Performance of KDF® 55 Filters for Control of Bacteria Growth

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Introduction

Bacteria can occur as free living organisms in aquatic and terrestrial ecosystems or as monolayers or biofilms attached to solid substrates. Adhesion of bacteria to surfaces and the development of attached communities are common survival phenomena. Attachment to solids is controlled by physiochemical interactions between the bacteria surface and the substrate. There are two forms of adhesion, reversible; those controlled by VanderWaals forces and irreversible, hydrophobic interactions, dipole moments and H-bonding. Several environmental factors influence bacteria attachment including; pH, electrolyte species and concentration and the presence of heavy metals. This may be through modifying the physiochemical interactions or through shifts in bacterial activity and growth. The USEPA has listed KDF 55 process medium as a “Pesticidal Device.” This means that the medium controls but does not eliminate bacterial populations. There is evidence that there are several mechanisms behind the medium’s bacterial control properties. These are theorized to be; ORP shift, radical and hydrogen peroxide formation and zinc dissolution into the effluent water.

Bacterial Physiology

Several hypotheses were put forward as to how the medium controls bacterial populations. The central theme of these is that the electrochemical reactions described previously are involved in many ways. Before these mechanisms can be elaborated on a general understanding of bacteria must be established as to their structure and metabolism.

Bacterial Structure

Structurally bacterial cell consists of the following:

1. A cell membrane usually surrounded by a cell wall and sometimes by an outer membrane.
2. An internal cytoplasm with ribosomes, a nuclear region, granules and vesicles.
3. A variety of external structures, such as capsules, flagella and pilli.

The semirigid cell wall lies outside the cell membrane and is present in nearly all bacteria and performs two functions. First, it maintains the characteristic shape of the cell. Second, it prevents the cell from bursting when fluids flow into the cell by osmosis. Though the cell wall surrounds the cell membrane it is extremely porous and does not play a major role in regulating the entry of materials into the cell. The cell membrane, on the other hand, is a dynamic, constantly changing entity that forms a boundary between a cell and its environment and regulates what moves into and out of a cell by transport mechanisms. In bacteria the membrane also performs certain functions such as synthesis of cell wall components, DNA replication, secretion of proteins, respiration, and captures energy in ATP.

Bacterial Metabolism

Metabolism is the sum of all the chemical processes carried out by a living organism. It

includes anabolism, reactions that require energy to synthesize complex molecules and catabolism, reactions that release energy by breaking complex molecules. Anabolism is needed for growth, reproduction and repair of cellular structures. Catabolism provides organisms with energy for its life processes including movement, transport, and anabolism.

All catabolic reactions involve electron transfer that allows energy to be captured in high-energy bonds in ATP and similar molecules. Electron transfer is directly related to oxidation and reduction. When substances lose electrons, oxidation, energy is released, but at the same time another substance must gain electrons, be reduced. The various ways that bacteria capture energy are classified as; autotrophic, self-feeding, and heterotrophic, other feeding. These two classifications include the sub groups of phototrophs that obtain energy from light and chemotrophs that obtain energy by breaking down chemical substances either organic or inorganic. Nearly all the infectious microorganisms are chemoheterotrophs. Metabolic processes in chemoheterotrophs include; glycolysis, fermentation, and aerobic respiration. Glycolysis and fermentation do not require oxygen as an electron acceptor and transfers only a small amount of the energy in a glucose molecule to ATP. Aerobic respiration does require oxygen as an electron acceptor and captures a relatively large amount of the energy in a glucose molecule in ATP.

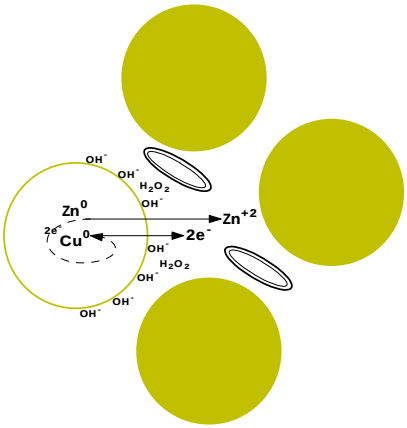
Electron Acceptor Consumption

Redox reactions affect the speciation and mobility of dissolved constituents of water, especially metals and organic compounds. Microbially mediated redox processes proceed sequentially so that electron donors and acceptors are converted to final product with transient intermediate products being formed [Chapelle, et al., 1995]. The patterns of electron acceptor consumption and final product accumulation do not always unequivocally define the distribution of redox processes due to continuous replenishment of the acceptor. For example, during the initial steps of anaerobic decomposition of organic matter a wide variety of fermentive microorganisms produce H_2. As rapidly as H_2 is produced, respiratory microorganisms that use oxidized compounds as terminal electron acceptors consume it. The most notable electron acceptors in aquatic environments are oxygen, manganese dioxide, nitrate, ferric iron, sulfate and carbon dioxide.

