

BioFil™ Filter

The BioFil is a water quality stewardship tool that provides a unique platform for reducing coliform indicator bacteria and ammonia. The BioFil Filter targets the colloidal particles that cause water turbidity, provide transport to coliform bacteria, heavy metals, and other pollutants, and degrade habitat for fish and other aquatic life. The open water deployment creates microbial habitat in the most oxygenated portion of the water column, enhancing aerobic processes such as nitrification, the oxidation and removal of ammonia.



BioFil Front Views

The standard BioFil is 60” wide and extends 60” into the water column. The filter has panes that can move independently, enhancing self cleaning and also serving as a pressure relief mechanism in high flow events. The panes are designed to have limited side to side movement, maintaining their position. Custom sizes are available.

BIOFILM BASED FILTRATION

The BioFil has a buoyant top that floats at or near the water’s surface and a filter media that extends down vertically into the water column. The filter media has a high degree of tortuosity, an almost endless maze of narrow channels, turns, switchbacks, and dead ends, which are covered with a sticky, adhesive substance, a component of biofilm called extracellular polymeric substances (EPS). When a surface is placed in water, bacteria will immediately move to colonize it by first excreting EPS, providing the initial adhesion of bacteria to the surface in the development of biofilm. In most biofilms, the microorganisms account for less than 10% of the dry mass, whereas EPS can account for over 90% (Flemming 2010).

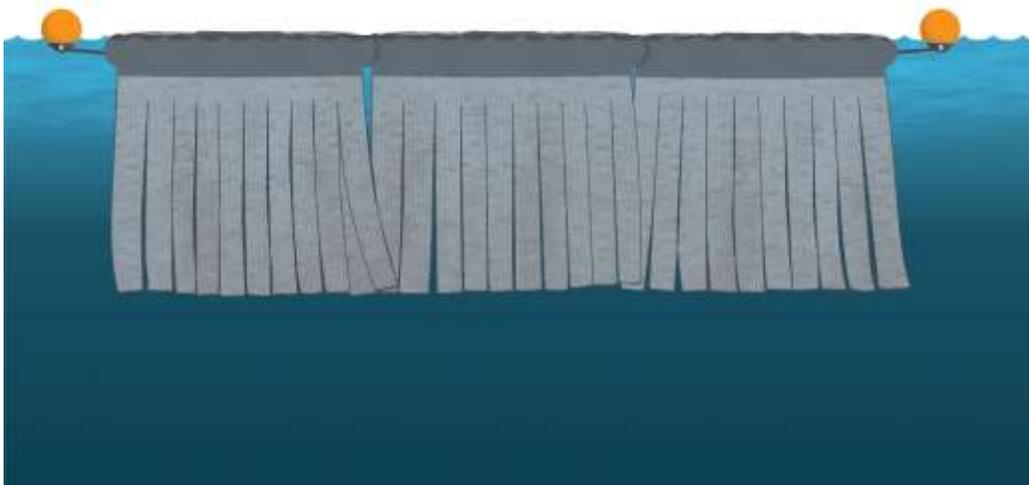
EPS make the hi-tech filter media active in many ways other than just being sticky. EPS contain apolar regions, groups with hydrogen-bonding potential, anionic groups and cationic groups (Hullebusch 2004) and act as a molecular sieve, sequestering cations, anions, apolar compounds and particles from the water (Flemming 2002). Owing to this stickiness of the EPS matrix, particles and nanoparticles can be trapped and accumulated (Flemming 2010).

EPS are mainly polysaccharides, proteins, nucleic acids and lipids; the biofilm matrix acts as an external digestive system by keeping extracellular enzymes close to the cells, enabling them to metabolize dissolved, colloidal and solid biopolymers (Flemming 2010). EPS incorporate both organic compounds and inorganic ions via sorption, and facilitate redox activity in the biofilm-acting as electron donor or acceptor.



BioFil

Biofilms have a self-cleaning/ regeneration ability via sloughing. This anti-fouling process entails the removal of unwanted biofilms after which new biofilms develop. The Biofil's strategically placed slits allow a gentle movement that facilitates mass transfer. The slits allow the filter to “open” in high flow events, acting as a pressure relief mechanism.



