NSW GOVERNMENT Department of Primary Industries

Ozone in recirculating aquaculture systems

Introduction

Recirculating Aquaculture Systems (RAS) provide potential advantages over pond or cage-based forms of aquaculture. These include flexibility in site selection, reduced water usage, lower effluent volumes, better environmental control, and higher intensity of production. However, as stock densities and levels of water re-use increase, wastes accumulate rapidly and environmental control becomes more difficult. Sophisticated systems capable of removing both particulate and dissolved organic wastes become necessary.

Conventional means of solids removal, such as microscreen filters and sedimentation tanks address the removal of coarse settleable and filterable solids, but not the removal of fine colloidal solids. Similarly, bacterial nitrification in biofilters removes dissolved ammonia and nitrite, but not other dissolved wastes. As the organic loading increases with intensity of production, the bacteria that convert nitrite to nitrate operate less efficiently, resulting in increased nitrite levels. The accumulation of fine colloidal solids, dissolved organics and nitrite in RAS can impair biofilter function, and increase biochemical oxygen demand and stress levels in the cultured stock. The net effect of this residual organic waste is a less stable, less productive system.

Increasing the daily water exchange rate in an RAS will remove accumulated colloidal solids, refractory organics and nitrite, to the detriment of water budgets and the cost of heating or cooling the system. The alternative method of removal is to breakdown these organic wastes using an oxidizing agent, such as ozone. Ozone is also widely used to sterilise supply and effluent water for RAS to remove pathogens. This advisory paper discusses use of ozone in RAS aquaculture, including methods of application, benefits of usage and potential risks.

The Chemistry of Ozone

Ozone is a clear, blue coloured gas that produces an easily detected pungent odour at concentrations as low as 5 parts per million (ppm) in atmospheric air. At higher concentrations of ozone the air becomes acrid and extremely hazardous to health. Ozone (O_3) is formed when an oxygen molecule (O_2) is forced to bond with a third atom of oxygen (O). The third atom is only loosely bound to the molecule, making ozone highly unstable. This property makes ozone an excellent oxidising agent and ideal for use in water treatment. However, storage is difficult and dangerous. For these reasons ozone must be generated on-site and used immediately.

Uses of Ozone

Ozone can be used in water treatment for the following purposes:

Removal of fine and colloidal solids

Fine and colloidal solids consist of particles 1-30 microns (m m) and 0.001-1 m m respectively. The small size of the particles enables the solids to remain in suspension and avoid most mechanical methods of separation. The accumulation of fine and colloidal solids can impair biofilter nitrification efficiencies and stress fish stocks.

Ozone removes fine and colloidal solids by causing clumping of the solids (microflocculation), which facilitates removal by foam-fractionation, filtration and sedimentation.

Removal of dissolved organic compounds

Dissolved organic compounds (DOC's) or refractory organics, give the water a characteristic tea-coloured stain. DOC's are non-biodegradable and accumulate according to feed input, water exchange rate and the rate of solids removal. High levels of DOC's can stress fish and reduce nitrification efficiencies of the biofilter.

Ozone removes dissolved organics by:

- oxidation into products that are more readily nitrified in the biofilter;
- including precipitation, which enables removal of waste particles by conventional filtration or sedimentation.

Removal of Nitrite

Nitrite can accumulate as production intensifies and organic loadings on the biofilter increase. Bacteria that process ammonia into nitrite (*Nitrosomonas* spp) operates more efficiently under high organic loadings than bacteria that process nitrite to nitrate (*Nitrobacter*) and levels of nitrite rise accordingly.

High levels of nitrite can be toxic to fish. Data available for silver perch, *Bidyanus bidyanus* indicates levels of nitrite as low as 2.8 parts per million (ppm) can reduce growth of fingerlings by 5%.

Ozone removes nitrite by:

- direct oxidation to nitrate;
- reducing organic loading, which improves biofiltration efficiency and nitrification.

Disinfection

The high stocking densities, associated fish stress and increased nutrient loads found in RAS create an ideal environment for fish pathogens. An important step in reducing the risk of disease outbreaks in RAS is the use of standard quarantine procedures for any fish introduced. Facilities using surface waters, including RAS and flow-through hatchery systems, are also interested in reducing the pathogen load introduced via the source water. The disinfection of effluent waters before introduction to the environment is also crucial to prevent the translocation of exotic diseases.

Ozone can effectively inactivate a range of bacterial, viral, fungal and protozoan fish pathogens. The effectiveness of ozone treatment depends on ozone concentration, length of ozone exposure (contact time), pathogen loads and levels of organic matter. If high levels of organic matter are present, the demand created by oxidising the organic matter can make it difficult to maintain enough residual ozone for effective disinfection.

Production of Ozone

Most commercially available ozone generators use either corona discharge or an ultraviolet (UV) light source to produce ozone. In corona discharge generation, a high-energy electric field is established between two metallic plates and dried air or oxygen gas is fed through the plates. The electrical energy excites a proportion of the oxygen molecules creating atoms of oxygen (O). The oxygen atoms then bond to oxygen molecules in the feed gas to create ozone.

