Enhanced Electrokinetic (EK) Technology: A comparative study for inactivation of Clostridium perfringens spores and Reovirus in anaerobically digested biosolids

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ABSTRACT

Enhanced Electrokinetic (EK) Technology: A comparative study for inactivation of Clostridium perfringens spores and Reovirus in anaerobically digested biosolids

Elham Safaei Takhtehfouladi

Municipal wastewater treatment facilities convert soluble organics into biosolids. The governmental agencies encourage biosolids recycling. Millions of tons of this nutrient-rich material are being added to soil each year. USEPA regulations for the use and disposal of sludge, defines two types of biosolids with respect to pathogen reduction: Class A and Class B. In Quebec, biosolids are defined according to C-P-O classification and the standard regulation governing them is similar to USEPA. Class A status can be achieved by decreasing helminthes, viruses and pathogenic bacteria counts below threshold levels or maintaining certain conditions of e.g. time, temperature or pH.

Clostridium perfringen is a spore-forming thermophilic bacterium and has been suggested as an indicator for inactiavtion mechanisms other than temperature (as it is resistant to temperature). This organism is found in densities of 10^6 colony forming units (CFUs) per gram of solids in raw biosolids and has been suggested as an excellent surrogate for the *Ascaris* ova in biological systems such as composting and anaerobic digestion. *C. perfringens* spores exhibits similar resistance to physical and chemical agents and is hardier than *Ascaris* in high temperatures.

Reovirus (RV) belongs to the virus family Reoviridae which can be readily detected in feces. Reovirus is strongly resistance to common disinfectants can persist for long periods of time. Due to characteristics of this virus, it is considered as an indicator of enteric virus contamination.

The aim of this study was to assess enhanced electrokinetic (EK) technology impact on inactivation of *C. perfringens* and Reovirus with reference to criteria for Class A biosolids. A series of twenty seven (27) EK reactors in two experimental phases, filled with anaerobically digested sludge were investigated in bench scale in an attempt to simulate possible future full scale process.

A number of factors including electric field strengths, duration of exposure and response to different enhancement agents were investigated. The pH, electrical parameters, as well as quality of catholyte and anolyte were measured daily. The physicochemical characteristics of biosolid were analyzed after each treatment. The enumeration of *C. perfringens* spores in treated biosolids and Reovirus were carried out by a series of microbiological tests.

Result showed a relationship between *C. perfringens* and Reovirus inactivation degree and type of enhancer and electric field. A decrease of nutrients contents was also observed in EK reactors. It was found that electrokinetic phenomena expressed catalyze characteristics in respect to chemical enhancers and improved biocide effects. Enhanced EK demonstrated to be a successful technique for biosolids disinfections.

In the name of God

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A great thank to all.

Elham Safaei Takhtehfouldi
22, August 2007.
31, Mordad 1386.
Montréal, Canada.

To those who support me
with their love,
My Parents

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ACRONYMS AND ABBREVIATIONS

a_w	Water activity
BNQ	Bureau de Normalisation du Québec
CWA	Clean Water Act
DC	Direct current
DNA	Deoxyribonucleic acid
DOE	Design of Experiments
Eh	Oxidation potential
EK	Electrokinetic
EDL	Electrical double layer
EPA	Environmental Protection Agency
FRs	Fertilizing residuals
FC	Fecal Coliform
kGy	Kilo gray
LR	Log reduction
MPN	Most Probable Number
MOE	Ministry of Environment
NRC	National Research Council
ORP	Oxido-reduction potential
pI	Isoelectric point
RNA	Ribonucleic acid
RV	Reovirus
TS	Total solids

VFAs	Volatile Fatty Acids
WEF	Water Environmental Federation
WWT	Waste water treatment
WWTP	Waste water treatment plant

I-INTRODUCTION

1-1- Problem

The development of appropriate policies for the treatment and disposal of sewage sludge is a major issue for most countries throughout the world. For example, the amount of produced sewage sludge or biosolids in Canada is about 388,700 dry tons per year (Apedail, 2001). Similarly, the US Environmental Protection Agency (USEPA) has reported a production of 7 million dry metric tons of biosolids during the treatment process of 0.69 m^3 of sewage per person per day (CGER, 1996; Tenebaum, 1997). Generation is expected to double by the year 2010 (USEPA, 1995). Approximately 60 percent of biosolids is used for land application, and 40 percent disposed of in landfill or by incineration (NRC, 2002).

Through the proscription of ocean dumping of biosolids in 1992, the use of sewage sludge as soil amendments or for land reclamation has been increased to reduce the volume of sewage sludge that must be landfilled, incinerated, or disposed of at surface areas. Land application of biosolids was based on the belief that biosolids should be considered a resource rather than a waste (Ponugoti et al, 1997). However, biosolids may contain significant quantities of steroid hormones, alkyl phenols, heavy metals and pathogens which are a threat to environment and human health. In many communities heavy metals and toxic compounds may be reduced through source control before application (BEST, 2002). Nevertheless, biosolids originating from urban areas contain many human pathogens (Sidhu et al, 2001). Therefore, complex regulatory systems have been developed for reusing of biosolids with the intention of protecting human, animal and plant health, ground and surface water quality, enduring soil quality and soil

biodiversity. The system of managing sewage sludge in USA is known as the Round One Sewage Sludge Regulation (USEPA, 1995). Part 503 regulation categorizes biosolids as Class A or B, depending on the level of pathogenic organisms in the material, and describes specific processes to reduce pathogens to these levels. Each class meets defined standards; Class B has low levels of pathogens which rapidly die-off when applied to soils, basically becoming pathogen-free within a short period following application; Class A biosolids are essentially free of pathogens prior to land application (USEPA, 1999). In Québec biosolids is classified according to C-P-O classification and the standard regulation governing them is similar to USEPA.

The part 503 rule permits a combination of monitoring and "processes to significantly reduce pathogens (PSRP)", and "processes to further reduce pathogens (PFRP)" method for controlling pathogens in biosolids (Wright, 2001). Each of these technologies has its own advantages and disadvantages. For example, using radiation to produce Class A biosolids is an effective technology; however, it increases the energy costs. More importantly, the processes to reduce pathogens have no effect on heavy metals, and may not have effects on the level of the other trace pollutants in biosolids.