Deployable at the surface or just below the surface

COLIFORM BACTERIA

The contamination of surface water bodies by coliform indicator and pathogenic bacteria and the resulting health hazards to human and animal populations are a major problem all over the world. Two dominant factors controlling the survival of these pathogens in the aquatic environment are sunlight and protozoan predation. The BioFil represents a unique platform for reducing pathogenic microorganisms via:

- i) filtration of colloidal particles that cause turbidity and provide transport of pathogenic microorganisms,
- ii) enhanced penetration of sunlight into the water column due to reduced turbidity,
- iii) robust habitat for protozoan predators, and
- iv) wave dampening effect reduces re-suspension of sediments.



Side View

The mechanisms and processes involved in eliminating coliform bacteria in aquatic ecosystems are well documented. Numerous investigators have documented the dominant roles of sunlight (Davies , 1991; Sinton, 1999, 2002; Abhirosh , 2006) and protozoan predators(McCambridge and McMeekin, 1981; Anderson 1983; Barcina 1992; Hahn 2001; Duhamel 2006). When both naturally occurring microbial predators and solar radiation are applied together, they killed significantly more E. coli than the sum of when sunlight and predators are operating independently (McCambridge and McMeekin, 1981).

Colloidal particles, ubiquitous in natural waters, are microscopic particles with size in the range 1 μm to 1 nm (Stumm and Morgan, 1996). Because of their small size, they do not settle out by gravity. The surfaces of these micro particles play a key role in regulating the concentrations of most reactive elements and of many pollutants in natural waters (Stumm and Morgan, 1996). Colloidal particles are commonly called suspended solids.

Increased turbidity from colloidal particles reduces the deactivation rates of UV sterilization on pathogenic organisms by restricting the depth that sunlight can penetrate. Colloidal particles often contain and provide transport for coliform bacteria and other pathogens. Microorganisms attached to suspended particles and sediment are the main contributor for the elevated level of bacteria in the surface water. (Davies 1995; Crump and Baross, 1996)

Stormwater runoff is a major contributor to the transport of pathogens from urbanized watersheds to surface waters. A number of stormwater best management practices (BMPs) have been established to achieve total maximum daily load limits for indicator bacteria. One such BMP is wet ponds. A number of studies have shown poor wet pond removal of indicator bacteria and that effluent concentrations may exceed inflow concentrations (Davies 2000, Hathaway 2009). The poor performance of the wet ponds in removing indicator bacteria was attributed to poor removal of fine clay particles, to which the bacteria were “predominately absorbed.”(Davies 2000)

A CalTran report noted, “a central conundrum of roadway runoff BMP development is that the bioavailability, toxicity, and mobility of contaminants generally decrease with particle size while the ability of BMPs to remove contaminants from water generally increases with particle size”(Grant 2003). Said another way, BMPs are generally designed for large particles and the majority of the pollutants are small particle associated. The BioFil is an economical, efficient, and easy to use solution to the many issues associated with colloidal particles in stormwater ponds and other water bodies.

Reduction of suspended solids significantly improves habitat for fish and other aquatic life.
Reduction of coliform bacteria contributes to a safe growing environment for shellfish farming.

Ponds and lakes used for recreation often suffer discoloration from fine clay particles in storm water runoff. The filter is useful in restoring and maintaining the aesthetic and recreational water qualities of water bodies.

Sunlight Effects on Coliform Bacteria

Sunlight is considered the most important factor in bacteria inactivation. By reducing turbidity, the BioFil greatly expands the depth to which sunlight can kill coliform bacteria. The bactericidal action of sunlight in water is dependent on dissolved oxygen levels (Curtis 1992; Reed, 1997). Photons of light are absorbed by photosensitizers, which become electronically excited and react with neighboring oxygen molecules leading to the production of highly reactive oxygen species, which react with DNA causing both strand breakage and base changes (Acra 1990). The 320 to 280 nm component of light inflicts direct photobiological DNA damage. Photochemical mechanisms are dominant in the 400 to 320 nm range.

Protozoan Predation

Periphytic biofilms, such as the ones that grow on the BioFil, contain abundant numbers of protozoa that prey on coliform bacteria. The role of protozoan grazing as a dominant factor regulating the bacterial population in aquatic environments is well documented (McCambridge and McMeekin, 1981; Anderson 1983; Barcina 1992; Hahn and Hofle, 2001; Duhamel 2006).

