Next Generation Oxidant;

The Best Molluscocide in the Industry

JC 9465

Figure 1

Before JC 9465

Figure 1:
This pipeline has been infected with biofilm and zebra mussles that limited the flow of liquid.

Figure 2

After Treatment With JC 9465

Figure 2:
After the simple addition of JC 9465, all biofilm, zebra mussels, and most mollusides were eliminated.

JENFITCH INC

The following information about zebra mussels is extracted from the EPA manual: Alternative disinfectants and oxidants, also the US Corp of Engineer ZM chemical control guide.

Control of Nuisance Asiatic Clams and Zebra Mussels

The Asiatic clam (*Corbicula fluminea)* was introduced to the United States from Southeast Asia in 1938 and now inhabits almost every major river system south of 40° latitude (Britton and Morton, 1982; Counts, 1986). Asiatic clams have been found in the Trinity River, TX; the Ohio River at Evansville, IN; New River at Narrows and Glen Lyn, VA; and the Catawba River in Rock Hill, SC (Belanger et al., 1991; Cameron et al., 1989a; Matisoff et al., 1996). This animal has invaded many water utilities, clogging source water transmission systems and valves, screens, and meters; damaging centrifugal pumps; and causing taste and odor problems (Sinclair, 1964; Evans et al., 1979; Smith, 1979).

Cameron et al. (1989a) investigated the effectiveness of several oxidants to control the Asiatic clam in both the juvenile and adult phases. As expected, the adult clam was found to be much more resistant to oxidants than the juvenile form. In many cases, the traditional method of control, free chlorination, cannot be used because of the formation of excessive amounts of THMs. As shown in Table 2-6, Cameron et al. (1989a) compared the effectiveness of four oxidants for controlling the juvenile Asiatic clam in terms of the LT50 (time required for 50 percent mortality). Monochloramine was found to be the best for controlling the juvenile clams without forming THMs. *(Ozone data is an addition and extrapolated from EPA data)* Further research showed that the effectiveness of monochloramine increased greatly as the temperature increased (Cameron et al., 1989b). Note that the temperatures in this study reflect conditions in the Lynchburg Reservoir, Houston, Texas. Clams can tolerate temperatures between 2 and 35°C (Cameron et al. 1989a).

Table 2-6. The Effects of Various Oxidants on Mortality of the Asiatic Clam (*Corbicula fluminea*)

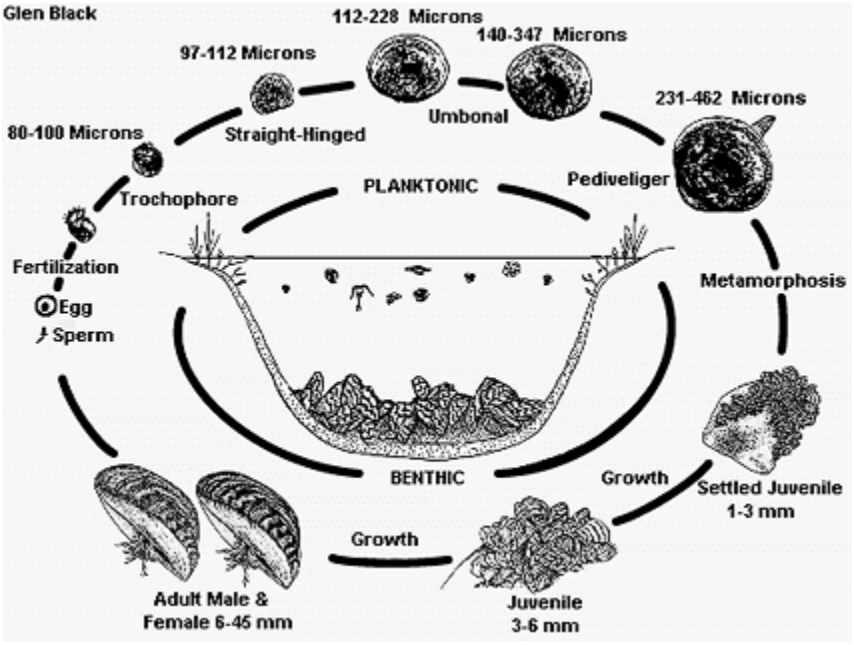
Chemical	Residual mg/L	ORP (mV)	Temperature (°C)	pH	Life Stage	LT50 (Days)
Free chlorine	0.5	NA	23	8.0	A	8.7
	4.8		21	7.9	A	5.9
	4.7		16	7.8	J	4.8
Potassium Permanganate	1.1	NA	17	7.6	J	7.9
	4.8		17	7.6	J	8.6
Monochloramine	2.6	NA	28	7.9	J	0.6
	10.7		17	7.9	J	0.5
Chlorine dioxide	1.2	NA	24	6.9	J	0.7
	4.7		22	6.6	J	0.6
Ozone	0.5	NA	17	7.0	A	4
	0.5		17	7.0	J	0.2
JC 9465	**0.5**	**~+650 mV**	**17**	**7.0**	**A**	**4**
	0.5	**~+650 mV**	**17**	**7.0**	**J**	**0.2**

A= Adult
J= Juvenile

In a similar study, Belanger et al. (1991) studied the biocidal potential of total residual chlorine, monochloramine, monochloramine plus excess ammonia, bromine, and copper for controlling the Asiatic clam. Belanger et al. (1991) showed that monochloramine with excess ammonia was the most effective for controlling the clams at 30°C. Chlorination at 0.25 to 0.40 mg/L total residual chlorine at 20 to 25°C controlled clams of all sizes (LT50 below 28 days) but had minimal effect at 12 to 15°C (as low as zero mortality). As in other studies, the toxicity of all the biocides was highly dependent on temperature and clam size.

The zebra mussel (*Dreissena polymorpha*) is a recent addition to the fauna of the Great Lakes. It was first found in Lake St. Clair in 1988, though it is believed that this native of the Black and Caspian seas, was brought over from Europe in ballast water around 1985 (Herbert et al., 1989). The zebra mussel population in the Great Lakes has expanded very rapidly, both in size and geographical distribution (Roberts, 1990). Lang (1994) reported that zebra mussels have been found in the Ohio River, Cumberland River, Arkansas River, Tennessee River, and the Mississippi River south to New Orleans.

