: Harwood Academic Publishers; 2000. p. 333–60.
  51. Ehlers LJ, Bouwer EJ. RP4 plasmid transfer among species of *Pseudomonas* in a biofilm reactor. Water Sci Technol 1999;7:163–171.
  52. Roberts AP, Pratten J, Wilson M, Mullany P. Transfer of a conjugative transposon, Tn5397 in a model oral biofilm. FEMS Microbiol Lett 1999;177:636.
  53. Hausner M, Wuertz S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Appl Environ Microbiol 1999;65:3710–13.

54. Ghigo J-M. [Natural conjugative plasmids induce bacterial biofilm development.](#) Nature 2001;412:442–5.
55. Xie H, Cook GS, Costerton JW, Bruce G, Rose TM, Lamont RJ. [Intergeneric communication in dental plaque biofilms.](#) J Bacteriol 2000;182:7067–9.
56. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. [The involvement of cell-to-cell signals in the development of a bacterial biofilm.](#) Science 1998;280:295–8.
57. Stickler DJ, Morris NS, McLean RJC, Fuqua C. [Biofilms on indwelling urethral catheters produce quorum-sensing signal molecules in situ and in vitro.](#) Appl Environ Microbiol 1998;64:3486–90.
58. Yung-Hua L, Lau PCY, Lee JH, Ellen RP, Cvitkovitch DG. [Natural genetic transformation of \*Streptococcus mutans\* growing in biofilms.](#) J Bacteriol 2001;183:897–908.
59. Murga R, Forster TS, Brown E, Pruckler JM, Fields BS, Donlan RM. [The role of biofilms in the survival of \*Legionella pneumophila\* in a model potable water system.](#) Microbiology 2001;147:3121–6.
60. McLaughlin-Borlace L, Stapleton F, Matheson M, Dart JKG. Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. J Appl Microbiol 1998;84:827–38.
61. Stewart PS, Camper AK, Handran SD, Huang C-T, Warnecke M. [Spatial distribution and coexistence of \*Klebsiella pneumoniae\* and \*Pseudomonas aeruginosa\* in biofilms.](#) Microb Ecol 1997;33:2–10.
62. Raad II, Sabbagh MF, Rand KH, Sherertz RJ. Quantitative tip culture methods and the diagnosis of central venous catheter-related infections. Diagn Microbiol Infect Dis 1992;15:13–20.
63. Wirtanen G, Alanko T, Mattila-Sandholm T. Evaluation of epifluorescence image analysis of biofilm growth on stainless steel surfaces. Colloids and Surfaces B: Biointerfaces 1996;5:319–26.
64. Buswell CM, Herlihy YM, Lawrence LM, McGuigan JTM, Marsh PD, Keevil CW, et al. [Extended survival and persistence of \*Campylobacter\* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining.](#) Appl Environ Microbiol 1998;64:733–41.
65. Camper AK, Warnecke M, Jones WL, McFeters GA. Pathogens in model distribution system biofilms. Denver: American Water Works Association Research Foundation; 1998.
66. Hood SK, Zottola EA. [Adherence to stainless steel by foodborne microorganisms during growth in model food systems.](#) Int J Food Microbiol 1997;37:145–53.
67. Watnick PI, Kolter R. [Steps in the development of a \*Vibrio cholerae\* El Tor biofilm.](#) Mol Microbiol 1999;34:586–95.
68. Stark RM, Gerwig GJ, Pitman RS, Potts LF, Williams NA,

- Greenman J, et al. [Biofilm formation by \*Helicobacter pylori\*](#). Lett Appl Microbiol 1999;28:121–6.
69. Gilbert P, Evans DJ, Brown MRW. Formation and dispersal of bacterial biofilms in vivo and in situ. J Appl Bacteriol Symposium Supplement 1993;74:67S–78S.
70. Boyd A, Chakrabarty AM. [Role of alginate lyase in cell detachment of \*Pseudomonas aeruginosa\*](#). Appl Environ Microbiol 1994;60:2355–9.
71. Brading MG, Jass J, Lappin-Scott HM. Dynamics of bacterial biofilm formation. In: Lappin-Scott HM, Costerton JW, editors. Microbial biofilms. Cambridge: Cambridge University Press; 1995. p. 46–63.
72. Characklis WG. Biofilm processes. In: Characklis WG, Marshall KC, editors. Biofilms. New York: John Wiley & Sons; 1990. p. 195–231.
73. Korber DR, Lawrence JR, Lappin-Scott HM, Costerton JW. Growth of microorganisms on surfaces. In: Lappin-Scott HM, Costerton JW, editors. Microbial biofilms. Cambridge: Cambridge University Press; 1995. p. 15–45.
74. Donlan RM. [Biofilms and device-associated infections](#). Emerg Infect Dis 2001;7:277–81.