Conversely, some new technologies have upgrading effects on reducing heavy metals, pathogens, and other contaminants, as well as the volume of biosolids.

Electrokinetic (EK) treatment is one of these new technologies which has been previously applied to soil remediation. This technology is based on application of direct current (DC) within the contaminated matrix for removing pollutants through several EK phenomena. EK can be considered as a unique remediation method for the dewatering process, and removal of pathogens and metal through oxidation and reduction zones

which affect cell wall of microorganisms. This research investigated the enhancement of EK method through the introduction of various enhancers in order to develop a new technique for pathogen inactivation.

1-2- Purposes of the study

Based on a literature review (Chapter 2) viruses and *C. perfringens* spores (Payment et al., 1993, 1985; Venczel, 1997) can be inactivated using mixture of strong oxidants e.g. chlorine dioxide, hydrogen peroxide and other short-lived oxidants. On the other hand, electrokinetic treatment has been already successfully applied for the removal of metal (Esmaeily et al., 2006), dewatering (Esmaeily et al., 2006; Huang, 2007), inactivation of *Salmonella* and Fecal Coliforms (FC) (Esmaeily et al. 2006; Esmaeily, 2002; Huang, 2007) in biosolids. In most of these cases an amphoteric enhancer (ammonium phosphate) was also applied. It is necessary to verify how disinfecting oxidants affect the developed electrokinetic technology for biosolids management. Therefore, the main objective of this research was to assess the inactivation of *Clostridium perfringens* spores and Reovirus in electrokinetic management of anaerobically digested biosolids under different experimental conditions. Along with this primary objective, the following secondary objectives were taken into account to be fulfilled as a matter of course.

- An assessment of the inactivation by applying of different enhancement agents, exposure time and voltage gradient.
- To observe impact of EK and enhancement agents on characteristics of biosolids e.g. total solid, pH, ORP, volatile suspended solids, volatile fatty

acids (VFAs), nitrogen-ammonia, phosphate, chloride, sulfate, nitrate and nitrite.

- Defining optimum conditions for the best inactivation state.

II- LITERATURE REVIEW

2-1-Biosolids generation and characteristics

Due to regulatory and environmental pressures waste water treatment plants are moving away from unsustainable practices and towards the development of more valuable and sustainable alternatives. A by-product of these treatment plants is sewage sludge, recently called biosoilds. The term of biosolids was coined by the Name Change Task Force at Water Environmental Federation (WEF) in order to make distinction between raw, untreated sewage sludge, and treated, and tested sewage sludge that can be used as soil amendment (Tenebnaum, 1997).

In general, biosolids is by product of wastewater treatment activity which removed during the treatment process from the bottom of the settling tanks, bioreactors, and lagoons. Figure 2-1 illustrates biosolids production process (BEST, 2002).

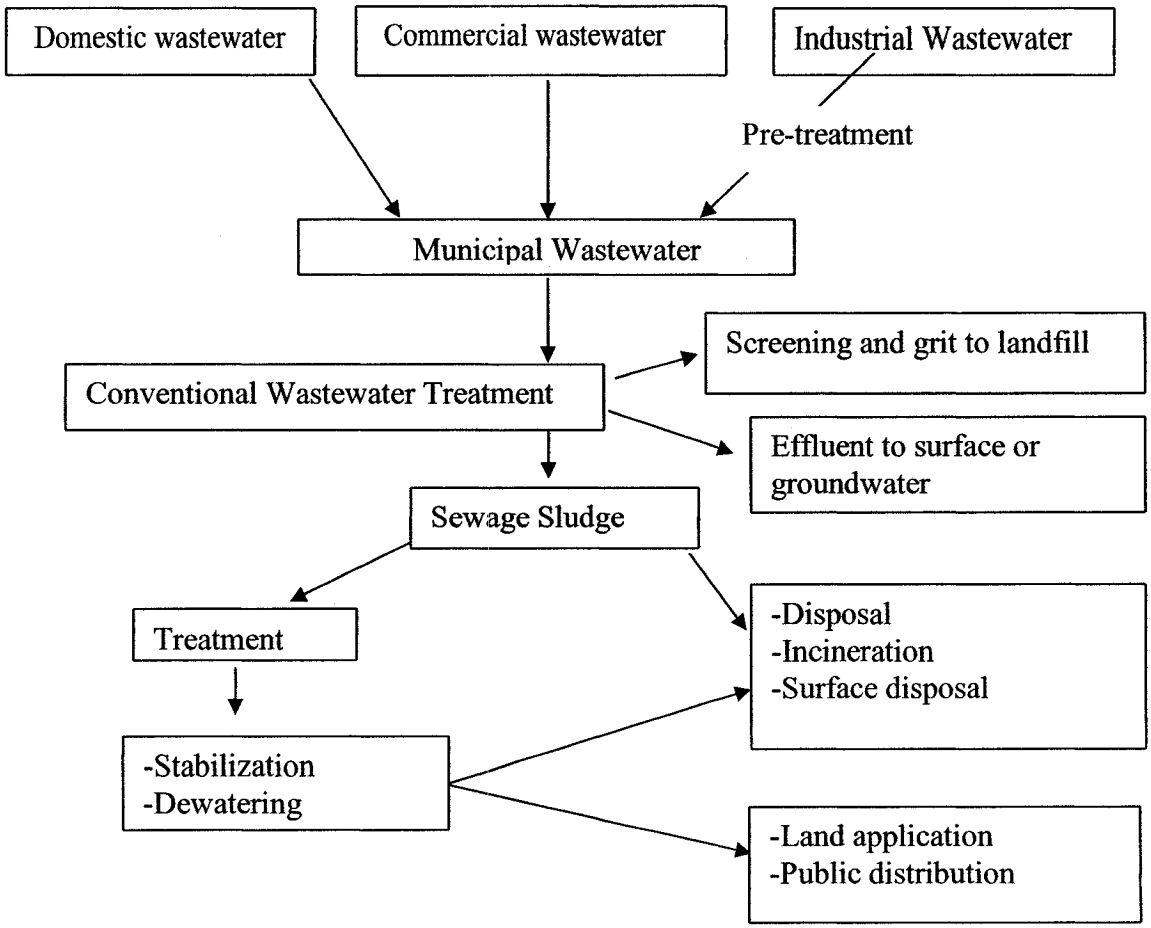


Figure 2-1: Biosolids production process (BEST, 2002)

Very often biosolids contains inorganic material, plant nutrients, trace elements, organic compounds as well as heavy metals, some pathogens and toxic organics. However, after passing through special treatments most of complex organic molecules are decomposed, and most of the pathogens killed (Tenebaum, 1997). A typical biosolids material may contain between 10 and 30% colloids (Karathanasis, 2006). Figure 2-2 shows the colloidal structure of biosolids.