Life Cycle



There are three main periods in the zebra mussel life cycle: the larval, juvenile, and adult stages. The larvae are planktonic (float in water column) during their initial three life stages: trochophore, straight-hinged veliger, and umbonal veliger. Larvae eventually settle on a substrate during their pediveliger stage, and move only by crawling during their plantigrade stage. The pediveliger is considered by some to be the final larval form, with the plantigrade as a stage between larval and juvenile stages (Ackerman et al. 1994). These stages are identified primarily on morphology and behavior and are somewhat variable and overlapping in physical dimensions. The amount of time required for a fertilized gamete to develop into a fully developed juvenile is longer at colder water temperatures and thus can range from 8 to 240 days (Nichols 1996).

The zebra mussel's clam-like shape throughout much of its larval stage is replaced by a more triangular or mussel-like shape as it enters the juvenile stage. Mussels are considered adults when they become sexually mature. Adult mussels range from approximately 6 to 45 mm and generally live to be 2-3 years old in temperate climates.

Klerks and Fraleigh (1991) evaluated the effectiveness of hypochlorite, permanganate, and hydrogen peroxide with iron for their effectiveness controlling adult zebra mussels. Both continuous and intermittent 28-day static renewal tests were conducted to determine the impact of intermittent dosing. Intermittent treatment proved to be much less effective than continuous dosing.

Hypochlorite Reaction

Chlorine treatments have relied on the use of pressurized gas; liquid sodium hypochlorite is the chlorine source of choice because of safety concerns. Sodium hypochlorite, NaOCl, is considered a safe and versatile chlorinating liquid. Claudi and Mackie (1994) described the reaction that takes place when sodium hypochlorite is added to water, with hypochlorous acid (HOCl) formed as the oxidizing agent in this reaction. As a "weak" acid, hypochlorous acid tends to undergo partial dissociation, to produce a hydrogen ion (H^+) and a hypochlorite ion (OCl^-). Hypochlorous acid has more biocidal effect than the hypochlorite ion because of its ability to penetrate cell walls (White 1986). The FAC is the combined amount of HOCl and OCl^- in the water.

The hydrogen peroxide-iron combination (1–5 mg/L with 25 percent iron) was less effective in controlling the zebra mussel than either permanganate or hypochlorite. Permanganate (0.5–2.5 mg $KMnO_4$/L) was usually less effective than hypochlorite (0.5–10 mg Cl_2/L).

Van Benschoten et al. (1995) developed a kinetic model to predict the rate of mortality of the zebra mussel in response to chlorine. The model shows the relationship between chlorine residual and temperature on the exposure time required to achieve 50 and 95 percent mortality. Data were collected for chlorine residuals between 0.5 and 3.0 mg Cl_2/L and temperatures from 0.3 to 24°C. The results show a strong dependence on temperature and required contact times ranging from two days to more than a month, depending on environmental factors and mortality required.

Brady et al. (1996) compared the efficiency of chlorine to control growth of zebra mussel and quagga mussel (*Dreissena bugensis*). The quagga mussel is a newly identified mollusk within the Great Lakes that is similar in appearance to the zebra mussel. Full-scale chlorination treatment found a significantly higher mortality for the quagga mussel. The required contact time for 100 percent mortality for quagga and zebra mussels was 23 days and 37 days, respectively, suggesting that chlorination programs designed to control zebra mussels should also be effective for controlling populations of quagga mussels.

Chlorine Dioxide

Chlorine dioxide (ClO_2) has been an effective disinfectant in the water industry for over 50 years (Claudi and Mackie 1994). Unlike the hypochlorite reaction, its by-products are primarily sodium chloride and sodium chlorite and does not lead directly to the formation of

trihalomethanes. Opinions differ as to its effectiveness in zebra mussel control. The use of chlorine dioxide may not offer significant advantages over sodium hypochlorite when cost and ease of use are considered. Chlorine dioxide must be manufactured on-site with the use of specialized equipment. Chlorine dioxide control methods may be beneficial if a chlorine dioxide system is already in place or the formation of trihalomethanes is a serious problem.

Matisoff et al. (1996) evaluated chlorine dioxide (ClO_2) to control adult zebra mussels using single, intermittent, and continuous exposures. A single 30-minute exposure to 20 mg/L chlorine dioxide or higher concentration induced at least 50 percent mortality, while sodium hypochlorite produced only 26 percent mortality, and permanganate and hydrogen peroxide were totally ineffective when dosed at 30 mg/L for 30 minutes under the same conditions. These high dosages, even though only used for a short period, may not allow application directly in water for certain applications due to byproducts that remain in the water. Continuous exposure to chlorine dioxide for four days was effective at concentrations above 0.5 mg/L (LC50 = 0.35 mg/L), and 100 percent mortality was achieved at chlorine dioxide concentrations above 1 mg/L.

Chloramine

Chloramines are produced in the reaction of free available chlorine with various forms of nitrogen containing compounds occurring in the water such as ammonia, nitrites, nitrates, and amino acids. Chloramines are formed naturally when chlorine or sodium hypochlorite is added to raw water. The chloramines include monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3), together designated as TCC (Claudi and Mackie 1994). The more ammonium that is present, the higher the level of chloramines that are formed. Claudi and Mackie (1994) stated that chloramines are considered less powerful as oxidants than hypochlorous acid. At sites where the formation of trihalomethanes is a concern, the use of chloramines offers some advantages. Chloramine treatments are applied by co-injection of ammonium as either ammonium gas or ammonium hydroxide and sodium hypochlorite. Exact dosing requirements for effective zebra mussel control is unknown.

Factors Influencing Chlorine Effectiveness

A number of raw water parameters influence the effectiveness of chlorine treatments. These factors include organic and inorganic compound concentrations, temperature, and pH (Claudi and Mackie 1994, EPRI 1992). The physical state of the zebra mussel and the extent of infestation will also influence the effectiveness of the chlorine treatment (Claudi and Mackie 1994).

Water chemistry has a very important impact on the toxicity of chlorination to zebra mussels. Claudi and Mackie (1994) stated that waters rich in organic and inorganic compounds have high chlorine demand, consuming larger amounts of chlorine residuals through oxidation-reduction reactions. The presence of reducing agents, such as S^{2-}, Fe^{2+}, Mn^{2+}, and NO^{2-}, accelerate the chlorine decomposition rate and should be taken into account to ensure expected zebra mussel mortality.

