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THE EFFECT CHLORINE DIOXIDE AS A DISINFECTANT HAS ON UJAMS EFFLUENT

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Submitted In Partial Fulfillment Of The Requirements For The Award Of The Degree Of Bachelor Of Science In Natural Resources (Fisheries And Aquatic Sciences)

Declaration

"I hereby declare that that this work is the product of my own research efforts, undertaken under the supervision of Mr A Samakupa (The Effect Chlorine Dioxide as a Disinfectant has on Ujams Effluent) and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly and appropriately acknowledged."

Signature…….

Names……..

Student number.....

Certification

This is to certify that this report has been examined and approved for the award of the degree of Bachelor of Science in Natural Resources (Fisheries and Aquatic Sciences) of the University of Namibia.

External Examiner ….……

Internal Examiner ……..……

Supervisor ……..

Head of Department …..……

Acknowledgement

First and foremost I would like to thank God for giving me the opportunity to be able to complete my first degree at The University of Namibia. He has provided me with the strength to face anything (Phil 3:14), and He has placed His favor on me during my tests, assignments, presentations and examinations.

I would like to wholeheartedly thank, all staff members at Gammams Scientific Laboratory from the Chemistry, Microbiology and the sampling personal who actively participated in the completion of the project. This includes assistances with the sample collection, sample processing and sample analysis, especially Mr. G J Menge. I would like to thank him for his valuable supervision and guidance in carrying out this work and for the guidance, support and enlightenment.

Special thanks to Mr A Samakupa who gave undivided attention, advice and guidance, despite all other pending lecturing activities. Without you I wouldn't have been able to submit this project.

Dedication

I dedicate this paper to my sisters Helga, Alina, Sonja and Linekeela. Thank you so much for the encouragement and love, you always believed in me even during times that I didn't believe in myself. My success is all for you. Love you and GOD BE WITH YOU ALWAYS.

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ABSTRACT

The effect chlorine dioxide as a disinfectant has on Ujams effluent was investigated at two dosage levels (2 mg/l and 5 mg/l) and two contact time levels (3 and 7 hours) in a 2 factor experiment. The study was conducted on effluent sample collected from Ujams Wastewater Treatment Plant, by determining the feacal coliform and *C. perfringens* counts after chlorine dioxide treatment. Four replicates were set up for the four treatments: 2 mg/l at 3 hours, 2 mg/l at 7 hours, 5 mg/l at 3 hours and 5 mg/l at 7 hours. After the samples were treated, the microbiological analysis was done using Mf-c Agar for feacal coliform and Perfringens Agar Base (TSC) according to The American Public Health Association: Standard Methods for the Examination of Water and Wastewater. The bacteria were counted and results were obtained. Clostridium perfringens interaction was $p = 0.577$, contact time $p = 0.391$ with the mean of 3 hours is 42 438 and 7 hours is 40 688, dosage level $p = 0.001$ and the means for the two levels are 2 mg/l is 45 938 and for 5 mg/l is 37 188. For feacal coliform, interaction was $p = 0.984$, contact time levels $p = 0.700$ with the mean of 3 hours is 97 744 and 7 hours is 5 135, dosage level $p = 0.002$ and the means for the levels are 2 mg/l is 616 7869 and for 5 mg/l is 37 188. It was found that there is no interaction between the contact time levels and dosage levels for both C. perfringens and feacal coliform counts. The results show that the contact time of 7 hours is more superior to 3 hours. Dosage level of 5 mg/l removes more bacterial counts compared to the 2 mg/l dosage level.

CHAPTER ONE

INTRODUCTION

Namibia is one of the most arid countries in Africa, with only two perennial rivers in the North or South, of the country. Most parts of the country mainly depend on water supply from boreholes and surface dams in ephemeral rivers including the City of Windhoek. Due to water shortages from these supplies, it has prompted the City of Windhoek to look for alternatives to augment the water supply (Menge, 2006). The increase in the population has placed great pressure on the water demand, and it is for this reason that authorities had to find a way to reduce the pressure.

In 1968 the first reclamation plant called the Old Goreangab Reclamation Plant (OGRP) started its operations. This has a capacity of producing ca. 4,800 m^3 of water per day. Severe droughts experienced in 1992 and 1997 led to overhaul of the reclamation process (Haarhoff and van der Merwe,1995). In September 2002 the New Goreangab Reclamation Plant (NGRP) was commissioned with a capacity of producing 21,000 $m^3$of water per day (Menge, 2006).

A country like Namibia decision's to directly reclaim its effluent is faced with many challenges like quality targets, economic considerations, micro pollutants research, biological and virological testing. The success of Windhoek's reclamation plant is due to policies put in place and ongoing research with tests done by external laboratories or by the City of Windhoek Scientific Services. The domestic and industrial effluent in Windhoek is treated separately with the former at The Gammams wastewater treatment plant (GWTP) and Otjomuise wastewater treatment plant (OWTP) while the later at Ujams Wastewater Treatment Plant.

In general, the water used by the industries and domestically becomes contaminated and therefore requires treatment or purification (Pybus, 2002). On the other hand different industries have effluents with different characteristics and some are more difficult to treat than others. Domestic effluent is usually treated with disinfectants such as ozone and chlorine.