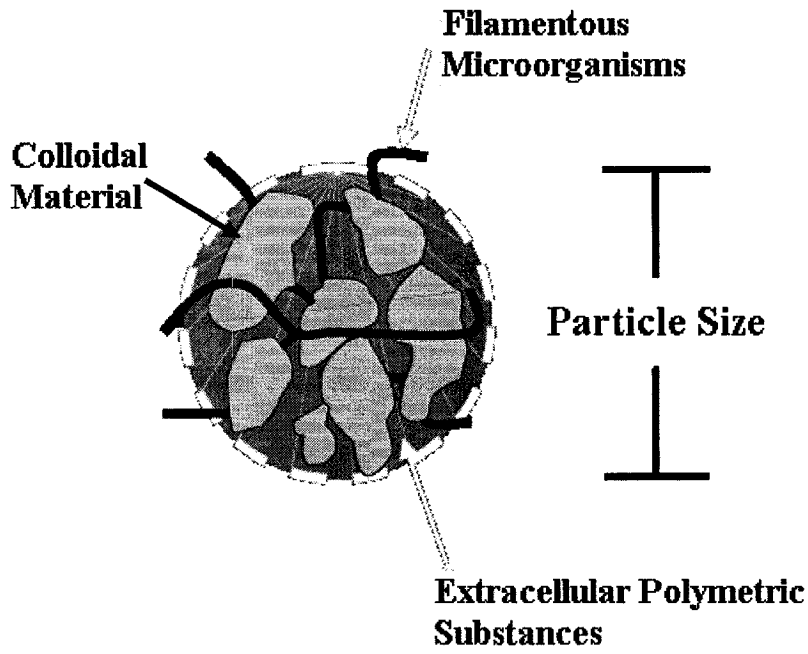


Figure 2-2: Colloidal structure of biosolids (Merlo et al., 2004)

The primary importance of colloids stems from their surface reactivity and charge characteristics. Characterization of size, shape, surface area, surface charge density and changes in surface charge permits to understand the processes of adsorption, flocculation, dispersion, transport within sludge and the resultant changes in sludge hydraulic properties as well as chemical migration (Hiemenz, 1997). For example, colloids are reactive not only because of their total surface area, but because of enhanced reactivity related to rough surfaces and highly energetic sites, as well as the effects of electrostatic charge (Summer et al., 1996).

The quantity and characteristics of biosolids produced at a wastewater treatment plants (WWTP) vary widely depending on the quantity and characteristic of the wastewater entering to plant, the type of plant, method of operation and the type of subsequent treatment applied to the biosolids. Higher levels of treatment increase the

concentration of contaminants in biosolids. Also adding chemicals during treatment to improve the quality of treatment increases the concentration of those chemicals in biosolids (CGER, 1996). At large, the characteristic of biosolids can be broken down in three domains: physical, chemical and biological. The physical specification consists of total solid and organic content; it gives general idea on biosolids processability and handleability. The chemical characteristics are pertinent with presence of the trace elements, toxic materials and metal concentrations. The biological properties are defined as the amount of pathogens and microbial activity in biosolids (Epstein, 2003).

2-1-1- Wastewater treatment plant

The facility where the raw wastewater is treated is called a wastewater treatment plant (WWTP). There are three typical stages involved in each WWTP called primary, secondary and tertiary treatment (CGER, 1996). The whole process consists of separation of solids from the wastewater stream, converting dissolved biological matter into a solid mass with help of indigenous, water-borne bacteria, and at the end, neutralizing, and disposal of the biological solids (BEST, 2002). The addition of chemicals can also help to make the flux of sludge more stable, and to reduce the amount of nutrients (e.g. phosphate). The final effluent of these processes after disinfection can be discharged to a receptor (e.g. river, streams and lake) or reused.

The sludge produced during the treatment process must be treated, and disposed of in a safe way. The main idea of this stage of treatment is to reduce the amount of organic matter and the number of disease causing microorganisms existing in the sludge to the standard levels (USEPA, 1995). To do so, the most common treatment choices consist of anaerobic digestion, aerobic digestion and composting (NGSMI, 2003).

Choosing a suitable biosolids treatment method is reliant on the amount of solids produced and site-specific conditions. For large-scale facilities, anaerobic digestion is more common; however, for small-scale treatment plants, composting and aerobic digestion are more preferable (PDEP, 2006). The typical process of treating wastewater is illustrated in Figure 2-3.