Water temperature effects both the dissociation of hypochlorous acid into the hydrogen and hypochlorite ions and the metabolic rate of zebra mussels. As water temperatures rise, the

concentration of the more effective hypochlorous acid decreases as the concentration of the dissociated ions increase. Higher temperatures also seem to escalate the intake of chlorine compounds as the zebra mussel's metabolic rates increases. As a result, even though higher temperatures lower the toxicity of the chlorine, the increased uptake of chlorine compounds increases the overall chlorine effectiveness.

Water pH strongly influences the dissociation of hypochlorous acid into the hydrogen and hypochlorite ions. Claudi and Mackie (1994) presented a graph showing dissociation of hypochlorous acid versus pH, showing that when the pH of the chlorinated water is approximately 7.5, 50 percent of the chlorine concentration present will be undissociated hypochlorous acid and the remainder, the hypochlorite ion. A 100 percent hypochlorite ion concentration is attained at a water pH of 10. Conversely, at pH 5, 100 percent of the chlorine concentration will be the more effective undissociated hypochlorous acid.

Chloramine formation is pH-dependent; a lower pH will yield a higher concentration of dichloramines, whereas a higher pH will yield a higher concentration of monochloramines. Dichloramines are more potent disinfectants than monochloramines (Claudi and Mackie 1994). Maximum (100 percent) dichloramine concentrations occur at pH 4.5. At pH 8.5, 100 percent monochloramine concentrations exist.

Toxicity studies have shown that mature zebra mussels are slightly more resistant to chlorine than are various veliger stages (Claudi and Mackie 1994). Chlorine treatments are more effective at the end of a growing season due to the physiologically exhausted state of the mussel following the reproductive effort. There is an inverse relation between the population biomass and the treatment effectiveness. Larger populations, particularly individuals farther away from the surface layer, are less vulnerable than are single layer colonies (Claudi and Mackie 1994). Thus, multiple applications or multiple treatment methods may be necessary in problem infestations.

Application and Effect Ranges for Chlorine Treatments (McMahon, Lussery, and Clarke 1994)

Treatment	Application	Effect
Chlorination (adults)	0.5 ppm for 7 days 0.3 ppm for 14 to 21 days	75% kill
Chlorination (adults)	2 ppm continuous flow	>95% Kill
Chlorine Dioxide	0.5 ppm for 24 hr	90% Kill
Chloramine	1.2 ppm for 24 hr	100% veliger kill
Ozone	0.5 ppm for 5 hr 0.5 ppm for 7 to 12 days	100% veliger kill
JC 9465	**+650 mV or higher for 5 hr +650 mV or higher 7 to 12 days**	**100% veliger kill 100% adult Kill**

Nontarget Effects of Chlorine

Chlorine, chloramines, and chlorine dioxide are nonselective and highly toxic to nontarget fish and invertebrates. Claudi and Mackie (1994) have provided a detailed information on the impacts of chlorination on fishes, invertebrates, and phytoplankton, which can be consulted for guidance. Fish seem to be more negatively affected than are other aquatic organisms (Claudi and Mackie 1994), though literature related to the effects on other aquatic organisms (i.e., invertebrates and phytoplankton) is less abundant. Following chlorine treatment, phytoplankton populations may drastically decrease; however, their recovery is generally rapid.

Besides killing the nontarget organisms, sublethal life parameters of nontarget species that chlorine may affect include behavior, reproduction, growth and mutagenesis. Claudi and Mackie (1994) stated the most important aspect of behavior affected by chlorination is avoidance, and fishes have received more attention in the literature with regard to their avoidance of chlorine. Reproduction is a sensitive indicator of sublethal toxicity. Chlorination adversely affects the reproduction of certain nontarget aquatic organisms and its presence inhibits the growth of both plant and animal species. Chlorine can also react with dissolved organic material to form chlorinated organics, some of which are suspected mutagens.

Dechlorination

Federal and state statutes regulate the concentrations of chlorine that can be released into the environment and require that water samples be analyzed accurately for the presence of free and residual chlorine. A major concern when using chlorine in fresh waters is that it will combine with various organic compounds to form trihalomethanes, which are considered carcinogenic. Stringent requirements are also placed on the level of total residual chlorine allowed in the discharge. Facilities unable to meet TRC water quality limits must dilute the discharge with raw water or neutralize the chlorine prior to release. Sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), sodium metabisulfite ($Na_2S_2O_5$), or sulfur dioxide (SO_2) may be used. The most convenient chemical used to neutralize residual chlorine is sodium bisulfite (sometimes called "liquid sulfite"), with the dosage requirement being a concentration of 1.8 to 2.0 units of sodium bisulfite for each unit of TRC (Claudi and Mackie 1994). Sodium bisulfite can be fed directly into the discharge prior to reintroduction into the waterbody because the reaction of chlorine with sulfite is almost instantaneous.

Ozone

Ozone is a well-known bacterial agent, used in Europe to disinfect drinking water and industrial and municipal wastewater (EPRI 1993). Ozone also improves taste, odor, and color of drinking water and can be used to prevent biofouling. Ozone out performs chlorine in terms of contact time at comparable residual levels. Lewis, VanBenschoten, and Jensen (1993) indicated that at 15^0C to 20^0C, a minimum of 5 hr contact time was required at 0.5 mg/L for a 100-percent mortality of veligers and post- veligers in the water column

Ozone residuals of 0.5 mg/L or greater for 7 to 12 days will cause 100-percent mortality of adult zebra mussels. Time to death is inversely related to both on concentration and ambient temperature.

[1] (Lewis, D., Van Benschoten, J.E., and Jensen, J. N. (1993). "A study to determine effective ozone dose at various temperatures for inactivation of zebra mussels," unpublished report, Ontario Hydro, Toronto, Canada.)

Ozone is highly explosive, especially when solutions are warmed. Commercial ozone is not available due to shipping problems, and ozone used in water treatment is always generated on site (EPRI 1993). Ozone is a powerful natural oxidant in the atmosphere not occurring naturally in surface waters. When released in natural waters, residual ozone concentrations quickly dissipate. Dissipation in raw water is so rapid that, if injected in pipe intakes or forebays, no ozone residual can be found in facility discharge.