UV light of a certain wavelength (140-190nm) can be used to excite and break the oxygen molecules to generate ozone in a similar way. UV generators are less expensive to purchase than corona discharge generators, but at present is a much less energy efficient way of producing ozone.

Use of oxygen as the feed gas increases the yield of ozone from both UV and corona discharge generation substantially when compared with dried air, but has an associated cost.

Ozone Application

System specifications

The design of the ozone reactor or contact vessel is very important for safe, successful ozonation. There is a range of reactors available using various designs to transfer ozone to the water. Designs include fine bubble diffusers, turbine contactors, injectors, deep u-tube reactors, packed columns, static mixers and spray contact chambers. Some designs are also used for oxygen transfer or aeration. Each design has advantages and disadvantages not discussed here.

Important considerations when choosing a reactor include:

- ozone transfer efficiency;
- leak-free design and construction;
- construction with ozone resistant materials.

Materials used in an ozone treatment system must be highly resistant or inert to ozone. Use of improper materials can lead to erosion of the unit and cause dangerous and costly leakages. Such systems are not suitable for the long-term application of ozone and require on-going, high replacement costs. The generation of ozone in systems with substandard materials is also less efficient as ozone is lost as the materials of the reactor are oxidised. The use of some plastics, such as polyvinyl chloride (PVC) and polycarbonate is not recommended for long-term applications for this reason. Galvanised steel is also not recommended.

Stainless steel contact chambers and piping are recommended for use with ozone. Valves should be made of stainless steel, with gaskets and membranes of Teflon or similar.

Treatment Regimes

Ozone can be applied continuously, as a series of treatments per day or as a single batch treatment per day. Application in most situations can be linked to the feeding strategy employed in the culture system. Three to four hours after feeding fish, the concentrations of ammonia, dissolved organics and other wastes products reach a maximum. If fish are fed several times during the day, a series of ozone treatments can be introduced after each feed to target the associated rise in waste levels. If feed is introduced 24 hours per day, water quality degrades continuously and so ozone application should be continuous. A single batch ozone treatment can be used to target rises in waste levels in the system associated with a moderate feed event or to treat batches of exchange or inlet water from the supply source.

Continuous ozonation is beneficial when compared to batch and serial treatments because water quality remains relatively stable. However, the lower costs of serial and batch ozonation make these treatments regimes viable management options.

The required amount of ozone for treatment in an RAS is usually calculated according to the daily feed rate. Rates of 10-15 g of ozone per kilogram of feed are generally recommended to reduce accumulated organics. Any background organic loadings of the source water used for the RAS should also be taken into account.

If disinfection is the primary goal of ozonation, the amount of ozone necessary is largely dependent on the background organic loading of the water to be treated. In pure water, residual concentrations of 0.01-0.1 ppm ozone for periods as short as 15 seconds can be effective in reducing bacterial loads. However, in water with organic loadings the residual ozone concentration and/or contact time of ozone must be increased to produce significant disinfection. Natural waters (seawater, brackish and freshwaters) generally require residual concentrations of between 0.1-0.2 ppm ozone and contact times of 1-5 minutes for disinfection. Aquaculture effluent generally requires between 0.2-0.4 ppm residual ozone for 1-5 minutes for significant disinfection to occur after oxidation of organics.

The optimum rate of ozone for disinfection is highly variable and represents the sum of ozone demands from dissolved organics, colloidal solids, nitrate and disinfection. In many situations in RAS, the cost of production of sufficient residual ozone for complete disinfection after all other ozone demands are met is prohibitive. However, some reduction in pathogen loads can be achieved using moderate levels of ozone, and water quality improvements are considerable.

Disinfection of exchange and effluent water is more cost effective than treating the entire system due to the relatively small volumes treated. Disinfection of source water with ozone, in combination with quarantine procedures for incoming stock, reduces the risk of disease outbreak within the system.

Site of Application

Ozone is reported to be toxic to a wide range of fresh and salt-water organisms at residual concentrations between 0.01 ppm and 0.1 ppm. When deciding where to introduce ozone the effect of residual concentrations from the reactor on either the biofilter or fish stocks should be carefully considered. There are several locations in a RAS where ozone may be added depending on the desired outcome.

- *Oxygen feed gas* - A common method of introduction uses existing oxygen transfer systems to add ozone with the oxygen feed gas. This generally occurs after the biofilter and just prior to the culture tank. Due to the proximity to the culture tank this method carries a moderate risk of exposing fish to residual concentrations of ozone. By retaining water in a contact chamber (de-ozonation unit) for several minutes before it passes to the culture tanks, this risk can be reduced. Advantages of this option include reduction of pathogen loads and nitrite levels immediately prior to contact with fish stocks.
- *Pre-biofilter* - Addition of ozone before the biofilter is also popular. This method carries a lower risk of exposing fish stocks to residual ozone. Any residuals present must first pass through the biofilter and are used in the oxidation of biofilms. In this way the biofilter effectively buffers the fish stocks from toxic effects of ozone. However, if levels of residual are too high performance of the biofilter may be affected, leading to decreased nitrification. An advantage of applying ozone before the biofilter is that the oxygen produced as a reaction end product of ozone increases dissolved oxygen levels in the biofilter. This is particularly beneficial in submerged biofilters. However, for trickling biofilters any by-product oxygen is effectively lost to the atmosphere.
- *Incoming water supply* - Ozone can be introduced into the incoming supply water as it enters the building to achieve disinfection. This prevents entry of pathogen loads to the system and enables isolation of healthy stock. For RAS relying on surface water supplies disinfection at this point is very important. It should be noted that ozonation of incoming water alone would not address the build-up of organics within the system.
- *Effluent* - Conversely, ozone can be used to disinfect effluent exchange water before it leaves the building to prevent exotic disease loads from entering the environment. This can be done most effectively on bath loads of effluent prior to release to irrigation or discharge systems.