Flagellates and ciliates are considered the most significant protozoan predators targeting bacterial populations in aquatic ecosystems (Pace, 1998; Alonso et al., 2000; Wcislo and Chrost, 2000). Due to their mobility and generally high abundance, protozoa can track down 'hot spots' of bacterial growth.

Sediment Resuspension

Aquatic sediments are a well-recognized reservoir for coliform bacteria. Due to the accumulation of pathogens in bottom sediments, resuspension of sediments can result in coliform contamination of the overlying surface waters. Resuspension may be caused by storm water events or wave action. The Biofil both dampens waves and acts as a buffer in high flow environments thus inhibiting resuspension (Huang 2007).



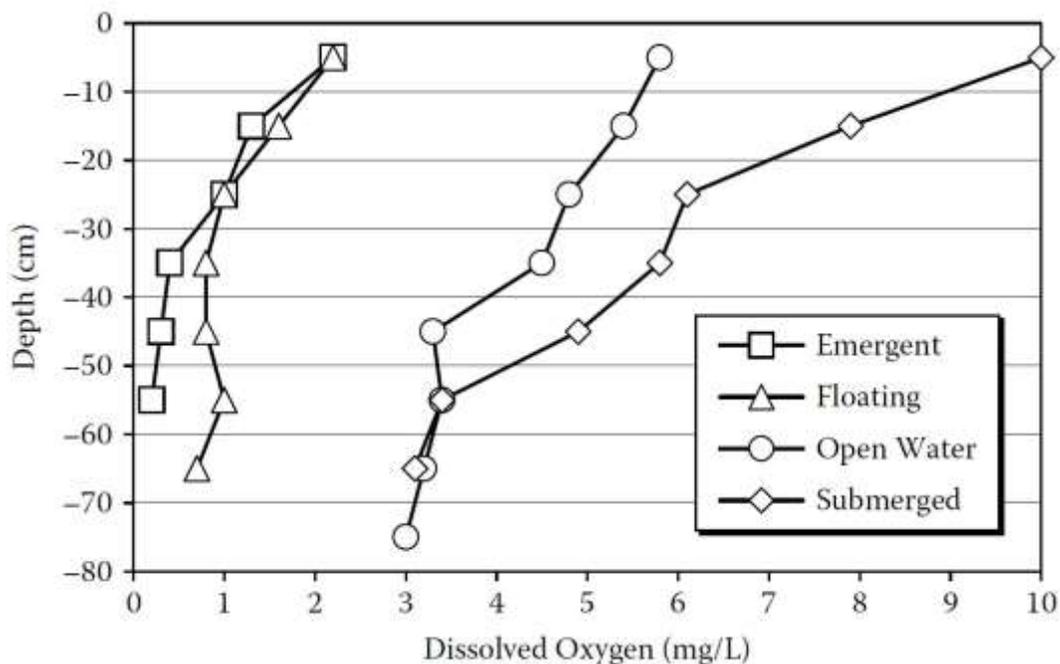
Reduces Erosion

REMOVAL OF AMMONIA

The BioFil creates microbial habitat in the most oxygenated portion of the water column, enhancing aerobic processes such as nitrification, the oxidation and removal of ammonia. The following outlines the relationship between dissolved oxygen levels and nitrification rates, demonstrating that the BioFil's design aligns its microbial habitat with the basic science for nitrification.

Nitrification is a two step process, where ammonia is first oxidized to nitrite and then nitrite is oxidized to nitrate. A biofilter requires three things in order to function: habitat for nitrifying bacteria, a substrate (ammonia), and oxygen. Nitrifying bacteria are considered autotrophic bacteria, or bacteria that utilize CO₂ as their carbon source for growth. During nitrification, 4.33 mg O₂ is consumed for every mg of ammonia oxidized to nitrate (Grady 1999).

The BioFil vertical deployment places nitrifying bacteria in the most favorable oxygen conditions in the water column. Vertical profiles of dissolved oxygen levels in various types of Free Water Surface wetlands (Dodkins 2014, Chimney 2006, Kadlec & Wallace 2009) indicated that DO levels in open water range from approximately 6.0 mg/l at 5 cm below the water surface to approximately 3.0 mg / l at 75 cm below the surface. The BioFil further supplements the DO levels via oxygen generated by photosynthesis in its periphytic algal community.