Microbial Control Mechanisms

Contact with the Medium Surface

KDF 55 process medium produces up to a 500-mV drop in the oxidation-reduction potential (ORP) as measured in the medium bed [Wireman, 1990]. This is caused by the previously discussed galvanic cell formation the by-product of which is negatively charged electrons being made available at the medium's surface. Bacteria that directly contacting these electrons could have their cellular structures



disrupted through depolarization. Also, as water molecules are involved in the initial galvanic reaction through dissociation into hydroxide ions, a concentration gradient is formed at the interface. The concentration of water in the bacterial cell would be greater than that surrounding the medium resulting in rapid diffusion leading to lysis, rupture, of the bacterial cellular membrane.

Radicals and Hydrogen Peroxide

Studies conducted at the University of Notre Dame in South Bend, Indiana, on the ability of KDF process medium to reduce iron concentrations of water showed that hydroxyl radicals and hydrogen peroxide are produced during the oxidation of ferrous iron to ferric iron by the medium [Schmelling and Gray, 1994]. Other research also indicates that oxidation reactions involving dissolved oxygen in groundwater systems may include an intermediate product such as hydrogen peroxide [Barcelona, et al., 1989]. This would help explain the media's ability to control organisms that lack catalase, the obligate anaerobes, but not the coliforms. Also, due to the reactivity of hydrogen peroxide and radicals, this effect would only be evident in the medium bed and any residual affect in effluent waters would be minimal.

Zinc Intolerance (Toxicity)

Bacteria that are zinc intolerant will be impacted by the release of zinc by the medium. Intolerance for a metal ion is dependent upon whether or not an organism has an active transport mechanism to regulate its concentration within its cellular structure. Zinc, and other heavy metals denature proteins through the disruption of hydrogen and disulfide bonds resulting in the functional shape of the protein being destroyed [Creager, et al., 1990]. Since much of a cell as well as all its enzymes are proteins the regulation of heavy metals into and out of cells is important. In the instance of KDF process medium, the zinc concentrations may not be sufficient to permanently denature proteins, bactericidal, but only temporarily, bacteriostatic. Again, different species will have different levels of tolerance therefore not all bacteria are effected equally.

As other studies have shown a residual affect of the medium on bacterial populations the actions of zinc as an enzyme inhibitor may have to be taken into consideration as a primary factor [Wireman, 1990]. Since one of the similarities in the coliforms is that they are mixed acid fermentors, it is likely that zinc may interfere with this process as an enzyme inhibitor. Enzyme inhibition takes two forms, competitive and noncompetitive. As a competitive inhibitor the zinc would bind to an enzyme at an allosteric site blocking the normal substrate from attaching to the enzyme thus stopping its further reaction. Substrates are the substances upon which enzymes react forming products organisms use for their metabolism. Noncompetitive inhibition would involve the zinc ion binding in a place other than the allosteric site that then changes the shape of the enzyme enough to inhibit the normal substrate from attaching to the enzyme.

Affects on Attachment

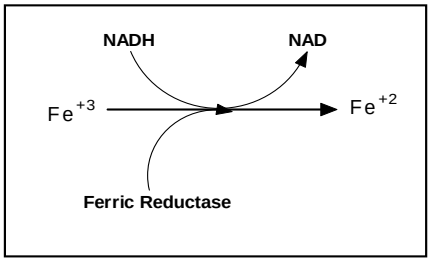
Zinc has been shown in at least one study to lower the ability of bacteria to attach themselves to substrate [McEldowney, 1994]. The outer membrane of gram-negative bacteria, of which coliforms are a member, is structurally dependent on metallic cations. Metal binding to bacteria surfaces can influence attachment physiochemistry through

polymer condensation and increased water structure at the surface causing greater hydrophobicity [McEldowney 1994]. Normally increasing the hydrophobicity of the cell surface would lead to a higher degree of attachment. However, zinc binding to the surface causes an increase in the anionic binding capacity leading to compaction of the diffuse electric double layer [McEldowney, 1994]. This in effect decreases bacterial attachment through modification of cell surface physiochemistry. Once the spatial distribution of bacteria between the solid, attached, and liquid, free, phases stress is created in the population leading to a crash in total bacterial counts [McEldowney, 1994].