In Namibia and South Africa respectively, effluent released into the environment has to comply with general standard (DWAF) to ensure that it is safe and will not cause any harm to the environment. Ujams, wastewater treatment plant (WTP) was built in 1973 to treat industrial effluent from the Northern Industrial area as well as small section of the residential development within the drainage basin in Windhoek. The wastewater stream is generated inter alia by abattoirs, beer brewery, a leather tannery. These industries usually produce high levels of organic and inorganic compounds that make treatment of the effluent in the ponds at Ujams treatment plant ineffective. Since the effluent is not properly treated it does not meet the required standard and is also harmful to the environment.

Chlorine has been used as disinfectant to treat effluent. However there are by-products that are produced as a result. The use of other disinfectant such as chlorine dioxide has been proposed as it does not result in by-product and it is more effective than chlorine. Chlorine dioxide is also preferred as it is cheaper than ozone. In order to improve the safety of the effluent at Ujams, a study was conducted to determine the effects of chlorine dioxide as a disinfectant on, faecal coliform and *Clostridium perfringens* counts.

LITERATURE REVIEW

1.1 OVERVIEW OF WATER PURIFICATION SYSTEMS IN WINDHOEK

In Windhoek there are three water purification plants namely Gammams wastewater treatment plant, Otjomuise wastewater treatment and Ujams wastewater treatment plant. The first two plants treat domestic wastewater while the latter treats industrial effluent. According to Menge (2006) the following is the description of the purification plants:

1.1.1 The Gammams Wastewater Treatment Plant

The Gammams wastewater treatment plant (GWTP) process consists of: primary settling and anaerobic digestion with drying beds. Stream a: biofilters with secondary settling and 3 maturation ponds. Stream b: biological nutrient removal activated sludge plant (UCT or modified Johannesburg or Ludzack-Ettinger configuration) and 8 maturation ponds. Especially in winter months the nitrification in the biofilter system is low. To produce sufficient effluent of high quality for drinking water reclamation and irrigation, the biofilter system was integrated into the activated sludge system in 2002.

1.1.2 The Otjomuise Wastewater Treatment Plant

The Otjomuise wastewater treatment plant (OWTP) process consists of: No primary settling, biological nutrient removal activated sludge plant (UCT configuration), no maturation ponds. The waste streams of NGRP are pumped to OWTP, contributing to one third of its influent. It contains a high concentration of iron from $FeCl^3$ dosing, thus phosphate is removed to below <1.0 mg/l. Nitrification is complete with very good denitrification.

1.1.3 Ujams Wastewater Treatment Plant

Ujams Wastewater Treatment plant however uses the oxidation ponds system. Oxidation ponds are the simplest aerobic biological treatment used to treat wastewater. Within an oxidation pond, heterotrophic bacteria degrade organic matter in the sewage which results in production of cellular material and minerals. These products support the growth of algae which allows further decomposition on the organic matter by producing oxygen. The production of this oxygen replenishes the oxygen used by the heterotrophic bacteria (Atlas, 1995).

Soluble organics in the pond are metabolized by the bacteria and the end product of that metabolism, such as carbon dioxide, ammonium and nitrate ions, and phosphate ions, become available for growth of the algae. As the autotrophic algae generate new cells using solar energy, oxygen is produced which can be used by the heterotrophic bacteria. Surface aeration due to wind action rarely meet the oxygen needed for the oxidation of the organic matter in the pond, and the oxygen produced is essential to maintain aerobic conditions.

The term “aerobic” does not completely describe the microbial reactions that take place in an oxidation pond. While ample dissolved oxygen may exist in the upper portion of the pond, there may be little or no dissolved oxygen in the lower depths. Oxidation ponds are therefore designed and loaded so that the anaerobic conditions in the lower portions have little impact on the quality of effluent from the ponds (Noyes, 1991). The effluent however may contain undesirable concentrations of algae and, especially in the winter when less oxygen is liberated by photosynthesis which may produce unpleasant odours. Usually increase in load from the community, lack of surface wind, and reduction in mixing led to the pond being overloaded resulting in obnoxious odors from the ponds (McKinney, 1968).

1.2 DISINFECTANTS USED IN WASTEWATER TREATMENT

Disinfection was first used in 2000 B.C. where it was found that exposing to water to sunlight, boiling and filtering water through charcoal was means of disinfecting water (Aly, 1998). Disinfectants used were electrolysis, copper and silver, in 1908 the first large-scale chlorination was used in public water in the United States (Aly, 1998).

Chlorination was the first disinfectant that was used at the beginning of the 20th century (Bitton, 1999). Eventually, other disinfectants where also discovered such as chlorine dioxide, ozone, potassium permanganate, peroxone, chloramines and UV light. A treatment plant may decide to use one or a combination of disinfectants.

1.2.1 Chlorine

Chlorine is a very effective disinfectant and, it is relatively easy to handle. The capital cost of chlorine installation are low, cost effective, simple to dose, measure and it has a relatively good residual effect. Due to its oxidizing power, chlorine has been used to controls taste and color, prevent algal growth, and remove iron and manganese. It is also an effective biocide. However, high dosage can cause taste and odour problems as well produce trihalomethanes (THM) which are known to be carcinogenic. Moreover, the by-product and chlorine gas formed are hazardous and corrosive (Pieterse, 1988).