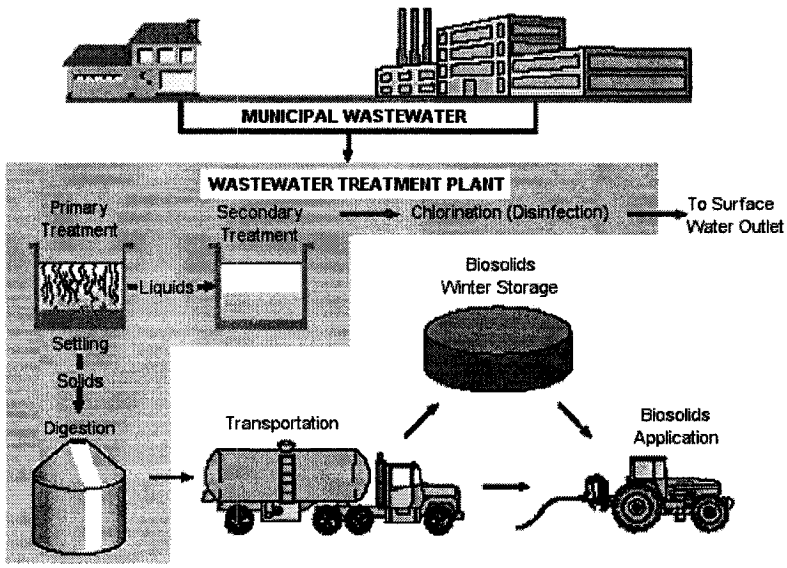


Figure 2-3: A pictorial procedure of a typical WWTP (OMAFRA, 2005)

2-2- Biosolids constituents

Typical compositions of untreated sludge and digested biosolids are important factors in considering ultimate disposal methods of biosolids; they consist of organic content, nutrients, pathogens, metals and toxic organics. Some of the most concerned components of biosolids are given in the following section (CGER, 1996).

2-2-1- Metals in biosolids

Biosolids contain trace elements, including heavy metals. Metals can enter wastewater from discharges of commercial and industrial drains, residential dwellings and groundwater infiltration. Considering their high specific surface area and metal sorption capacity, it is expected that the vast majority of the metal load will be associated with the colloid fraction of biosolids (USEPA, 1999).

Typical metals include cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu) and zinc (Zn). The most important source of Cu in biosolids is from pipes, taps, and roofs. Some business activities (e.g. car washes) followed by households are the dominant source of Cd and Pb. In USA, and Canada the amount of metals entering from industrial drains is regulated by the governmental pretreatment program, and control regulations. In general, source control programs have evidently reduced the amount of metals found in biosolids (Epstein, 2003; Raihan, 2006). Table 2-1 sets the standard level of different metals in biosolids.

Table 2-1: Standard level of metals in biosolids (NGSMI, 2003)

	Unit	**Class A**	**Class B**	**Fertilizer Act of Canada**
Arsenic	mg/kg total solid	41	75	75
Cadmium	mg/kg total solid	39	85	20
Chromium	mg/kg total solid	1,200	3,000	-
Copper	mg/kg total solid	1,500	4,000	-
Lead	mg/kg total solid	300	840	500
Mercury	mg/kg total solid	17	57	5
Molybdenum	mg/kg total solid	Under review	75	20
Nickel	mg/kg total solid	420	420	180
Selenium	mg/kg total solid	36	100	14
Zinc	mg/kg total solid	2,800	7,500	1,850

2-2-2- Principal pathogens in biosolids

The source of pathogens in biosolids is mostly human excreta (Wright, 2001). The concentration of pathogens in biosolids is related to the type and concentration of pathogens in the source of sewage as well as treatment efficiency of WWTP for removing or eliminating pathogens. On the whole, human excreta-related pathogens are classified in four groups consists of viruses, bacteria, protozoa, and worms (helminthes) (Gerba, 2002; Wright, 2001). There are a large number of recognized pathogens of concern in domestic sewage sludge shown in Table 2-2. In addition to recognized pathogens a list of newly identified and emerged hazards has been added to be considered in the risk assessment studies. This list includes (Warnes et al., 2004; Wright, 2001):

- *E.coli* O157:H7
- Viruses generally
- *Camplyobacter*
- *Listeria* spp. and *Listeria monocytogenes* in particular
- *Salmonella typhimurium* phage type DT 104
- *Cryptosporidium*
- *Giardia*
- Bovine Spongiform Encephalopathy (BSE) agent

Table 2-2: Principal pathogens of concern in biosolids (Wright, 2001)

Group	Group
Bacteria *Salmonella* spp. *Shigella* spp. *Yersinia* spp. *Escherichia coli* (pathogenic strains) *Pseudomonas* *Clostridium* *Bacillus* *Listeria* *Vibrio* *Mycobactrium* *Leptosperia* *Camplylobacterium* *Staphylococcus* *Streptococcus*	**Viruses** Polioviruses Coaxackieviruses A and B Echoviruses Reoviruses Adenoviruses Rotaviruses Astroviruses Calciviruses Coronaviruses Norwalk agent and other Small round viruses (SRSV)
Protozoa *Crypotosporidium* *Entamaeba* *Giardia* *Balantidium* *Toxoplasma* *Sarcocystis*	**Fungi** *Aspergillus* *Phialophora* *Geotrichum* *Trichophyton spp.* *Edidermophyton*
Nematodes *Ascaris* *Toxocara* *Tricuris* *Ancylostoma*	**Yeast** *Candida* *Cryptococcus* *Ttrichosporon*
Cestodes *Tania* *Diphyllobothrium* *Echninoccus*	**Phytopathogens** *Botrytis cinerea* *Clavibacter michigansis pv. sepedonicus* *Globodera pallida*

2-2-2-1-Indicator pathogens

Detection of every pathogenic organism in the biosolids is a difficult and time consuming work due to presence of the wide array of the microbes. As a solution, indicator organisms are used because they are easy to detect. Indicator bacteria are

selected groups of microorganisms which are found to indicate the likelihood of the presence of special case of pathogens (Fujioka, 1985).

The part 503 USEPA regulation, considers FC as indicator organisms in two ways. FC density can be used as an indicator of health hazard to classify Class A biosolids. Moreover, FC density is applied to assess if *Salmonella* spp. has repopulated when Class A biosolids are stored before land application (USEPA, 1999). In the other word, it is an indicator of WWT efficiency. Although FC can be considered as an indicator of treatment efficiency; however, since they are capable of regrowth, their use as an indicator for public-health hazard is less justified. Furthermore, some other pathogens are more resistance than FC, emphasizing the potential for understanding a specific health hazard (Byamukama et al., 2005).