Properties of ozone offer both advantages and disadvantages. Ozone treatments do not exhibit downstream environmental impacts, making it attractive for use in once through systems. This characteristic, however, is undesirable when considering control of downstream zebra mussel settlement and growth. Maintaining sufficient residual ozone levels required to kill adult zebra mussels in an extensive piping system is very difficult and expensive, requiring multiple injection points would be required.

These experiences all show that the dose required to induce mortality to these nuisance organisms is extremely high, both in terms of chemical dose and contact time. The potential impact on DBPs is significant, especially when the water is high in organic content with a high propensity to form THMs and other DBPs.

Treatment Strategies

The applied chemical treatment strategy is as important as the type of chemical used. There are five different chemical treatment strategies proposed by Claudi and Mackie (1994) for zebra mussel infestations: end-of-season, periodic, intermittent, continuous, and semi-continuous. A chemical zebra mussel control strategy may consist of a single treatment scenario or a combination of treatments used in concert. The treatments most applicable to a particular facility depends on the extent of zebra mussel infestation, the degree of permissible infestation, water quality, existing facility systems, economics, permit requirements and environmental regulations. An effective chemical treatment design allows for flexibility in treatment applications in accordance with the facilities entire zebra mussel control program for each facility.

End-of-Season Treatment

End-of-season treatment is generally a reactive strategy, acceptable in systems that can tolerate limited macrofouling. Limited macrofouling can be anticipated if chemical treatments are applied once during the year, usually after the spawning season or at the end of the growing season. Treatments after the spawning season increase chemical effectiveness and reduce required concentrations as individuals are fatigued and weakened. Also, shells and soft tissue debris of young-of-the-year mussels more easily pass through facility systems.

Mitigation of established mussels by end-of-season treatments requires higher dosages of chemicals over an extended period of time (2 to 3 weeks) (Claudi and Mackie 1994). Chemical concentrations and exposure times are dependent on the chemical used, water quality, and health of the mussels. Defining absolute levels applicable to all locations at all times is very difficult. Byssal threads remaining after end-of-season treatment can promote the settlement of veligers, and cause corrosion, and add surface friction.

Periodic Treatment

Periodic chemical treatment, like end-of-season treatment, is usually a reactive treatment (usually conducted on a regular basis, such as every 2 months) designed to eliminate adults that have accumulated since the previous application. Again, limited infestations must be tolerable, but because treatments are more frequent, infestations will be proportionally smaller. The chemical concentration and exposure time should be comparable to end-of-season values, though the total removed biomass will be smaller.

Intermittent Treatment

Intermittent chemical use is designed to prevent initial zebra mussel infestation at facilities that cannot tolerate macrofouling. Dosing at frequent intervals (e.g., 6, 12, 24 hr) destroys post-veligers that have settled since the previous treatment. Post-veligers are more susceptible to oxidizing chemicals than are adults; thus, the concentration of the chemical and exposure times will be considerably less than if adults were the target. Because post-veligers with shells about 250 mm long can easily pass through the system, disposal and under-deposit corrosion is eliminated.

Semi-continuous Treatment

Semi-continuous treatment is a preventive control method developed by Ontario Hydro. Because zebra mussels will stop filtering and close their shell when exposed to a toxic substance, the utility postulated that frequent on-off cycling of chlorine was more effective than continuous chemical treatments. Treatment schedules can be adjusted to 15 min on and 15 to 45 min off. Chlorination treatments consisting of 15 min on and 15 or 30 min off at the 0.5-mg/L level have been as effective as continuous treatment (Claudi and Mackie 1994). Semi-continuous treatment is ideal for facilities where several discrete systems need to be treated and results in less chemical usage, than continuous chlorination.

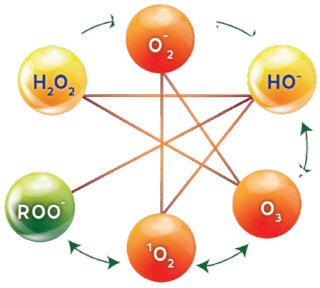
Continuous Treatment

Continuous chemical treatment is designed for facilities that cannot tolerate any level of macrofouling. Low chemical concentrations, applied continuously, prevent any post-veliger settlement and is stressful enough to either kill adult mussels or cause them to detach and move out of the system. Continuous treatment should be carried out for the entire zebra mussel breeding season.

JC 9465

INTRODUCTION

The overwhelming obstacle to the use of conventional chlorine oxidants is the nature of surface water. Surface water is the most common method of water collection and distribution in this country.Lakes, ponds and flowing sources have been the mainstay of water sourcing in this country for decades. By the very nature of surface collection, the concentration of contaminants within these sources is expected.One must consider the type of surface supply, location, and realize that all runoff feeding the body of water will be reflected in the following:



1. Surface-Natural Content
   a. TSS/TDS: Suspended solids: silt, dirt, large organic particles
   b. Organics: natural scouring, humic acids
   c. Organics: suspended growth
   d. Nutrients: inorganic minerals, detritus
   e. Minerals: non-nutrient
   f. Parasites & Pathogens: cephalopods, algae, viruses, bacteria

2. Surface Contaminants
    a. Fertilizers
    b. Pesticides
    c. Organic growth
    d. Industrial waste
    e. Residential run off
    f. Pathogen multiplication
    g. Nitrates

Profiles of both Ground and Surface water sources are, in 1999, beginning to reflect similarities in content. This has been the result of intrusion of surface water sources (storage and surface disposal of waste streams, both solid and liquid) into ground aquifers and water sands. Very significant increases in nutrient levels, nitrates, and general organic growth are evident at levels exceeding 1,000 feet depth. Growth on well screens at depths exceeding 1,000 feet are common as well as "aerobic slime layers" on all internal pipe surfaces feeding all types of process consumption. These living slime layers generate toxins, and microbial growth, which are released into the source water, fed to the specific consumer.

Chlorine Compounds

Chlorine, at pH of 4-5, produces hypochlorus acid, which is the desired chlorine derivative that is active against all microorganisms. To use chlorine correctly a water stream must be prepared to receive a chlorine compound to achieve optimum effectiveness.