Depletion of Electron Acceptors

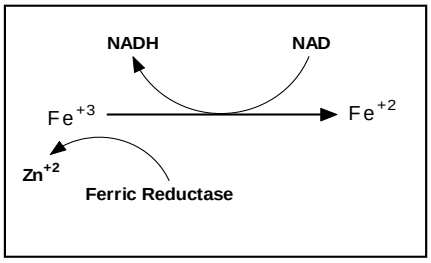
Each reaction involving an electron acceptor requires a single type of bacteria to carry it out. As long as the acceptor is present in the water the population of the bacterial species that uses it flourishes. Once the acceptor is used up, that particular species dies out and another takes its place depending upon availability of acceptors.

Normal Electron Transfer



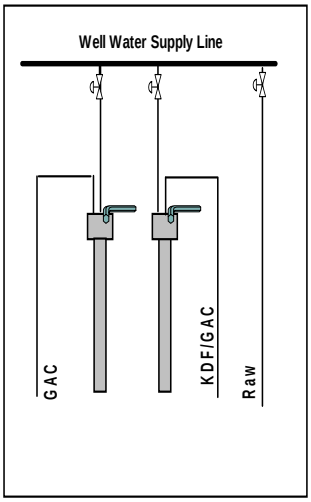
Zinc ions have been proven to have an inhibitory effect on bacterial activity during cell growth in Azobacter vinelandii [Huyer and Page, 1989]. This is by acting as a mixed type inhibitor of the enzyme ferric reductase. Ferric reductase is an enzyme used by some bacteria in electron transfer reactions for the production of energy by the conversion of NADH to NAD. Zinc in effect prevents this conversion from occurring. This inhibition is illustrated in the diagram below on the left.

Zinc Inhibited Electron Transfer



Experimental

Test Apparatus



The diagram and picture on the left illustrate the apparatus designed to test the effectiveness of filters for bacterial control. A challenge water supply conforming to ANSI/NSF 42-1988 Standards feeds the filters, one containing a combination of KDF 55 process medium and carbon (KDF/GAC) and one containing carbon alone (GAC). The same water supply line feeds both filters. Two sets of filters were tested. The discharge lines for the

filter and the raw supply line empty into a basin and water samples for testing were drawn directly from these discharge lines. A timer was used to cycle the filters for 90% off 10% on for 16-hours per day with an 8-hour per day rest period. Samples for testing were drawn at the end of the 8-hour rest period and represents the volume of water contained within the filters. The raw sample grabbed represents the majority of the volume of stagnant water in the test lines prior to the on-cycle. The test filters were run at a flow rate of 0.50-gpm that calculates to 45-gallons per day with the on-off cycle used. The following is a list of the analytes measured during testing: total heterotrophic bacterial, zinc (Zn), and copper (Cu).

Bacteriological Examination

Membrane Filtration (MF) techniques, HACH Method 8242, using m-TGE broth with TTC indicator was used to determine total heterotrophic bacteria. This method is USEPA approved and requires that no additional confirmation tests be performed.

The water samples generated were filtered through a 0.45 μm cellulose membrane and then transferred to petri dishes containing the prepared broth suspended on an absorbent pad. Once the petri dishes were prepared they were then incubated at 35 ± 0.5 ^{o}C for 48-hours. At the end of the incubation periods all colonies on the pads were counted and reported as total colonies per 200-mL. All effort was made throughout testing to conform to quality assurance practices as outlined in Standard Method 18th Edition Part 9000.

Data/Results

Bacteria (Colonies/200mL)						
	Filter Set #1			Filter Set #2		
Gallons	Raw	KDF/GAC	GAC	Raw	KDF/GAC	GAC
0	469	50	1088	800	35	1080
250	413	63	1320	1440	743	1390
500	1270	76	1400	1200	960	1560
750	1540	68	2550	1320	980	2400
1000	1080	426	2000	1440	840	2440
1250	1800	619	2200	1320	936	2640
1500	1200	600	3460	724	488	1200
Mean	983	159	1864	1136	513	1
ANSI/NSF 42-1988 Sec. H Effluent Mean not to Exceed Influent Mean with 20% precision						

The data presented above helps support the claims of KDF Fluid Treatment Inc., that KDF 55 process medium is bacteriostatic as described in ANSI/NSF 42-1988. This description states that a bacteriostatic unit is a drinking water treatment unit designed to limit the passage and/or growth of heterotrophic bacteria so that the bacterial population is no higher in the effluent than in the influent. Section H of this standard defines acceptance as: the geometric mean of the heterotrophic plate counts of the effluent samples from each test unit shall be no greater than the geometric mean of the

heterotrophic plate counts of the influent samples within a measurement precision of 20 percent. Section H goes on to state that an active agent (or it's degradation product) if any shall not be at a level of toxicological significance. Zinc and copper elution from the filters, the degradation products, are within the USEPA set MCLs of 5.00 mg/l and 1.30 mg/l respectively.