Clostridium perfringens has been suggested by US National Research Council (USNRC) as a better indicator for monitoring day-to-day regulatory compliance, and to evaluate the effectiveness of biosolids disinfection processes as a tracer for less strong indicators, and for absence of protozoan parasites and viruses. It can be fund in the sewage sludge in numbers several orders of magnitude greater than those in soil. It counts of approximately 10^6 spores /g total solid (TS) in untreated sludge and 10^1 to 10^2 spores per mL in effluents from WWTP (Fujioka, 1985; Hill, 1993).

C. perfringens spores have been demonstrated to be useful replacement for monitoring the quality control of WWTP for removal not only *Crytosporidium* oocysts but also *Giardia* cycts and viruses as well as *Ascaris* eggs in biosolids (Reimers et al., 1991; Venczel et al., 1997;Warners, 2004).

The *Ascaris* inactivation is employed to verify if a disinfection process produces Class A biosolids; however, the direct method of measuring *Ascaris* ova presently needs recovering the eggs from biosolids, incubating them in a culture for 21-28 days, and then microscopically scrutinizing the ova for viability. The method is expensive, time-consuming, and ova not present in all biosolids constantly (Bean et al., 2003). Rather using an easy, inexpensive technique to screen for inactivation of helminth eggs by replacing microbial spores would be useful. *C. perfringens* is a good indicator for *Ascaris* inactivation by anaerobic digestion (Bean et al., 2003; BEST, 2002; Mackes et al., 2004; Payment et al, 1993).

Payment et al. (1985, 1993) used *C. perfringens* and coliphages as indicators of treatment efficiency of water, and observed that they can correlate with *Cryptosporidium*, *Giardia lamblia* and human enteric viruses.

2-2-2-1-1- Characteristics of Clostridium perfringens

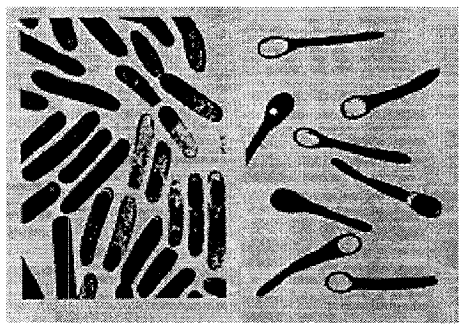
Clostridium perfringens is a gram-positive, rod-shaped, non motile, sulfate-reducing anaerobe which ferments lactose with stormy gas production (Fuioka et al., 1985; Skanavis et al., 2001). It is part of normal flora of the human digest tract with population of 0.5 percent of the fecal microflora (ICR, 1996; McNamara et al., 1998; Payment et al., 1993).

C. perfeingens has ability to form environmentally stable endospores which are mostly formed in the intestine tract of humans and animals. It produces single oval subterminal spores less than 1 μm in diameter during adverse conditions (ICR, 1996). The process of sporulation consists of eight stages from 0 to VII according to morphological changes in the cell (Paustian, 2001). The endospore that develops is highly

refractile body formed within the cell. Spores are resistance to heat, drying, and chemical disinfectants which would kill the vegetative cell (Adams et al., 2000; Bates, 1997; Hook et al., 1996; McClane, 1997). Figure 2-4 demonstrates the *Clostridium* spore (a) along with its schematic structure (b). The spore consists of three parts (Paustian, 2001):

- Core: which is dehydrated cytoplasm containing deoxyribonucleic acid (DNA), enzymes and other part of cytoplasmic system
- Cortex: is an altered cell wall/ peptidoglycan layer that is not cross-linked any more. Production of cortex takes place at the stage III of sporulation cycle followed by synthesis of small acid soluble spore proteins (SASPs) and glocuse dehydrogenase in the stage IV. These compounds play an important role in protection of the spore from UV and other chemical stress.
- Coates: consist of several different layers of impermeable proteins outside of cortex. This coat forms in the stage V of sporulation period, and like SASPs is responsible of spores' resistance.

(a)



(b)

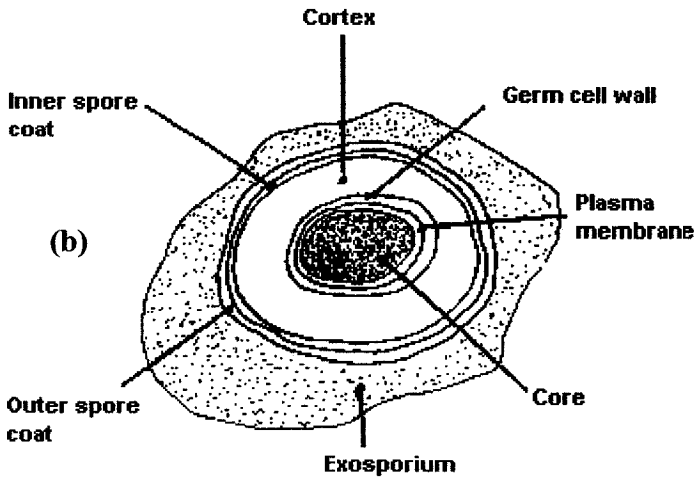


Figure 2-4: Clostridium spores (a) (GIUES, 2007) with its schematic structure (b) (Heritage, 2006)

C. perfringens plays an auxiliary role in water examination. Clostridial spores survive longer than coliforms, *Escherichia coli (E.coli)* or enterococci, consequently they are used as an indicator of past fecal pollution (ICR, 1996). The organism is insensitive to oxidation with ozone (McClane, 1997; Roberts et al., 1996). Vegetative forms of *C. perfeingens* can growth in the temperature range of 12 – 50°*C* while the optimum growth has been shown in 43 – 47°*C* (Duncan et al., 1967; Hook et al., 1996). Boiling temperature kills rapidly the growth form of bacterium while spores are heat resistance, and some survive at boiling temperature for 1 hour (McClane, 1997). Sublethal heat will

activate many spores of *C. perfringens* strains, and increase germination under conditions not permissive for germination of dormant spores (Craven et al., 1985; Levinson et al., 1978). D values (Decimal reduction time) for spores at $100°C$ vary among strains from 0.31 min to more than 38 min. D time for vegetative cells at $60°C$ is $5.4-14.5$ min (Roberts et al., 1996). Vegetative cells also are very sensitive to freezing temperature. Freezing temperature at $-17.7°C$ for 30 days kills 93.5% of bacteria; they decline more slowly under refrigeration (Smith et al, 2004). Spores; however, survive both refrigeration and freezing. Cells will not grow at less than $12°C$. Generally heat resistance strain of *C. perfeingens* can be activated by 10-20 min at $75-80°C$ (Doyle, 2002). Different spores show D value from 1.2 to 3.4 kGy (Kile gray) to radiation depending on strains (Doyle, 2002; Murrel, 1989; Roberts et al, 1996).