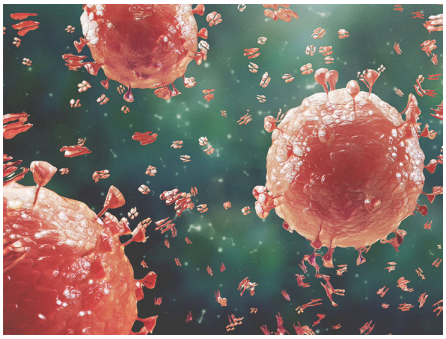
Factors affecting Chlorine Activity:

1. pH: MUST be adjusted to pH of 4-5,. By adjusting the pH, the bicarbonates become carbonates and cease to scavenge oxidants (bicarbonates are oxidant scavengers).
2. Temperature: chlorine reactivity is temperature sensitive. Cold water slows down and requires more chlorine than warmer water (well vs surface, summer vs winter).
3. Organics: Organics will consume chlorine to form *chloramines and THMs'*
4. Ammonia & Nitrates: these two compounds will slow the bacterial kill action of chlorine, increasing the contact time required for chlorine to work

Reactivity of Chlorine Compounds

Chlorine was first discovered to be a disinfectant in 1787 and has been universally accepted and utilized ever since. The reactivity of chlorine has been well studied and general guide lines for its use are as follows:

1. Inorganics: Chlorine, used by itself, requires a concentration of *6 mg/L per 1.0 mg/L of inorganic at a pH of 8.5 –9.0*. If used in conjunction with permanganate this ratio will drop to below 1.0 mg/L per 1.0 mg/L of inorganic but will require tight instrumentation controls.
2. Pathogens: At a pH of 4-5, chlorine is effective as a disinfectant when used at a ratio of 1-3 mg/L per 0.5 mg/L pathogen *and requires a residence time of 45 – 60 minutes (12 time that for Ozone).*



3. Pathogens: A 500 mg/L chlorine concentration will normally be effective against vegetative bacteria. At this concentration, chlorine has limited effect against viruses and is poorly effective against bacterial spores and fungi (adverse effects on body chemistry/ metabolism).

4.Pathogens: a 1000 mg/L chlorine concentration is effective against all bacteria, viruses and fungi yet requires 25-30 minute residence time (adverse effects on body chemistry/metabolism).

5. Organics: practical applications of chlorine, even at 1000 mg/L have little effect on oxidizing organic material other than generating chlorinated organics, hence chlorine is not recommended for this use.
6. Residual: A chlorine residual can be provided only after the demand for chlorine by inorganic, bacteria and organics has been met

Chlorine can be effective for oxidizing inorganics and bacteria when pH, concentration and residence time are allowed for, **but** the chlorinated organic by-products are inevitable and only some may be filtered out. For agriculture applications, the important consideration for utilizing chlorine compounds is the amount of chlorides that will end up being consumed by an animal, the impact of the chlorinated organics on the animals' performance and toxic residues absorbed into the tissues of the animal.. If excess chlorides are produced, necessary steps should be taken to remove them as a last step in a water treatment program.

JC 9465

The use of chlorine dioxide and ozone, though not cost effective, reveals the effectiveness of reactive oxygen species (ROS) for dealing with organic loading and the target pests.

JC 9465 is a solution that delivers a family of ROS in a liquid and provides oxidation potential almost equal to that of ozone but with the added benefit of providing residual oxidation energy. To understand this oxidation potential, it helps to understand how ROS behaves against microbes and as a water treatment vector.

The spectrum of aerotolerance displayed by all life should be considered as the result of two contending sets of tendencies: (a) those that predispose to oxygen tolerance and (b) those that predispose to oxygen sensitivity. Some microorganisms have an aerotolerance measured in the ppb range, whereas as life forms become more complex, aerotolerance goes as high as 21%. Atmospheric oxygen concentration is 21%. If it should increase, the increase in oxygen concentration would interfere with the gas exchange in our lungs and all mammalian life would become extinct. When considering atmospheric ROS, the maximum contaminant level is 0.1 ppm because any higher concentration is damaging to lung tissues.

These tendencies are then defined by :

1. Possession or lack of specific protectives against toxic metabolites of oxygen, i.e., enzyme scavengers.
2. Different degrees of reliance on oxygen sensitive cell constituents.
3. Differences in the supply of reducing power for oxygen scavenging.
4. Differences in the rates and mechanisms of oxygen consumption (which may alleviate or aggravate the threat posed by reactive oxygen species [ROS]).

All living cells are prone to oxygen toxicity. However, some bacteria (microaerophiles), while aerointolerant, require a little oxygen for growth. This suggests that degrees of oxygen

tolerance must be displayed even by anaerobes. This conclusion awakened bacteriologists to the realization that among aerointolerant bacteria there exists a complete spectrum of oxygen tolerance ranging from the most exacting, extremely oxygen sensitive (EOS) obligate anaerobes, through moderate obligate anaerobes which are able to survive brief exposure to low concentrations of oxygen, to microaerophiles which can survive molecular oxygen at an atmospheric concentration less than 20% v/v (equivalent to 7 ppm dissolved oxygen) for several days.

Though a range of aerotolerance is observed, it is difficult to establish a table of anaerobes in invariant order of increasing oxygen sensitivity. The difficulty is due to the inability of present techniques to quantify differences in oxygen sensitivity and that the organisms themselves exhibit a range of oxygen sensitivities among species of the same genus, in different strains of the same species, in the same organism harvested in different life cycle phases or the same organism cultured in different media. However, generalizations can be made about the more and less oxygen sensitive species among moderate anaerobes and differences in the response to oxygen of various EOS anaerobes can be discerned, e.g., *Methanobacterium AZ* ceases growth and methane production at 0.01 ppm O_2 but survives exposure for several days to 7 ppm dissolved oxygen.

OXYGEN AS TERMINAL ELECTRON ACCEPTOR

Anaerobes develop and sustain a low oxidation-reduction potential, (-200 mV to -50 mV) in their growth media, e.g., gastrointestinal tract. Reducing agents that affect the conversion of O_2 to water aid growth by facilitating the establishment of an agreeable redox potential. The addition of oxidants, raising E_h to as high as +500 mV, may retard or prevent the establishment of the desirable E_h value but will not necessarily be toxic to the organism. At best, an E_h is an imperical indicator of the prevailing balance between numerous contending oxidizing and reducing agents. The low E_h maintained by anaerobes is, therefore, evidence that they grow best under "reducing conditions" where there is minimum uncontrolled drainage from the organisms of reducing power which they could otherwise employ more productively for energy yielding or biosynthetic purposes.