Vertical profiles of dissolved oxygen in various types of FWS (Free Water Surface) wetlands, Florida. Data from 141 profiles collected over a 2½ year period. Data from Chimney et al. (2006), Figure from Kadlec & Wallace (2009). FTWs are most readily compared to floating plant systems. Figure 3 from Dodkins 2014 page 12.

While nitrification may occur at lower dissolved oxygen (DO) levels, the availability of adequate DO for the nitrification processes has a direct effect on removal efficiency. Ammonia oxidation becomes inhibited at DO <2.0 mg/l and nitrite oxidation becomes inhibited at DO <2.5 mg/l (Paredes, 2007). The optimal dissolved oxygen concentration for growth of nitrifying bacteria is in the region of 3-4 mg / l (Prosser, 1989). Note that the optimal DO range for nitrifier growth overlaps the open water DO levels in the 5cm to 75 cm depth range (Dodkins 2014, Chimney 2006, Kadlec & Wallace 2009).

The essential nutrients for nitrifying bacteria within the biofilm are ammonia and oxygen, which are supplied by a diffusive process, thus the nitrification rate is dependent on the diffusion rates of ammonia and oxygen (Saucier). (Riley 2001) reported that higher DO levels enhance nitrite production by the activation of dormant nitrifiers deeper in the biofilm, based on increased oxygen diffusion into the biofilm at higher DO levels. In order for the dormant nitrifiers to function, both oxygen and ammonia must reach them via diffusion into the biofilm, which allows one to relate oxygen concentration to ammonia concentration. Models using stoichiometry and diffusion coefficients indicate that oxygen is the limiting substrate if the oxygen to ammonia concentration ratio in the bulk liquid is less than 2.72(Saucier; Tanaka and Dunn 1982). Oxygen to ammonia ratios greater than 3 mg O₂/ mg NH₄-N are necessary before nitrification rate stops increasing (Tanaka and Dunn, 1982).

Wheaton (1989) reported that in activated sludge systems, DO concentrations of approximately 4 mg/liter were found to remove 80% of ammonia in a 6-hour detention time, while 2 mg O₂/liter removed only 40%.

As noted above, nitrite oxidation becomes inhibited at a higher DO level than ammonia oxidation inhibition DO levels, (< 2.5 mg /l versus < 2.0 mg/l). Consequently, low oxygen conditions can result in nitrite (NO₂-) being produced instead of completing the process toward nitrate. The consequence of this is that in the later denitrification stage, some of the nitrite is converted into nitrous oxide (N₂O), a potent greenhouse gas. Aboobakar (2012) found that combined nitrous oxide emissions when nitrification occurred at DO levels of 1.0 mg /l and below were 3.5 times greater than emissions at DO levels of 2.0 mg /l and above.

The BioFil does not have plants, which is beneficial to nitrification in two ways. The absence of plant litter means oxygen is not consumed by microbial decomposition activities, thus making more oxygen available to nitrifiers. Secondly, the lack of organic carbon food source from plant litter inhibits fast growing heterotrophic bacteria from dominating slow growing autotrophic nitrifying bacteria. While plants are beneficial in many ways, the absence of plants in the BioFil establishes a microbial habitat that favors autotrophic nitrifiers over heterotrophic bacteria.

COMPLETE NITROGEN REDUCTION SOLUTION

It is well understood that aerobic/anoxic zones may exist within a biofilm, allowing both nitrification and denitrification to occur in the same environment. While the the BioFil supports denitrification at some level, the fact that it has the optimum conditions for the aerobic nitrifying processes also means it is not the optimum tool for the anaerobic denitrification processes. For total nitrogen removal, pairing BioHaven islands with the BioFil creates a complete solution based on optimum efficiencies for both processes. BioHaven islands create an anoxic zone based on blocking diffusion of oxygen from the air/water interface and by blocking oxygen generation from algal photosynthesis. Oxygen consumption by microbial metabolism associated with the islands further reduces DO, creating the anoxic environment required for nitrate removal (Dodkins 2014). The other requirement for nitrate removal is a source of organic carbon, which is supplied by the litter form the island plants. Thus, the BioFil Filter and BioHaven islands create a synergistic pairing for highly efficient total nitrogen reduction.