1.2.2 Chlorine Dioxide

Chlorine dioxide is excellent for the control of phenolic taste and odour problems in wastewater. It is also effective in the oxidation of manganese and iron. It is a better and more superior to chlorine as it does not result in the formation of trihalomethanes (THMs) viricide. Chlorine

dioxide is easy to generate, provides residuals that enhances to the clarification process and oxidizes iron, manganese and sulfides. The only problem is that chlorine dioxide is generated on-site due to the costs involved in training, operation and maintenance. Chlorine dioxide is a small volatile and very strong molecule that reacts with other substances by way of oxidation rather than substitution; it is a yellowish-green gas that has a similar odour to that of chlorine, with a molecular weight of 67.46. It is explosive in air at concentrations greater than 10% and this property prohibits chlorine dioxide from being transported and so it must be generated on-site (Benarde, 1965). Chlorine dioxide used in the experiment is in a solution form which can be generated in three ways:

Sodium chlorite + chlorine = chlorine dioxide + sodium chloride

Sodium chlorite + hypochlorous acid = chlorine dioxide + sodium chloride + sodium hydroxide

Sodium chlorite + hydrochloric acid = chlorine dioxide + sodium chloride + water

The antimicrobial activity of chlorine dioxide is extremely broad spectrum. It is highly effective against gram negative and gram positive, aerobic and anaerobic, spore forming and non spore forming pathogenic and saprophytic bacteria as well as bacterial spores (Benarde, 1965**).** Chlorine dioxide is more superior oxidant over other disinfectants in that its oxidation is more selective and according to the U.S. Environmental Protection Agency (EPA) Oxidant Manual Guide (1999), chlorine dioxide has two disinfection mechanisms. The first kind of disinfection mechanism, chlorine dioxide readily reacts with amino acids tyrosine, tryptophan, cysteine, rhethionine. The first two amino acids are aromatic and the last two contain sulphur. The reaction between the aromatic acids and the chlorine dioxide might be responsible for the observed destruction of cellular structure components. The second kind of disinfection mechanism is the physiological functions. It has been suggested that the primary mechanism for

inactivation was the disruption of protein synthesis. Chlorine dioxide affects the cell membrane by changing membrane proteins and fats and by prevention of inhalation. Chlorine dioxide penetrates the cell wall; viruses are eliminated when chlorine dioxide reacts with peptone, a water-soluble substance that originates from hydrolysis of proteins to amino acids – by prevention of protein formation. Chlorine dioxide is more effective against viruses than chlorine and it removes biofilms and kills pathogenic microorganisms, and this prevents biofilm formation as it remains active in the system for a long time.

1.2.3 Ozone

Compared to chlorine, ozone reacts rapidly and it is effective in reducing colour and odour thereby improving suspended solids removal. The biocidal activity is not influenced by pH; it is more effective than chlorine, chloramines, and chlorine dioxide for inactivation of viruses and protozoan, (Cryptosporidium and *Giardia*). However the capital cost of ozone and is very high and must be generated on site (Masten & Davies, 1994; Isaac, 1996). Ozone like chlorine forms DBP and it is highly corrosive and toxic. Also it provides no residual.

1.2.4 Potassium permanganate

Potassium permanganate ($KMnO_4$) is used to control taste and odors, remove color, control biological growth and remove iron and manganese. Potassium permanganate may be used to control the formation of THMs and other DBPs (Hazen & Sawyer, 1992). It is easy to transport, store, and apply, and it controls nuisance organisms. However long contact time is required when using potassium permanganate. Moreover potassium permanganate gives water a pink color, and it so toxic that it irritates the skin and mucous membrane.

1.2.5 Peroxone

Peroxone is effective in oxidizing the difficult-to-treat organics, such as taste and odor compounds. It has shown to be effective in oxidizing halogenated compounds. Oxidation is much faster when using peroxone compared to ozone. Moreover, it is a strong oxidant and contact with personnel is extremely dangerous. It can be stored on site, but deteriorates gradually even when stored correctly. Its ability to oxidize iron and manganese is less effective than ozone.

1.2.6 UV light

Pathogenic organisms are more susceptible to UV than they are to chlorine (Sobsey, 1989; Kaur et al, 1994). It is easy to handle and there are no chemical additives. However the capital cost of operation is high and it produces disinfection by-products (DBP). Moreover, it has a short-lived residual and it is not easy to measure (Freese & Nozaic, 2004).

1.3 FACTORS THAT INFLUENCE THE EFFICIENCY OF DISINFECTANTS

Several factors have an influence on the disinfection process of water and wastewater. According to Bitton (1999), there are several factors that influence the efficiency of disinfection. These include amongst others type of disinfection, type of microorganisms, disinfection concentration and contact time, effect on pH, temperature, chemical and physical interference with disinfection and protective effect of macroinvertebrates.

1.3.1 Types of microorganisms:

Disinfectants interact differently with microbial cells. Their target sites include peptidoglycan layer, cytoplasmic membrane, outer membrane, structural proteins amongst other things. Pathogens appear in two forms, vegetative cell or in spore form with the spore form being very

resistant to disinfection. Resistance to disinfectants varies also among vegetative bacteria and among strains belonging to the same species. In general, the resistance to disinfection goes along the following order:

Vegetative bacteria < enteric viruses < spore-forming bacteria < protozoan cysts

Inactivation of pathogens with disinfectants increases with time and ideally should follow first-order kinetics.