The establishment and maintenance of these desirable equilibrium conditions call for a reducing contribution from the organisms themselves, but anaerobes differ in their capacity to generate the necessary disposable reducing power. Possibly those with the least capacity have a minimally flexible cycle of endogenous electron generation and acceptance which leaves little for diversion of electron flow for reduction of exogenous oxidants. Thus, the very reactivity of oxygen, very effective as an electron acceptor, poses a threat to anaerobes which may consume it nonproductively as a preferred oxidant. In doing so, they uselessly divert reducing power from other life-sustaining functions and shift internal redox couples to their fully oxidized condition with consequentially amplified effects on metabolism.

Many moderate anaerobes, e.g., species of *Bacteroides*, *Clostridium*, may be grown and survive in the presence of high concentrations of dissolved oxygen. For these organisms, such levels of oxygenation are bacteriostatic and the ill affects are readily reversed when anaerobic conditions are restored. During the bacteriostatic phase, preferential reduction of oxygen occurs at the expense of metabolic functions. One of the consequences of which is, although fermentation continues at a much inhibited rate, it preferentially yields only the more oxidized of the usual end products. This protective response (bacteriostic Phase 1 of oxygen action) has its counterpart in those aerobic bacteria that divert reducing power to

scavenge molecular oxygen to shield key oxygen-labile components from direct contact with oxygen.

If the reductive Phase 1 defenses are overwhelmed by excessive exposure to oxygen, then a bactericidal Phase 2 of oxygen toxicity supervenes wherein irreversible damage is suffered. Strict EOS anaerobes differ in not displaying the Phase 1 response of the moderate anaerobes. Some are immediately plunged into a Phase 2 situation while others might tolerate X concentration of oxygen, being bacteriostatic, and any increase above X concentration would overwhelm available reducing power resulting in a bactericidal environment. These organisms are totally dependent on exogenous oxygen scavenging and reducing systems to remove oxygen and to restore a congenial low redox potential.

CAUSES OF IRREVERSIBLE, PHASE 2 OXYGEN TOXICITY

Highly reactive and destructive by-products of oxygen reduction are invariably formed whenever oxygen is consumed by living cells and has the opportunity to react with many reduced cellular constituents such as iron-sulfur proteins, thiols, tetrahydropteridines, flavoproteins, i.e., most organic compounds with double and conjugated double bonds. Oxygen by-products are: hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical ($OH^\cdot$) and singlet oxygen ($^1O_2$).

$$O_2 + e^- \Rightarrow O_2^-$$

$$O_2^- + e^- + 2H^+ \Rightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \Rightarrow H_2O + OH^\cdot$$

$$OH + {}^\cdot e^- + H^+ \Rightarrow 2H_2O$$

Both peroxide (H_2O_2) and superoxide (O_2^-) are not of themselves dramatically cytotoxic, but are particularly dangerous products of oxygen consumption because they can generate the more devastating hydroxyl radical ($OH^\cdot$) via a biologically catalyzed sequence. The intracellular reduction of O_2 to $2H_2O$ requires addition of four electrons. This reduction usually occurs by single electron steps and the first product formed in the reduction of O_2 is *superoxide* anion, O_2^-.

Superoxide

Superoxide (O_2^-) is transiently produced by a one-electron transfer to oxygen by flavins, flavoprotiens, quinones, thiols and iron-sulfer proteins. If the superoxide anion is not intercepted and neutralized, it will cause oxidative destruction to biochemical components within the cell. This anion has the longest life of the oxygen by-products and may pass from one cell to another. However, in chemical terms, O_2^- is a poorly reactive radical in aqueous solution. The protonated form of O_2^-, hydroperoxyl radical, $HO_2^\cdot$, is somewhat more reactive than O_2^-. For example, $HO_2^\cdot$ can initiate peroxidation of fatty acids. The equilibrium of $HO_2^\cdot$ with O_2^- is pH controlled and since the pH very close to a membrane surface may be more acidic than the pH in bulk solution, $HO_2^\cdot$ formation will be favored. At a pH of 3.8, the ratio of O_2^- to $HO_2^\cdot$ is 1:10. At a pH of 5.8, the ratio shifts to 10:1 and at pH 6.8, the ratio leaps to 100:1. The pH beneath activated macrophages adhering to a surface has been reported to be 5 or less. Thus, a considerable amount of any generated superoxide will exist as $HO_2^\cdot$. Because a cell membrane rejects charged molecules, $HO_2^\cdot$ should be able to cross membranes as easily as H_2O_2. After penetration into the hydrophobic membrane interior,

conditions favor O_2^- which is highly reactive in organic media and will attack carbonyl groups of ester bonds that link fatty acids to the glycerol "backbone" of membrane phospholipids and are constituents of essential enzymes partly responsible for the biosynthesis of branch-chain amino acids

Hydroxyl

The hydroxyl radical, $OH^{\cdot}$, reacts with extreme high rate constants with almost every type of molecule found in living cells, i.e., sugars, amino acids, phospholipids, nucleotides and organic acids. With saturated complexes, OH will abstract a hydrogen to form water, leaving behind an unpaired electron on the carbon atom. This new carbon radical will undergo one or more additional reactions, e.g., alcohols will form a hydroxymethyl radical capable of a wide range of reactions dependent on what might be available and sugars of DNA produce a huge array of different products, some of which become mutagenic. With more complex structures, i.e., aromatics and compounds with conjugated double bonds, $OH^{\cdot}$ will proceed by addition to the ring or double bond which drastically alters the physical properties from what is necessary for biosynthetic processes. Thus, hydroxl radicals severely damage the bases and sugars of DNA and also induce strand breakage. If damage is repairable, mutations may result and if the damage is beyond repair, the cell will die.

The hydroxyl radical is a product from the endogenous reduction of peroxide by one of two methods.