Ammonia exists in equilibrium between its dissolved ammonium form (NH₄⁺) and its gaseous form (NH₃). While ammonia loss as gas is negligible at lower pH, losses due to volatilization can become significant at a pH around 9.3 (Vymazal, 2007). N removal rates due to ammonia volatilization have been measured at 2.2 g N/m²/d in wetlands (Stowell et al., 1981). Increased pH from Algal photosynthesis associated with the BioFil potentially enhances ammonia volatilization.

References

- Abhirosh, C., Hatha, A.A.M. 2005. Relative survival of *Escherichia coli* and *Salmonella typhimarium* in a tropical estuary. *Water. Res.* 39: 1397-1403.
- Aboobakar A, Cartmell E, Stephenson T, Jones M, Vale P, Dotro G., 2013. Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant. *Water Res.* 2013 Feb 1;47(2):524-34.
- Acra, A., Jurdi, M., Muallem, H., Darahagopian, Y., Raffoul, Z. 1990. Water disinfection by solar radiation: assessment and applications. Ont., Canada: International Development Research Center.
- Alonso, M.C., Rodriguez, V., Rodriguez, J., Borrego, J.J. 2000. Role of ciliate, flagellates and bacteriophages on the mortality of marine bacteria and on dissolved DNA concentration in laboratory experimental systems. *J. Exp. Mar. Bio. Eco.* 244: 239-252.
- Anderson, J.C., Rhodes, M.W., Kator, H.I. 1983. Seasonal variation in the survival of *E. coli* exposed in situ in membrane diffusion chambers containing filtered and nonfiltered estuarine water. *Appl. Environ. Microbiol.* 45: 1877-1883.
- Barcina, I., Arana, I., Astorga, A.F., Iriberry, J., Egea, L. 1992. Survival strategies of plasmid-carrier and plasmidless *Escherichia coli* strains under illuminated and non-illuminated conditions, in a fresh water ecosystem. *J. Appl. Bacteriol.* 73: 229-236.
- Chimney, M. J., L. Wenkert, & K. C. Pietro, 2006. Patterns of vertical stratification in a subtropical constructed wetland in south Florida (USA). *Ecological Engineering* 27: 322–330.
- Crump B and Baross J. 1996. Particle-attached bacteria and heterotrophic plankton associated with the Columbia River estuarine turbidity maxima. *Mar. Ecol. Prog. Ser.* 138: 265-273.
- Curtis, T.P., Mara, D.D., Silva S.A. 1992. Influence of pH, oxygen, and humic substances on ability of sun- light to damage faecal coliforms in waste stabilization pond water. *Appl. Environ. Microbiol.* 58: 1335-1343.
- Davies CM, Long J, Donald M, Ashbolt N. Survival of Fecal Microorganisms in Marine and Freshwater Sediments. *Applied and Environmental Microbiology*, May 1995, p. 1888–1896 Vol. 61.

- Davies, C.M., Bavor, H.J. 2000. The fate of storm water associated bacteria in constructed wetland and water pollution control pond systems. *J. Appl. Microbiol.* 89: 349-360.
- Davies, C.M., Evison, L.M. 1991. Sunlight and the survival of enteric bacteria in natural waters. *J. Appl. Bacteriol.*
- Dodkins I, Mendzil AF, 2014. Floating Treatment Wetlands (FTWs) in Water Treatment: Treatment efficiency and potential benefits of activated carbon. Sustainable Expansion of the Applied Coastal and Marine Sectors (SEACAMS)
- Duhamel, S., Domaizon-Pialat, I., Personnic, S., Jacquet, S. 2006. Assessing the microbial community dynamics and the role of bacteriophages in bacterial mortality in Lake Geneva. *Revue des Sciences de l'Eau* 19: 115-126.
- Flemming, H.-C. & Leis, A. in *Encyclopedia of Environmental Microbiology* (ed. Bitton, G.) 2958–2967 (Wiley, New York, 2002).
- Flemming H-C and Wingender J. The Biofilm Matrix. *Nature Review- Microbiology*; Volume 8, September 2010, 623 – 633
- Grady, C. P. L., Daigger, G. T., and Lim, H. C. (1999). *Biological Wastewater Treatment.*, 2nd edition. Ed., Basel. Marcel Dekker, Inc., New York.
- Grant SB, Rekh NV, Pise NR, Reeves RL, Matsumoto M, Wistrom A, Moussa L, Bay S, Kayhanian M. A Review of the contaminants and toxicity associated with particles in stormwater runoff, California Department of Transportation, August 2003
- Hathaway, J., Hunt, W., and Jadlocki, S. (2009). "Indicator Bacteria Removal in Storm-Water Best Management Practices in Charlotte, North Carolina." *J. Environ. Eng.*, 135(12), 1275–1285.
- Hahn MW, Höfle MG. Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiol Ecol.* 2001 Apr; 35(2):113-121.
- Horn, H. 1994. Dynamics of a Nitrifying Bacteria Population in a Biofilm Controlled by an Oxygen Microelectrode. *Water Science Technology* 29(10-11):69-76.
- Huang P, Han B, Liu Z. Floating-leaved macrophyte (*Trapa quadrispinosa* Roxb) beds have significant effects on sediment resuspension in Lake Taihu, China, Eutrophication of Shallow Lakes with Special Reference to Lake Taihu, China Developments in Hydrobiology Volume 194, 2007, pp 189-193
- Kadlec, R. H., & S. D. Wallace, 2009. *Treatment Wetlands* (2nd Edition). CRC Press, Taylor & Francis, Boca Raton, Florida, 1016 pp.