The minimum water activity (a_w) for growth is from 0.93 to 0.97 depending on the solute. Spore germination occurs in the same a_w range as growth. Spores are highly resistance to desiccation while vegetation cells are not very tolerant of low a_w (Murrel, 1989).

Optimum pH for growth of these bacteria is between pH 6.0 to 7.0. Growth is possible over the pH range of 5.5 to 9.0. Good sporulation occurs between pH 6.0 to 8.0. Cells will die after several days below pH 5.0 and above pH 8.3 (Veitch et al., 1996). Oxidation potential (Eh) values above 300mV inhibit the growth of cell (Murrel, 1989).

High concentrations of ethanol result in spore injury and death (Craven et al., 1985).

Chlorination of water can not inactivate clostridal spores; however, they are not considered as a hazard to health in potable water (Adams et al., 2000). An amount of 5

mg/liter free chlorine produced a 1.4 log unit reduction (LR) in 4 hours of exposure, while a mixed oxidant system resulted in a LR bigger than 3 at the same time (Venczel, 1997). Inactivation of *C. perfringens* spores by a mixed-oxidant disinfectant and free chlorine was examined by Venczel et al. (1997). They did a comparative study of inactivation kinetics of *Cryptosporidium parum* oocysts and *C. perfringens* spores exposed to a 5-mg total oxidant dose of mixed-oxidant solution. In their research they generated mixture of oxidants by the electrolysis of a solution of sodium chloride. They found an electrochemically produced solution of mixed oxidant at applied dose can inactivate both *Cryptosporidium parum* oocysts, and *C. perfringens* spores in pH 7.0 buffered water at $25°C$, with >2.3-$\log_{10}$-unit (>99.5%) inactivation after 4 hours exposure time.

❖ Small acid soluble spore proteins (SASPs)

There are different factors which play important role in spore resistance including low water content, spore metabolic dormancy and high impermeability of a spore's coat (Klobutcher et al., 2006). However, presence of SASPs as a protective coat around the spore DNA is a major factor to protect the spore from environmental stresses. They are among a large number of proteins which are synthesized during sporulation period, and do not exist in the vegetative cell (Raju et al., 2006).

SASPs can be divided in two types: α/β and γ type. In *C. perfringens* $3\ \alpha/\beta$ type of SASPs has been identified. Each type of SASPs seems to have different importance in spore resistance. For example, α type plays a major role in resistance of spore to UV radiation comparing to β-SASP (Setlow et al, 1992).

In vitro studies of behavior of SASPs have been shown the ability of these proteins to attach DNA, and shield it from damage. It has been shown an altered photochemistry of the spore DNA due to the saturation with SASPs. This change elevates resistance of spore to harsh environment conditions (e.g. heat, hydrogen peroxide, and UV) (Raju et al., 2007).

Presence of SASPs also plays a significant role in germination, and outgrowth of dormant spores. During germination, SASPs are degraded by a sequence specific protease GPR, and providing amino acids for outgrowing cell. Lack of SASPs slows down the process of germination (Hayes et al., 1998; Raju et al., 2007).

2-2-2-1-2- Characteristics of Reovirus

Reovirus (RV) belongs to the virus family Reoviridae, and its name is derived from respiratory enteric orphan viruses. A name "orphan virus" means a virus that is not associated with any known disease (Brewster, 2003). Even though Reoviroidea have been identified with various diseases, the original name still used. The genetic material of the viruses in this family is 10 double-stranded RNA segments which are enclosed in a double shell of protein (Coombs, 1994). The virus can be readily detected in feces, and many also be recovered from pharyngeal or nasal secretions, urine, cerebrospinal fluid, and blood (Theil et al., 1995). Three major RV serotypes have been described, which are represented by the prototype strains type 1 Lang (T1L), type 2 Jones (T2J), and type 3 Dearing (T3D) (Chappell et al., 2002).

The RV "core" or "inner capsid particle" (ICP) is one of the largest viral structures available with about 75 nm in diameter, and are composed of eight structural proteins (Arkhipov et al., 2006). Five of these ($\lambda 1$, $\lambda 2$, $\lambda 3$, $\mu 2$, and $\sigma 2$) has an

icosahedrally symmetric framework formed by the protein $\lambda 1$, and stabilized by a second protein, $\sigma 2$. A second layer of proteins ($\mu 1$, $\sigma 1$ and $\sigma 3$) forms the RV outer capsid, with $\mu 1$, and $\sigma 3$ comprising the bulk of this capsid, and $\sigma 1$ protruding from the 12 vertices of the icosahedrons (Shing et al., 1996). The $\sigma 3$ protein, whose crystal structure is known, is thought to serve as a protective cap for $\mu 1$ and cleavage of $\sigma 3$ by endosomal proteases during viral infection results in the loss of $\sigma 3$, and generation of infectious subvirion particles. Altogether, 480 protein units comprise the particle (Olland et al., 2001). Electron cryomicroscopy also shows that the $\sigma 3$ subunits project above the level of $\mu 1$, so that the virion surface appears to have rings of six knobs around the local 6-fold axes (Nason et al., 2001) (Figure 2-5).

RVs exist in three forms: the mature virion, intermediate (or infectious) subviral particle (ISVP), and core which vary in morphology, protein composition and physicochemical and biological characteristics (Smith et al., 2003). RV has an isoelectric point (pI) near 3.8, and becomes increasingly negatively charged as pH increases from this value (Moore, 1982).