1. H_2O_2 + e- + H^+ => H_2O + $OH^{\cdot}$

2a. M^{3+} -complex + O_2^- => M^{2+} -complex + O_2

2b. M^{2+} -complex + H_2O_2 => $OH^{\cdot}$ + OH^- + M^{3+} -complex

Where "M" is a metal, this reaction has become known as the *Haber-Weiss* reaction. Relating to bacteria, those species that produce superoxide dismutase (SOD), utilize metal chelates of iron and manganese in the process of building this enzyme. If concentrations of superoxide and peroxide are substantial enough to come in contact with a metal chelate, a hydroxyl radical can then be catalyzed. Especially among obligate anaerobes where the fermentative cycle requires substantial NADH, NADPH and thiol compounds, any interaction with metal ions and peroxide with these biosynthesis complexes will increase OH. formation. The observation of DNA damage induced by peroxide is hence mediated by some metal catalyzed Haber-Weiss reaction within the cell, i.e., H_2O_2 penetrates the plasma membrane and interacts with O_2^- generated intracellularly to form OH. , using metal ions bound to a weak metal chelate, metal containing enzyme or possibly to DNA.

Singlet Oxygen

Singlet oxygen is an excited form of molecular oxygen, annotated $^1O_2$, and is extremely reactive, via addition reaction with compounds containing carbon-carbon double bonds and conjugated double bonds, which are structural attributes of all biologically important substrates. A physical property which should be noted is that the decay rate of singlet oxygen to its ground state, O_2, competes with reactions of singlet oxygen with oxidizable substrates. In aqueous solutions, the decay rate is measured in micro-seconds, and in aprotic solvents (not containing hydrogen), the decay rate can be extended to 30 to 40 seconds.

In biologic systems $^1O_2$ is normally a by-product of specialized activity of cells associated with the immune system. Specialized enzyme reactions associated with saliva and phagocytosis produce singlet oxygen as part of the defense mechanism against invading microbes. Exogenous to a cell, sources of $^1O_2$ are usually the result of decomposition reactions whereby a free superoxide anion will attempt to react with anything it comes in contact with.

The cell wall of *gram-negative* bacteria has four layers. The outer coat is composed of *lipopolysaccharide* (LPS). LPS offers some protection from the toxic effects of exogenous agents. This capacity enables these bacteria to survive in hostile environments, i.e., gastrointestinal tract. LPS presents a physical/chemical barrier through which exogenous $^1O_2$ must pass to interact with vital targets. Primarily, LPS repells $^1O_2$, but some does penetrate this layer and becomes trapped among the unsaturated fatty acids and protein components wherein peroxidation will occur. All things not being equal, some strains fail to produce a significant LPS layer which increases their sensitivity to exogenous $^1O_2$. Most *gram-positive* bacteria have a bi-layer membrane with an outer coat of *peptidoglycan* (PG), which with greater frequency, allows substantially more $^1O_2$ to pass through than LPS. For both types of bacteria, when $^1O_2$ traverses the membrane layers any number of enzyme/protein deactivation reactions can occur and when enough enter within a bacterium, i.e., more than can be countered, death is certain.

The toxicity of $^1O_2$ is hence dependent on the number of molecules attacking a bacterium. Calculations have concluded that to achieve a 99% kill, 1.3×10^{-5} mol of singlet oxygen should reach a bacterium in 20 minutes. On average, gram-negative bacterium required 5×10^{10} molecules of $^1O_2$ per cell and gram-positive bacterium required 6×10^9 molecules $^1O_2$ per cell. This finding suggests a low probability of vital target-singlet oxygen interaction, so most collisions do not result in cell death. However, the probability that the inactivating collision will occur never changes. A single reaction of $^1O_2$ could conceivably have devastating global effects such as initiating lipid peroxidation and subsequent radical-mediated reactions. Single hit kinetics in this case are not likely due to lethal DNA damage, as $^1O_2$ does not readily react with bacterial DNA.

Conclusion

Both hydroxyl radical and singlet oxygen are indiscriminate reactive reagents and their cytology is enhanced when they are generated in an *aprotic* solvent, e.g., within lipid membranes wherein their half-lives would be extended and molecular oxygen would be more soluble. The bactericidal effects of these radicals are the result of breaks in DNA, lipid peroxidation, impairment of transport processes across membranes, destruction of key enzymes and co-factors of reductant and energy generating pathways and/or biosynthetic processes. The suggestion is that obligate anaerobes depend on metabolic processes made up of excessively oxygen sensitive enzymes and co-factors and are either void of or lack sufficient means to neutralize oxygen radicals as they may be produced endogenously or introduced exogenously.

A remedial DNA repair mechanism exists in most aerobes and some microaerophiles. As part of a Phase 1 bacteriostatic reaction, DNA *glycosylases* have been described for both purines and pyrimidines damaged by OH- attack. Again, the concentration of the invading radical will have an impact on the cell's ability to keep up with the damage or fall too far behind and the DNA replication process becomes oxidized itself.

JC 9465

Important to biochemistry, and biologic functions, is ionic equilibria. For the majority of biologically important reactions, very low concentrations of the hydronium ion, along with ions of Mn, Mg, Fe, and Ca are significant driving forces.

Biochemical ionic reactions are *acid - base* reactions that are pH and temperature sensitive, and follow the Bronsted definition of an *acid* as a proton donor and a *base* as a proton acceptor. Because water is amphoteric, it is important to understand the biochemistry of water and its contribution to biologic ion driven reactions.

Within biologic systems, oxygen species are generated to oxidize and be anti-oxidants. The production, delivery, and attenuation of oxygen specie reaction rates, is dependent on the biochemical properties of water and the other ions carried with it. Because water auto-ionizes, forms polymers, encapsulates (forms shells) simple and complex ions, it attenuates the oxidation potential and reaction rates of ion complexes.

JC 9465 is an ionic solution that mimics biochemical water in the production of oxygen species, and in the conservation of ionic energies (oxidation potential). MOxC, like biochemical water, has pH and temperature sensitivities identical to biochemical water.

The formation of metal oxy-complexes results in the loss of "normal" chemical reactions of metal ions in solution. The fact that metal ions do not behave as expected is an indication that these ions are present in very low concentrations. What would normally precipitate a metal ion from a solution, will not work with metal-oxy-complexes because the metal, while in an oxy-complex formation, is "deactivated" from its expected behavior. Metals are normally expected to precipitate as an oxide, but the formation of mineral-metal-oxy-ion, *shelled by water*, keeps the metal in solution, thus de-activating the metal from normal behavior. Under this condition, no precipitation occurs until the concentration of the metal ion rises to a value such that the solubility product of the insoluble salt is exceeded.