Disinfectants like chlorine dioxide are more efficient at high pH while chlorine is more efficient at the pH of 6. Pathogen and parasite inactivation increases as temperature increases.

1.3.3 Chemical and physical interference with disinfection

Organic and inorganic matter in water that cause turbidity can interfere with the detection of coliforms in water and it can reduce the disinfection efficiency of disinfectants. Chemical compounds interfering with disinfection are inorganic and organic nitrogenous compounds, ion, manganese, and hydrogen sulfide. Dissolved organic compounds also exert a chlorine demand: their presence results in reduced disinfection efficiency (Bitton, 1999).

1.3.4 Protective effect of macroinvertebrates

Macroinvertebrates may enter and colonize water distribution systems (Levy et al., 1984: Small and Greaves, 1968). Nematodes may ingest viral and bacterial pathogens and thus protect these microorganisms from chlorine action (Chang et al., 1960). *Hyalella azteca*, an amphipod, protects *E.coli* and *Enterobacter cloacae* from chlorination (Levy et al., 1984). Enteropathogenic bacteria are also protected from chlorine action when ingested by protozoa (King et al., 1988).

1.4 BACTERIAL INDICATORS

1.4.1 *Clostridium perfringens*

For the experiment two indicators of the disinfection quality where used. *Clostridium perfringens* is an important sulphite reducing clostridia and is normally present in human and animal faecal. It is produces both a resistant spore (survival phase) and a sensitive vegetative cell (growth phase). *C. perfringens* is mainly an indicator of contamination from point sources, as well as a conservative tracer of past fecal contamination (Ohio Water Microbiology Laboratory, 2005). This makes it a good indicator on the efficiency of a disinfectant. The resistant spores of *C.perfringens* survive in water and in the environment for much longer time than *E.coli* and other feacal indicator organisms. The resistant spores are not usually inactivated by chlorination. By their presence with the absence of vegetative cells suggest that the disinfection treatment was effective. However, the presence of spores of *C.perfringens* in water does not normally pose any health problem (www.microbiolodyprocedure.com/water). The spores are normal inhabitants of the large intestine of humans and other warm blooded animals like cows, sheep, and pigs and are constantly released through their feaces. The presence of these bacteria indicates specifically the recent feacal pollution as these organisms cannot survive for long periods in water. Current research suggests that *Clostridium perfringens* could be better suited as indicators of drinking water quality and treatment (Payment and Franco, 1993). *C.perfringens* can survive longer than coliform thus it was used in this experiment to determine the effectiveness of chlorine and chlorine dioxide.

1.4.2 Feacal coliform

Feacal coliforms are total coliforms that are able to grow at elevated temperatures and are often, but not always, of fecal origin. The group of bacteria that are passed through the feacal excrement of humans, livestock and wildlife and a common member is E.coli. Feacal coliform indicates only the presence of pathogens, especially viruses and parasites.

Coliform bacteria, specifically the subgroup of thermo tolerant or fecal coliforms, are widely used to evaluate the effect of wastewater disinfection processes. But other alternative indicators have been proposed, this includes fecal streptococci, acid-fast bacteria, yeasts, total plate counts, and Pseudomonas aeruginosa.. This has led the author of this research paper to choose *Clostridium perfringens* and feacal coliform as the bacterial indicators to be used in the experiment.

1.5 PREVIOUS STUDIES

Chlorine dioxide is used in the United States to counteract taste and odour problems and disinfect drinking water (Aly & Faust, 1998). Chlorine dioxide reacts more rapidly than chlorine, and requires, lower dosages than chlorine to achieve similar performance results. Chlorine dioxide has been found in a number of investigations to be equal or superior to chlorine as a disinfectant. Aieta et al., (1980) conducted an experiment comparing chlorine dioxide and chlorine in wastewater disinfection, it showed that at the dosage of 2 and 5 mg/l chlorine dioxide gives better results than chlorine and at the dosage of 10 mg/l, chlorine is more effective in reducing the bacterial counts then chlorine dioxide. The contact time used was 30 minutes.

1.6 PROBLEM STATEMENT

Ujams receives in the order of 4 000m³ of wastewater per day and it was designed around the oxidation ponds system. Within an oxidation pond, heterotrophic bacteria degrade organic matter in the sewage which results in production of cellular material and minerals. The production of these supports the growth of algae in the oxidation pond. Growth of algal populations allows further decomposition on the organic matter by producing oxygen. The production of this oxygen replenishes the oxygen used by the heterotrophic bacteria (Atlas, 1995). Ujams consists of 15 ponds of which four are anaerobic and the rest are facultative ponds; due to the high COD loading on the anaerobic ponds at Ujams Water Treatment Plant, the rate of biodegration is so intense that the sludge layer is often brought to the surface by the trapped gas to form a surface sludge layer. This has resulted in the pond's water having a red colour which is due to a very favorable bacterium that indicates that the system is overloaded organically and it is one that should have been aerobic but now functions anaerobically, meaning the effluent does not comply with the DWAF standard and so it pose as a health risk to the environment. The treatment process is ineffective as a result of the sludge build-up and it is related to poor quality of final effluent which causes fairly severe odour problems.