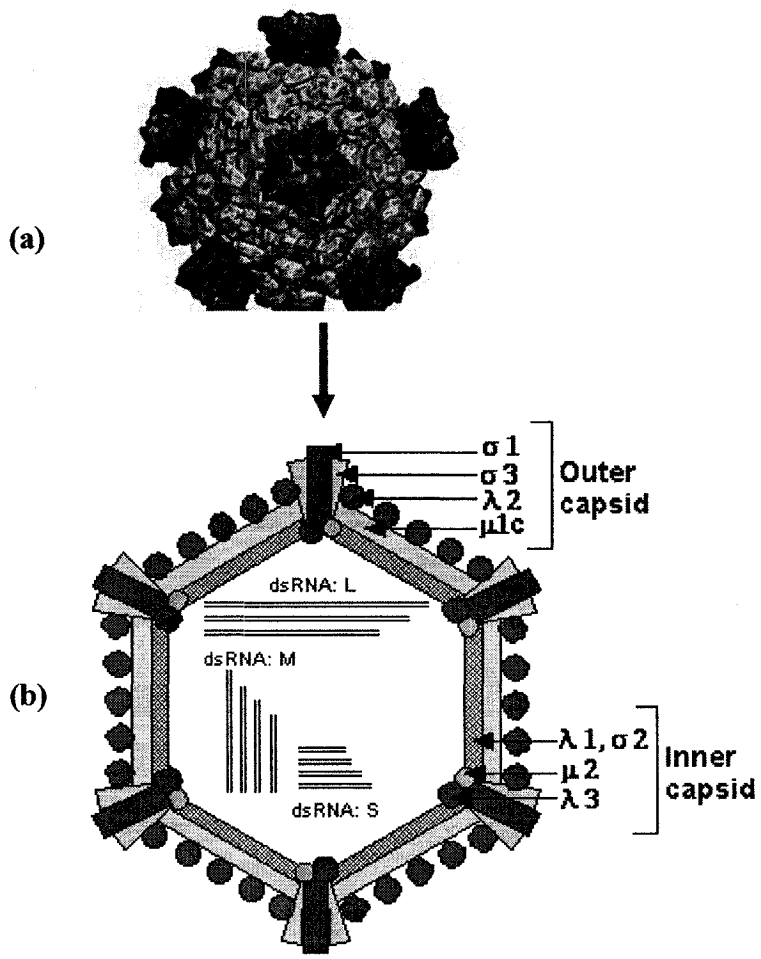


Figure 2-5: Cryomicroscopy of core (a), and schematic structure (b) of RV family (MICB, 2007; UIU, 2007)

2-3- Biosolids management practices

Until about three decades ago, biosolids were generally disposed of by landfilling, incineration or discharge into the ocean (Tenenbaum, 1997). Lately these practices have altered to point out further processing, higher levels of dewatering, lowering of pathogens, metals, and other substances that could undesirably affect the safety, and

acceptability of the biosolids product or limit its usage, and beneficial reuse rather than disposal (BEST, 2002). These changes have been the central point of several of scientific research studies. The results of these studies led to this fact that reliably managed biosolids are safe, and beneficial (USEPA, 1998). Furthermore, these results directed to develop human health-related guideline for using treated municipal and biosolids by Québec, United States, European countries and the World Health Organization (Wright, 2001).

With the advent of the regulation that encourages biosolids use, significant efforts have been directed to produce a "clean sludge" that meets heavy metals, and pathogen requirements, and is suitable for land application (CGER, 1996).

2-3-1- Management regulations

2-3-1-1- United States

On February 19, 1993 the USEPA was published "the Standards for The Use or Disposal of Sewage Sludge" (Title 40 of the Code of Federal Regulations [CFR], Part 503). Part 503 Rule consists of five categories or subparts as follows (USEPA, 1999):

- Subpart A: General provisions
- Subpart B: Requirement for land application
- Subpart C: Surface disposal
- Subpart D: Pathogen , and vector attraction reduction
- Subpart E: Incineration.

According to subpart B, the requirement of meeting land application of biosolids grouped in four options for meeting pollutant limits and pathogen, and vector attraction reduction operational standards. The options include:

- The Exceptional Quality (EQ) option; this group characterized by having low-pollutant concentration, virtual absence of pathogens limits, and low level of degradable compound that attracts vectors. This group is essentially unregulated for use.
- The Pollutant Concentration (PC) option: biosolids in this group meet the same low-pollutant concentration limits as EQ but they have certain amount of pathogens, and are subjected to site management practice.
- The Cumulative Pollutant Loading Rate (CPLR): this group exceeds at least one of the pollutant concentration limits for EQ, and PC biosolids.
- The Annual Pollutant Loading Rate (APLR): these biosolids meet APLR requirements, and must be accomplished by specific biosolids application rate information label when sold. (USEPA, 1995).

Table 2-3 illustrates the types of land onto which different kinds of biosolids may be applied.

Table 2-3: Type of land and related kinds of biosolids (USEPA, 1993)

Biosolids option	Pathogen Class	Type of land
EQ	A	All[a]
PC	A	All except lawn, and home garden[b]
	B	All except lawn, and home garden[b]
CPLR	A	All except lawn, and home garden[c]
	B	All except lawn, and home garden [b,c]
APLR	A	All, but most likely lawns, and home gardens

[a] Agricultural land, forest, reclamation sites, and lawns, and home gardens
[b] It is not possible to impose site restrictions on lawns , and home gardens
[c] It is not possible to track cumulative additions of pollutants on lawns , and home gardens.

Furthermore, according to 40 CFR Part 503 under section 405 of the Clean Water Act (CWA) for biosolids to be applied to land they must be subjected to one or two levels of treatment defined as Class A, and Class B. Class B has a FC density less than 2×10^6 MPN/ g Total Solids (TS), and its land application is restricted. Class A; the FC limit is less than 1000MPN/g TS or less than three *Salmonella* per 4 g, less than one virus per 4 g , and less than one viable helminth ovum per 4 g dry weight of biosolids. Class A needs a 3 to 5 $\log_{10}$-uint-inactivation for *C.perfringens* and viruses (Table 2-4) (USEPA, 1999).