Di-positive ions, (Ca, Fe, Mg, Mn) will form monohydroxy complexes and at pH above 9, these oxy-complexes are *continuously converted* to oxy-complexes having two or more hydroxyl groups. They also form interconnected matrices of a transient nature.

Ionized atoms/molecules do not share an electron with water (bonding), but rather conduct ionic interactions with the water shell around them. The formation of metal-oxy-complexes are accompanied by a *decrease* in ionic activity of the metal, and hence, an *increase* in its oxidation potential. In general, coordination with a donor group (oxygen species) increases the oxidation potential, and increases the relative stability of the higher valence state. It is these kinetics that defines the variability of JC 9465.

It is known that an ion has a negative contribution of entropy (randomness) which is due to the restriction of degrees of freedom of water molecules in the vicinity of the ion and that the effect is greater than the charge of the ion. When a metal in a simple ionic state is oxidized, the entropy contribution decreases. Conversely, an increase in valence of a metal ion, when it exists in a negative anion (oxy-complex), results in a decrease in the charge of the ion and in a corresponding entropy increase.

The increased entropy (randomness of structure) means that the oxidation state of both the metal and oxygen ions are maintained by the resonance of complex and rapidly changing matrixes of these ion complexes.

The *alteration in the rate of reaction*, through formation of metal-oxy-complexes always result in a *reduction in the rate of what is considered to be the "normal" reactions of these metals* in solution. Metal-oxy-complexes always result in a reduction in the rate of reaction. For example: when iron (a hexacoordinate bivalent ion) combines with oxygen anions (a tetradentate tetravalent anion), a water soluble anion, containing the metal and up to four oxygens (ferrate), is formed. In the presence of Ca, K, Cl, Mg, and or Mn, bridging of weak bonds are made between oxy-complexes of these compounds, forming transient matrixes. Although the solution may show no apparent changes accompanying the metal-oxy-complex formation, the reactivity of the metal is first limited to be a catalyst between the oxygen and a reactant (organic compound), and then secondly by its transitional oxidation state(s), when all oxygen molecules are released.

Water, by forming a shell around a metal-oxy-complex, enables the complex to *retain its energy* for extended times (residual ORP). This water shell, because of its hydrogen bonding with organics, becomes a bridge that delivers the metal-oxy-complex to a weak spot on the organic compound. Depending on the properties of the organic reactant, the water shell will either help the metal-oxy-complex regenerate (regain active oxygen species) or decompose (loose all active oxygen molecules).

The metal-oxy-complex has specific and significant reactions not shown by either metal cation or oxygen anion alone. Although the metal-oxy-complex is destroyed in the course of reactions, and all minerals and metals return to their ground state of oxidation, the net effect of the reduction of oxidative state, is said to be catalytic. This catalytic property lowers the activation energy required for the oxidation of many organic materials.

JC 9465 is a chelation of minerals with oxygen in liquid form. The molecules are purposely designed to be weakly bound together such that when it comes in contact with inorganics, microorganisms and organic matter, it readily gives off ROS that aggressively oxidizes all desired contaminants. The reactivity of mineral oxychlorides is closely matched to ozone but without the problems associated with dissolving a gas in water.

Reactivity of JC 9465

1. Inorganics: A concentration of less than 1.0mg/L per mg/L of inorganics.
2. Pathogens: A concentration of 1.0 mg/L per 1,000 – 10,000 mg/L pathogen
3. Organics: A concentration of 1.0 – 8.0 mg/L per 1.0 mg/L organics
4. Residual: The byproducts of mineral oxychloride are mineral oxides that are effective against bacterial recontamination hence providing a protective residue that kills bacteria.

One uniqueness of mineral oxychlorides is that they release single atoms of oxygen imbedded within the molecular structure of organic material and the molecular make up of pathogens. Therefore, mineral oxychlorides perpetuate the release of highly active atoms of oxygen.

As a preamble to EPA registration, a microbe specialty laboratory conducted a series of microbe kill assays. Two organisms were chosen, as species of Salmonella (an aerobic microbe) and a species of Staphylococcus (an anaerobic microbe).

Microorganism: Salmonella enterica 10708

Solution	PPM	CFU/ml : Time Zero	CFU/ml : 30 min	Log Reduction
Hypochlorite	10	2.00 E +07	1.89E +05	2.02
Hypochlorite	100	1.58 E +07	<5	6.49
JC 9465	10	2.00 E +07	<5	6.60

JC 9465 capitalizes on the properties and principles of Haber-Weiss reactions. This test is indicative of the effectiveness these reactions and of JC 9465s oxygen radicals in killing microbes versus the poisoning a microbe as with chlorine, i.e. more can be accomplished with less.

When you keep in mind that in most commercial and agricultural applications, it is uncommon to find microbial CFU densities above 10^3 – 10^4, but the range of non-microbial organic loading places added consumption of oxygen radicals (ORP energy).

When oxygen and its by-products overwhelm a bacterium, the following sequence of events takes place.

1. Oxidation of scavengers.
2. Peroxidation/disruption of membrane layers.
3. Oxidation of thiol groups.
4. Enzyme inhibition.
5. Oxidation of nucleotides.
6. Impaired energy production.
7. Disruption of protein synthesis.
8. Cell death.

In nature, an obligate anaerobe is generally found in association with fellow anaerobes and facultative aerobes. Some of its neighbors will act beneficially as harmless scavengers. Others may accentuate the oxygen threat by secreting peroxide and possibly superoxide as a consequence of their own encounters with oxygen. Microbial activities may, therefore, contribute to the exogenous threat with the ultimate establishment of a comprehensive, dynamic equilibrium between the microbial consortium and its oxygen input.

By mimicking nature, by utilizing high energy oxygen radicals, JC 9465 easily breaks down nature's defenses to kill microbes. And by utilizing metabolically friendly minerals and metals, JC 9465s residues readily break down and are consumed by nature.

Versus conventional disinfectants, JC 9465 can accomplish a desired disinfection with less product and because of the effectiveness of JC 9465, its residues fall well below FDA MCL (maximum contaminant level).