Referenced Documents

Standard Methods:

APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater; 18th Edition; APHA: Washington D.C., 1992.

HACH Company; Water Analysis Handbook, 3rd Edition; HACH Co: Loveland, 1997.

NSF International Standards:

ANSI/NSF 42-1988 Drinking Water Treatment Units-Aesthetic Effects.

Independent Studies:

Barcelona, M.J.; Holm, T.R.; Schock, M.R.; George, G.K.; Spatial and Temporal Gradients in Aquifer Oxidation-Reduction Conditions. Water Resour. Res. 1989, 25 (5), 991-1003.

Chapelle, F.H.; McMahon, P.B.; Dubrovsky, N.M.; Fujii, R.F.; Oaksford, E.T.; Vroblesky, D.A.; Deducing the Distribution of Terminal Electron-Accepting Processes in Hydrologically Diverse Groundwater Systems. Water Resour. Res. 1995, 31(2), 359-371.

Creager, J.G.; Black, J.G.; Davison, V.E.; Microbiology: Principles and Applications. Prentice Hall: New York, 1990.

Dean-Ross, D; Response of Attached Bacteria to Zinc in Artificial Streams. Can. J. Microbiol. 1990, 36, 561-566.

Huyer, M.; Page, W.J.; Ferric Reductase Activity in Azobacter vinelandii and Its Inhibition by Zn^{2+} Journal of Bacteriology. 1989, 171, 4031-4037.

McEldowney, S.; Effects of Cadmium and Zinc on Attachment and Detachment Interactions of Pseudomonas fluorescens H2 with Glass. Applied and Environmental Microbiology 1994, 171, 4031-4037.

Schmelling, D.C. and Gray, K.A. Laboratory Report. University of Notre Dame: South Bend, 1994.

Graphics and Statistics Software:

Column Statistics were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA.

WATER ANALYSES

SAMPLE IDENTIFICATION	
Client	KDFFT Well Water
Date Received	06/01/00
Technician	HM
Date Analyzed	06/01/00

TESTING PARAMETERS

Test	Method	EDL	Results	Units
PH	SM 18th 4500 B	NA	7.56	pH units
Conductivity	SM 18th 2510 B	NA	573	μS/cm
TDS	SM 18th 2510 B	NA	279	mg/l Total Ionic
Total Alkalinity	SM 18th 2320 B	10.00	289	mg/l as $CaCO_3$
Bicarbonate Alkalinity	SM 18th 2320 B	10.00	289	mg/l as $CaCO_3$
Carbonate Alkalinity	SM 18th 2320 B	10.00	0	mg/l as $CaCO_3$
Total Hardness	Calculated	10.00	270	mg/l as $CaCO_3$
Calcium	SM 18th 3500 Ca B	13.00	61.44	mg/l Ionic
Magnesium	SM 18th 3500 Mg B	0.190	28.26	mg/l Ionic
Sodium	SM 18th 3500 Na B	1.700	13.07	mg/l Ionic
Potassium	HACH Method 8049	NA	0.77	mg/l ionic
Iron, Total	SM 18th 3500 Fe B	0.020	0.215	mg/l Ionic
Copper	SM 18th 3500 Cu B	0.077	ND	mg/l Ionic
Zinc	SM 18th 3500 Zn B	0.018	0.173	mg/l Ionic
Lead	SM 18th 3500 Pb B (3113A)	NA		μg/l ionic
Arsenic, Total	SM 18th 3500 As B (3113A)	NA		μg/l ionic
Sulfate	HACH Method 8051	7.000	47.0	mg/l Ionic
Nitrate	HACH Method 8171	NA	ND	mg/l Ionic
Orthophosphate	SM 18th 4500 P E	0.010	0.32	mg/l Ionic
Chloride	SM 18th 4500 Cl^- B	10.00	62.0	mg/l Ionic
Silica	HACH Method 8185	NA	16.2	mg/l Ionic
Chlorine, Free	SM 18th 4500 Cl G	0.010		mg/l ionic
Chlorine, Total	SM 18th 4500 Cl G	0.010		mg/l Ionic
Monochloramine	HACH Method 10045	0.010		mg/l Ionic

Ammonia, Free	HACH Method 10045	0.010		mg/l Ionic