1.6.1 Rationale/Justification for the study

The effluent is transported to Klein Windhoek River via a stream. Downstream there is a settlement called Mix. The residents of the settlement use water from the stream for laundry and to bathe, this could lead to serious health complications to the people as *E.coli* is present in the water. Another reason for the need to treat the water with chlorine dioxide is that a new estate is being build that overlooks the Klein Windhoek river, but with the pungent smell the developers are complaining to the City of Windhoek to correct this problem.

1.7 OVERALL AIM

Chlorine dioxide has been proposed as a superior disinfectant. Therefore, the overall objective of the present study is to assess the effectiveness of chlorine dioxide on feacal coliforms and *Clostridium perfringens* as a disinfectant.

1.7.1 Specific objectives

- To determine which dosage level results in low bacterial counts.
- To determine which contact time level results in low bacterial counts.

1.8 HYPOTHESIS

H_{01}: There is no significant difference in the bacterial counts between the dosage levels.

H_{11}: There is a significant difference in the bacterial counts between the dosage levels.

H_{01}: There is no significant difference in the bacterial counts between the contact time levels.

H_{11}: There is a significant difference in the bacterial counts between the contact time levels.

CHAPTER TWO

MATERIALS AND METHODS

2.1 COLLECTION OF EFFLUENT

Sample of effluent were collected at the Ujams dams and placed in a sterile 5 litre plastic bottle. Samples were specifically collected at a stream flowing from the final pond at point 14 in figure 1 twice a week on Mondays and Wednesdays from 2 – 11 August 2010. Gloves were worn when the effluent was collected from the stream. The container was placed in a cool area during the transportation to the Gammams laboratory which was about 30 minutes drive from Ujams Water Treatment Plant. The sample was kept in a dark cool place and not allowing sun light as this could affect the results. On arrival to the laboratory the sample preparation immediately started. During the whole process of sampling sterile conditions will be maintained during collection, preservation, storage, and analysis of effluent samples for microbiological analyses.

Upon arrival at the lab, the water was poured into labeled beakers the different treatments where added to the labeled beakers. The beakers where placed on a stirring apparatus with stirring rods at slow stirring speed, this was done to ensure that the disinfectant is mixed with the sample. The stirring continued for 3 hours for that particular contact time and the other one was at the contact time of 7 hours. After 3 and 7 hours of slow speed stirring, 1 ml of thiosulfate was added and allowed ±1 minute of stirring, this stops chlorine dioxide from reacting with any bacteria in the sample.

2.2 EXPERIMENTAL DESIGN

Effluent from Ujams was used during the experiment. Sample used was collected from the plant on the morning of the experiment. The sample was subjected to two different dosage levels (2mg/l and 5 mg/l) of chlorine dioxide at two different contact times (3 hours and 7 hours), then the bacterial counts of fecal coliform and *Clostridium perfringens* were measured to determine the effects of chlorine dioxide on the counts in the sample. The treatments are presented in appendix 1.

2.3 STATISTICAL ANALYSIS OF THE DATA

The statistical software used is Genstat. In the statistical analysis of the data, a Complete Randomized Block Design (CRBD) and a one sample t-test were used; the treatments were assigned to experimental units with the principles of randomization. The two designs were used as they are the simplest designs and it's useful when the experimental units are essentially homogenous and it's normally used for laboratory experiments. The bacterial counts were analyzed using Genstat statistical software of which a two way ANOVA table was used to analyze the data. Data was analyzed using the Least Significant Difference (LSD) at a critical region, probability value as 0.05 to show whether there are any significant differences between compared means. The feacal coliform data was transformed by a log-10; this was to ensure that data are in the same range.

2.4 PREPARATIONS OF STANDARD SOLUTIONS

- Chlorine dioxide stock solution was prepared by adding 10 ml of hydrochloric acid and 10 ml of sodium chlorite into a 100 ml volumetric flask and filled it with milli-q water then mix. 5 ml of the stock solution is pipette into a 100 ml volumetric flask and fill it up with milli-q water. The standard solution was calibrated by pipetting 1.0, 2.0, 3.0 and 4.0 ml of chlorine dioxide into different 100 ml Erlenmeyer flasks filled with 100 ml of milli-q water. The colorimeter was used to determine the concentration of chlorine dioxide in each flask. Using the formula $C_1V_1=C_2V_2$, C_1 was calculated and the average C_1 of the stock solution was calculated. With the average C_1, the volume of the concentration to be added to the beakers was calculated using the intended concentration (2 and 5 mg/l) multiplied by 500, divided by the average stock solution concentration (USEPA, 1999).
- Sodium thiosulfate standard solution was prepared by diluting 10.0g of the 0.1M sodium thiosulfate to 100 ml with recently boiled distilled water (AWWA, 2005).

2.5 PREPARATION OF MEDIA AND RINGER SOLUTION

The following method of media preparations are adopted from The American Public Health Association: Standard Methods for the Examination of Water and Wastewater, 21st edition, 2005.

2.5.1 Feacal Coliform

52 grams of M-Fc Agar was weighed in a plastic beaker under a safety cabinet, and then poured into a 5 litre Erlenmeyer flask. 1000 ml of reagent water was added and a magnet stirrer was

placed in the flask and covered then it was allowed to boil to dissolve the agar completely. Then 10 ml of 1% Rosolic Acid and continue heating for one minute. The media was left to cool and then poured under a safety cabinet into Petri dishes. A third of the each Petri dish is filled with media; the dishes were placed in sterile plastic container and stored in the refrigerator at below 10°C.