- McCambridge, J., McMeekin, T.A. 1981. Effect of solar radiation and predacious microorganisms on faecal and other bacteria. *Appl. Environ. Microbiol.* 41:1083-1087.
- McHarness, D., Haug, R., and McCarty, P. 1975. Field Studies of Nitrification with Submerged Filters. *Journal of the Water Pollution Control Federation* 47(2):291-309.
- Pace, M.L. 1988. Bacterial mortality and the fate of bacterial production. *Hydrobiologia* 159: 41-49.
- Paredes D, Kusch P, Mbwette TSA, Strange F, Muller RA, Koser H, 2007. New aspects of microbial nitrogen transformations in the context of wastewater treatment- a review. *Eng Life Sci.* 7 (1), 13 – 25
- Prosser JI. Autotrophic nitrification in bacteria. *Advanc Microbiol Physiol* 1989; 30: 125-81.
- Reed, R.H. 1997. Sunshine and fresh air: a practical approach to combating waterborne disease. *Waterlines.* 15: 295-296.
- Riley, J., and D. Hagopian. 2001. Oxygen supplementation for aquaculture biofilters. *Maine Agricultural and Forest Experiment Station Technical Bulletin* 179.
- Saucier P, Zhu S, and Chen S. Nitrification Potential and Oxygen Limitation in Biofilters. Department of Biological Systems Engineering Washington State University, Pullman, WA 99164 USA. U.S. EPA's Development of Aquaculture Effluent Limitation Guideline Regulations and the Role of the Joint Subcommittee on Aquaculture and Other Stakeholders.
- Sinton, L.W., Finaly, R.K., Lynch, P.A. 1999. Sunlight inactivation of faecal bacteriophages and bacteria in sewage polluted seawater. *Appl. Environ. Micro- biol.* 65: 3605-3613.
- Sinton LW, Hall CH, Lynch PA, Davies-Colley RJ. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. *Appl Environ Microbiol.* 2002 Mar;68(3):1122-31.
- Stowell, R., R. Ludwig, & J. Colt, 1981. Concepts in aquatic treatment system design. *Journal of Environmental Engineering (ASCE)* 107: 919–940
- Stumm, W., Morgan, J.J., 1996. *Aquatic chemistry.* John Wiley and Sons. New York.
- Tanaka H, Dunn IJ. Kinetics of biofilm nitrification. *Biotechnol Bioeng.* 1982 Mar; 24(3):669-89.
- Van Hullebusch, E. D., Zandvoord, M. H. & Lens, P. N. L. Metal immobilization by biofilms: mechanisms and analytical tools. *Rev. Environ. Sci. Biotechnol.* 2, 9–33 (2004).
- Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. *The Science of the total environment* 380: 48–65

Wcisło, R., Chróst, R.J. 2000. Survival of *Escherichia coli* in freshwater. *Pol. J. Environ. Stud.* 9: 215-222.

Wheaton, F, Hochheimer J, and Kaiser G. 1989. *Biological Filters for Aquaculture*. Scientific article number A-4904. Contribution number 7946. Maryland Agricultural Experiment Station, University of Maryland, College Park