Ozonation of water prior to coarse solids removal is not recommended. Treatment of coarse solids by ozonation is prohibitively expensive as levels of residual ozone must be increased to address the additional ozone demand.

Direct treatment of the culture tank is not recommended. This method carries a high risk of exposing fish stocks to residual ozone concentrations.

Ozonation of brackish or seawater results in the production of different by-product oxidants to freshwater. Ozone reacts with bromide and chloride ions in saltwater to produce relatively stable oxidants that are toxic to aquatic organisms. Use of ozone in saltwater systems is usually restricted to batch treatment of water separate to the main recirculating flow. Activated carbon filtration can be used to remove residual ozone and other oxidants from ozonated saltwater.

Measuring Ozone in RAS

The direct measurement of ozone in a water sample is generally achieved using colorimetric test kits and spectrophotometry. However, these methods can be too coarse to detect the low residual levels lethal to some fish species and are unsuitable for continuous in-flow monitoring. A common way of providing some level of continuous in-flow monitoring for ozone is the use of oxidation-reduction potential (ORP) probes. Rather than measure ozone directly, an ORP probe measures the total capacity, in millivolts (mV), or various oxidants in a solution to oxidise an electrode. By keeping ORP measurements within a certain range, the levels of total oxidants can be controlled, which gives indirect control over ozone. A safe ORP level of freshwater fish culture is generally considered to be 300 mV.

Many systems automate ozonation by linking ORP measurement and the ozone generator, so that the generator switches off once the required ORP is reached and cuts back in when ORP drops again. Factors such as pH, temperature and species cultured will determine the exact targeted ORP level. However, due to the lack of direct measurement of ozone and because ORP probes can take several minutes to register a charge in ORP, any use of ORP to measure and control ozone application is approximate. For this reason it is recommended that ozone control using ORP measurements allows for some error and limits are set conservatively. Other water quality parameters, particularly nitrite, should also be monitored in close association to ORP and used to gauge the effect of ozonation.

The Risks

Ozone is a very effective oxidising agent for use in water treatment and reduction of pathogen loads in RAS. However, use of any chemical of this nature is accompanied by considerable risks. RAS viability may be threatened in several ways.

- The reduction of nitrite levels by ozone carries a risk. The biofilter receives less nitrite and the population of bacteria responsible for processing nitrite to nitrate diminishes. If any disruption to ozonation occurs, dangerous spikes in nitrite concentration can subsequently develop.
- High residual ozone concentrations are a risk to cultured fish stocks causing gross tissue damage and stock mortalities.
- High residual ozone concentrations are a risk to bacterial films on the biofilter. Disruption to biofilter performance can cause large fluctuations in ammonia and nitrite levels. This can have a lethal effect on fish stocks or at the very least reduce stock health and growth performance.

It is recommended that a de-ozonation unit be installed directly after ozone application in a RAS to prevent toxic residual levels. This should be done regardless of the location of ozone application in the system. A simple do-ozonation unit consists of a contact chamber to increase the retention time of water, allowing ozone to degrade. Alternatively, an in-line activated carbon filter or biofilter can also function as a de-ozonation unit. Degassing of residual ozone also occurs in packed column aerators and trickle filters. Any residual ozone gas should be vented from the RAS building and destroyed before release.

Ozone is extremely toxic and any exposure to humans constitutes a serious health hazard. Decrease in lung function, aggravation of asthma, throat irritation and cough, chest pain, shortness of breath and the inflammation of lung tissue are typical symptoms of ozone exposure. In cases of prolonged or severe exposure, chronic respiratory illnesses such as emphysema, chronic bronchitis and premature aging of the lungs may occur.

Exposure standards for residual ozone of various Australian and International occupational health and safety administrations range between 0.05 and 0.1 ppm for an 8 hour work period and a maximum single dosage of 0.3 ppm for less than 10 minutes. Workcover Australia has a maximum exposure standard for residual ozone of 0.1 ppm for 8 hours.

It is therefore important to repeat the requirements of a leak-free ozone reactor made of suitable ozone resistant materials. Venting of sheds or areas of a RAS where ozone is used is also highly recommended. Humans can detect low levels of residual ozone as a sharp, pungent odour, but continued exposure can quickly dull the senses. For this reason perceived odour should not be used as an indicator of ozone presence.

Test-kits for the detection of air-borne ozone are commercially available and are a useful tool in helping to ensure the safety of RAS operators.