2.5.2 *Clostridium perfringens*

23 grams of perfringens (TSC) agar base was added to a 5 litre Erlenmeyer flasks and 500 ml of boiled reagent water was added. A magnetic stirrer was placed in the flask and covered with aluminum foil. The flask is then placed into the autoclave at 121°C for 15 minutes. The media is left to cool at approximately 50°C. Then 25 ml of egg yolk emulsion was added and 1 vial of re-hydrate TSC supplement was added to the agar base and mixed well under a safety cabinet. A volume of the cooled media is poured into the plates to fill a third of the plates. After the agar has solidified the lids are closed and the plates are stacked and placed in a basket marked the type of medium, analyst, date prepared and expiry date. Plates are stored below 10°C.

2.5.3Preparation of Ringer Solution

One tablet was removed from the container using a clean spatula. The tablet was added to 500 ml of reagent grade water. The tablet was dissolved using a clean stirring rod. 95 mt was dispensed into 100 ml dilution bottles with loosely closed caps. The bottles were then sterilized by autoclaving for 15 minutes at 121°C.

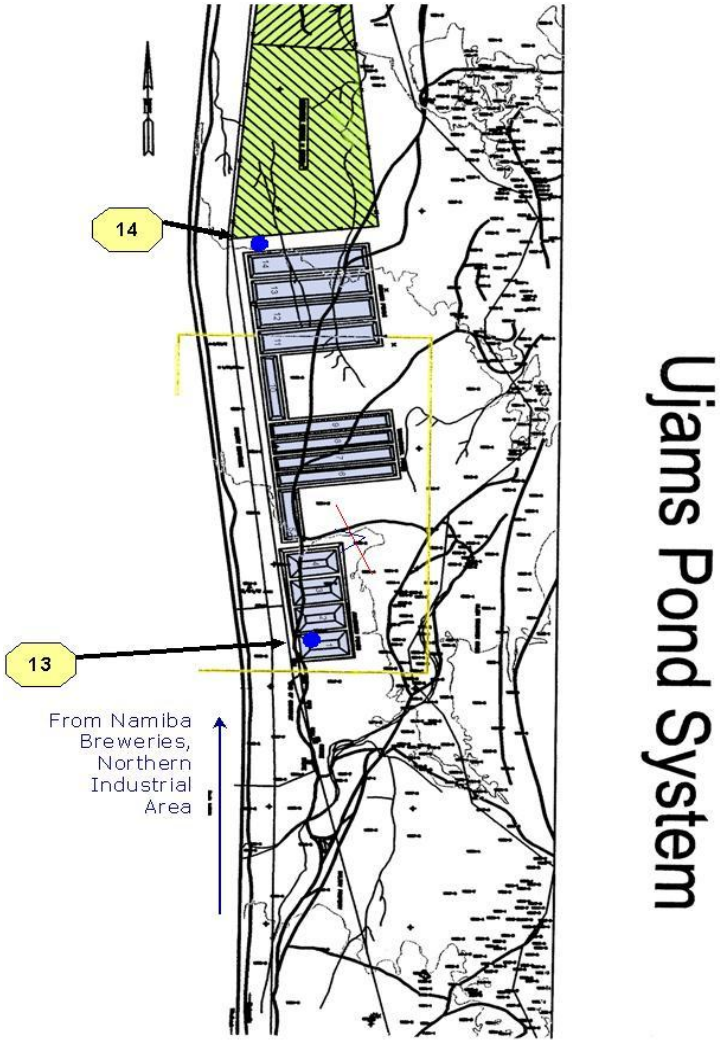

Figure 1: Ujams Pond Layout.

Source: Polytechnic of Namibia

2.6 PREPARATIONS OF SAMPLES AND ANALYSIS

2.6.1 Feacal Coliform and *Clostridium perfringens*

Membrane filtration involves passing a known volume of liquid through a cellulose acetate membrane, with pore sizes of 0.2µm or 0.4µm. Bacteria, yeast and moulds are removed from the liquid and precipitated on the membrane surface. When the membrane is transferred to a pad-soaked in a nutrient medium (growth medium) or an agar plate and incubated, nutrient diffuse through the membrane so that the organism can grow on the membrane surface giving visible colonies that can be counted. Clostridium cells (*Clostridium perfringens*) and feacal coliform are detected and enumerated using this method. The water samples are diluted at different dilutions. The samples are then filtered though a 0.4µm filter paper, the filter paper was placed on media in labeled Perfringens (TSC) Agar for clostridium cells and labeled M-FC Agar for feacal coliform. The clostridium plates are then incubated anaerobically for 48 ±3 hours at 35 ± 2°C. The feacal coliform plates were incubated for 24 ± 2 hours at 44.5 ± 1°C. Colony counting is done immediately after the incubation period.

CHAPTER THREE

RESULTS

3.1 BACTERIAL COUNTS

The following are the results obtained after the colony counting of feacal coliform and *C. perfringens* plates.

Table 1:. Results after colony counting.

	Contact time	**Clostridium viable (counts per 100 ml)**	**Feacal coliform (counts per 100 ml)**
2 August			
Control		45 000	185 000
2 mg/l	3	41 500	5 000
5 mg/l	3	38 500	950
2 mg/l	7	40 500	950
5 mg/l	7	36 000	1 300

4 August			
Control		45 000	105 000
2 mg/l	3	43 500	35 500
5 mg/l	3	39 000	0
2 mg/l	7	49 000	20 500
5 mg/l	7	39 500	50
9 August			
Control		75 000	340 000
2 mg/l	3	48 000	64 500
5 mg/l	3	34 000	1 000
2 mg/l	7	42 000	41 500
5 mg/l	7	30 000	0
11 August			
Control		55 000	105 5000

2 mg/l	3	56 500	675 000
5 mg/l	3	38 500	0
2 mg/l	7	46 500	500 000
5 mg/l	7	42 000	0

3.2 *C. perfringens*

Interpretations: Clostridium ANOVA Appendix 2 and one sample t-test Appendix 4

Interaction

Do not reject H_o. There is no significant interaction between contact time and dosage ($p = 0.577$). The mean count does not significantly change when at different combinations of contact time and dosage.

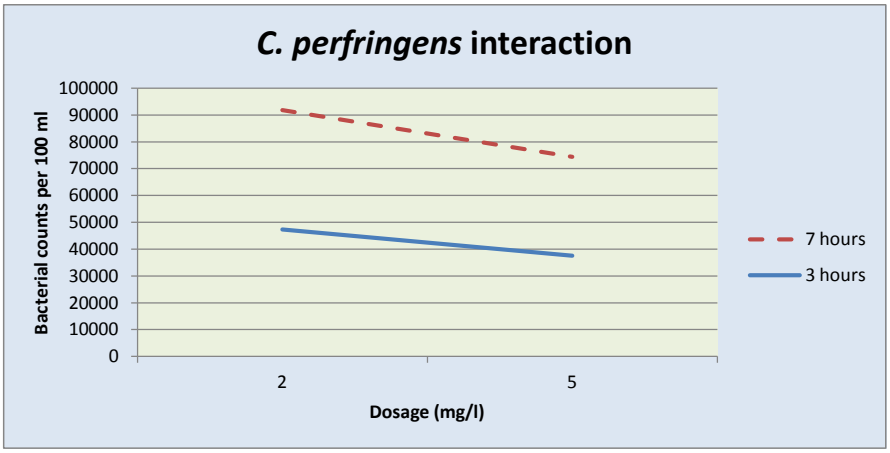

Figure 2: Dosage and contact time interaction of C. perfringens.

Contact time

Do not reject H_o. There is no significant difference in the mean Clostridium counts amongst the two contact time levels (p = 0.391). The clostridium counts are significantly higher at contact time of 3 hours (mean = 424 38) compared to contact time of 7 hours (mean = 40 688).

Dosage

Do not reject H_o. There is no significant difference in the mean Clostridium counts amongst the two dosage levels (p = 0.001). The clostridium counts are significantly higher at dosage 2 mg/l (mean = 45 938) compared to dosage 5 mg/l (mean = 37 188).

The F probability of the interaction is p = 0.577, meaning the clostridium count does not significantly change at different combinations of contact time and dosage. For the two contact

Comment [S1]: What you have presented here should is only the results. As such move this paragraphs to the results. When discussing explain what you think about your results and also compare it to previous findings; is your results unique or somebody has the same findings....discuss what needs to be considered as well.

time level p = 0.391, meaning there is no significant difference between the two contact time levels. The counts are significantly higher at the contact time of 3 hours (mean = 424 38) compared to 7 hours contact time (mean = 40 688). The dosage levels for the clostridium counts p = 0.001, the counts are significantly higher at dosage 2 mg/l (mean = 45 938) compared to dosage 5 mg/l (mean = 37 188).

3.3 FEACAL COLIFORM

Interpretations: Feacal coliform ANOVA Appendix 3 and one sample t-test Appendix 5

Interaction

Do not reject H_o. There is no significant interaction between contact time and dosage. The mean feacal coliform count does not significantly change when at different combinations of contact time and dosage (p = 0.984).

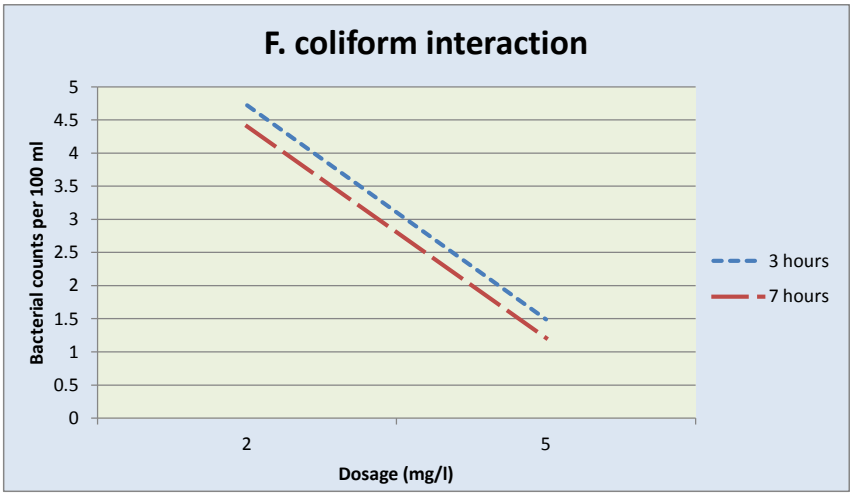

Figure 3: Dosage and contact time interaction graph – Feacal coliform.

Contact time

Do not reject H_o. There is no significant difference in the mean feacal coliform counts amongst the two contact time levels (p =0.700). The feacal coliform counts are significantly higher at contact time of 3 hours (mean = 3.11) compared to contact time 7 hours (mean = 2.80).

Dosage

Reject H_o. There is significant difference in the mean feacal coliform counts amongst the two dosage levels (p = 0.002). The feacal coliform counts is significantly higher at dosage 2 mg/l (mean = 4.56) compared to dosage 5 mg/l (mean = 2.80).

The mean feacal coliform count does not significantly change when at different combinations of contact time and dosage (p = 0.984), meaning there is no significant interaction between dosage and contact time. F probability for feacal coliform counts of the two contact time are not

significantly different (p = 0.700) the counts are significantly higher at contact time of 3 hours (mean = 97 744) compared to 7 hours (p = 5 135). For the dosage levels, the mean feacal coliform counts are significantly different. The counts at the dosage of 2 mg/l (mean = 167 869) are significantly higher to dosage 5 mg/l (mean = 37 188).

CHAPTER FOUR

DISCUSSION

The results show that chlorine dioxide is indeed effective as a disinfectant in removing a significant amount of counts of feacal coliform and *Clostridium perfringens.* For the interaction between the contact time and dosage it is evident that there is no significant interaction for both the bacterial indicators, which is unexpected as one would think that at the maximum dosage and contact time the counts will be significantly different from those of the minimum dosage and contact time .This occurrence can be due to the fact that the dosage levels are not too far apart as there is just a 3 mg/l difference. Feacal coliform dosages levels show that their results are significantly different which proves the findings of Aieta et al., (1980) that chlorine dioxide at the dosage 2 mg/l and 5 mg/l are quite effective in reducing the bacterial counts with the higher dosage of 5 mg/l showing to remove more bacteria's counts compared to the 2 mg/l dosage. The dosage and contact time level counts of *C. perfringens* showed that there is no significant difference in the results obtained, proving literature that clostridium can be resistant to disinfectants even though some of the counts were removed, this could probably be due that the dosage used was too low to remove significant amounts of the bacteria even though the contact time was sufficient. The results show that the contact time of 7 hours is more superior to 3 hours. Dosage level of 5 mg/l removes more bacterial counts compared to the 2 mg/l dosage level.

CONCLUSION

From the results obtained, one can recommend that instead of using 2 dosages and contact times, more could be used thus increasing the number of treatments in the end and eventually increasing the residual value in the ANOVA to more than the 9 value that was obtained. It is evident, from the study, that chlorine dioxide is effective and has the potential of being used as a disinfectant in improving the effluent of Ujams. However, it is important to note that the longer a disinfectant is contact with the sample the better the removal of microorganisms. Moreover, the more concentrated the disinfectant, the better the removal of microorganisms. In conclusion, chlorine dioxide removes more feacal coliform bacteria compared to viable clostridium bacteria. This is might be because clostridium vegetative cells survive longer then feacal coliform even after contact with a disinfectant. From the results one can conclude that chlorine dioxide is effective in removing a significant amount of feacal coliform bacteria from the sample this is for all the four treatments. Chlorine dioxide's effect on clostridium viable cells shows that this bacterium which is resistant to disinfectant was not significantly removed by the disinfectant. This means that although the clostridium counts where reduced, it was only by a small fraction compared to that of feacal coliform. There is a need for further research to find out the effects of chlorine dioxide on a sample which is set at different temperature intervals and pH levels; this will help us to know how the disinfectant reacts towards different temperatures and pH levels.

RECOMMENDATION

One can recommend that more than two dosages and contact times be used, this is to increase the number of treatment. A turbidity test should be conducted on the sample to determine the effects of the one disinfectant on the physical characteristics of the sample and a reduction in turbidity can mean microorganisms have been removed.

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APPENDICES

Appendix 1

Experimental design

Number of sets	Contact Time	Dosages
1	3 hours	2 mg/l
2	7 hours	5 mg/l
3		
4		

Appendix 2

Two way ANOVA for data analysis of *Clostridium perfringens* counts

ANOVA

Source of variation	**Degree of freedom**	**Sum of Squares**	**Mean Square**	**F probability**
Week	3	141312500	47104167	
Contact Time	1	12250000	12250000	0.391
Dosage	1	306250000	30620000	0.001
Contact Time x Dosage	1	5062500	5062500	0.577
Residual	9	136062500	15118056	
Total	15	600937500		

APPENDIX 3

Two way ANOVA for data analysis of Feacal coliform counts

ANOVA

Source of variation	**Degree of freedom**	**Sum of Squares**	**Mean Square**	**F probability**
Week	3	0.730	0.243	
Contact Time	1	0.371	0.371	0.700
Dosage	1	41.264	41.264	0.002
Contact Time x Dosage	1	0.001	0.001	0.984
Residual	9	21.082	2.342	
Total	15	63.448		