Class A must be treated by "processes to further reduce pathogens", and Class B by "processes which significantly reduce pathogens" (PSRP) (Hester et al., 2001). Biosolids treated by PSRP may be land applied if they meet certain limits with regard to crop production, animal grazing, and public access; however, there are no restrictions for application Class A biosolids (Gerba et al., 2002; Wright, 2001).

Table 2-4: Characteristics of Class B and A biosolids according to USEPA

	Fecal Colifoms / g TS	Salmonella spp. / 4g TS	Enteric Viruses / 4g TS	Helminths eggs / 4g TS
Class B	2×10^6 most probable number (MPN)	NA	NA	NA
Class A	>1000 MPN	<3	<1	<1 viable egg

2-3-1-2-Canada

In Canada, biosolids, as with all environmental legislation is governed by provincial jurisdiction, and the only national regulation that makes reference to biosolids is the Fertilizer Act (LeBlanc, 2007).

Regulations in some provinces refer to the USEPA definitions, while others have developed their own terminology and definitions (Lewis, 2006). These regulations will be discussed in details below.

British Columbia (BC):

There is no restriction and bans in BC for the use or disposal of biosolids. Regulation defines the Class A and B for biosolids according to USEPA (Van Ham et al., 2007).

Nova Scotia:

Since May 2004, Nova Scotia has its guidelines for land application and storage of biosolids. According to that, there are three categories of biosolids as EQ, Class A and Class B (NSEL, 2004).

Ontario:

In Ontario biosolids, even treated one in the processes such as digesting, composting defined as a waste, and there are no restrictions or bans for use or disposal of biosolids. Still before disposal of biosolids to a land the proponent must obtain approbation for a Waste Disposal Site- Organic Soil Conditioning from Ministry of Environment (MOE). Ontario also has developed guidelines for the utilization of biosolids and other waste for land application (Lewis, 2006).

Alberta:

Same as Ontario there are no bands or restrictions for the use or disposal of biosolids in Alberta; however, before applying biosolids to the land the proponent need to obtain an authorization from the Director of the local office of Alberta Environment, and written consent from all participating in the project (Lewis, 2006).

Québec:

In Québec biosolids put in the section of fertilizing residuals (FRs), and are mainly used for (EDQ, 2004):

- Land application on farms;
- Transformation into compost;
- The manufacture of commercial soil mixes (including compost or non-composted FRs);
- Revegetation of degraded sites;
- Application on forest soils.

In Québec, each year around 1.8 million wet tons of residuals are retrieved as fertilizing material (Table 2-5). Of this amount approximately 60% are being used for land application, and 40% for composting which is 20% higher than that for 1999 (Charbonneau et al., 2000).

Table 2-5: FR reclamation through land application or composting in 2001-2002 (Charbonneau et al., 2000)

FR	Land application	Composting	Total	
			Wet tons	%
Paper mill biosolids	720,000	250,000	970,000	54
Abattoir biosolids	46,000	54,000	100,000	6
Other agri-food biosolids	20,000	NA	20,000	1
Municipal biosolids	70,000	96,000	166,000	9
Forestry residual	NA	250,000	250,000	14
Various residuals	NA	65,000	65,000	4
Cement kiln dust	50,000	NA	50,000	3
Ashes	60,000	5,000	65,000	4
Lime residuals	25,000	NA	25,000	1
Alkaline residuals from paper mills	37,000	23,000	60,000	3
Other liming materials	25,000	NA	25,000	1
Total	1,053,000	743,000	1,796,000	100

NA: Data not available

Currently land application of sewage biosolids in Québec is affected by three sets of regulations as follows (Webber, 2003):

- Canadian standard/ Bureau de normalisation du Québec (CAN/BNQ) for pelletized biosolids,
- Provisional criteria for the beneficial use of residual fertilizer materials,
- Canadian Fertilizers Act.

A residual will be considered as a recyclable FR if it has fertilizing properties, and satisfy the criteria developed to protect the quality of the environment. Consequently,

every FR is classified according to C-P-O classification, where C stands for chemical contaminant content (C category), P for pathogen content (P category), and odor (O category).

Since there are two C categories (C1, and C2), three P categories (P1, P2, and P3), and three O categories (O1, O2, and O3), this means there are 18 possible classifications for FRs (Table 2-6) (Charbonneau et al., 2000).

"Chemical contaminants" covers metals, such as cadmium and other inorganic trace elements such as selenium, as well as organic contaminants such as dioxins, and furans.

Table 2-6: Different classifications for FRs in Québec

Classification	Classification
C1-P1-O1	C2-P1-O1
C1-P1-O2	C2-P1-O2
C1-P1-O3	C2-P1-O3
C1-P2-O1	C2-P2-O1
C1-P2-O2	C2-P2-O2
C1-P2-O3	C2-P2-O3
C1-P3-O1	C2-P3-O1
C1-P3-O2	C2-P3-O2
C1-P3-O3	C2-P3-O3

Based on the type of residual and pathogen category (P1, P2 or P3) different parameter options and criteria are used. For example, a municipal biosolids that contains less than 2,000,000 *E. coli*/g (geometric mean) and sludge that is more than 20 days old is

classified as P3. However, if it is incorporated into the soil in less than 6 hours, it may be considered P2 (Table 2-7) (Environnement Québec, 2004).

Table 2-7: Residual quality criteria for P categories (Environnement Quebec, 2004)

Residual	Category P1 option	Category P2 , and P3 option
Residual contaminated with: Domestic sewage Human fecal matter Abattoir residuals or manure Dead animals Egg residuals	a) *E. coli* < 1,000 MPN / g TS* , and *Salmonella* undetected with detection limit of < 3 MPN / 4g TS, drying at a minimum temperature of 80 °C , and dryness > 90% b) Any other combination that meets the USEPA Class A requirement for the reduction of pathogens, and vector attraction	**P2:** a) Lime to pH ≥ 12 for at least 2 hours, and maintain at pH ≥ 11.5 for at least 22 hours. b) *E. coli* < 2,000,000 MPN*/ g TS, and aerobic biological treatment, and O2 uptake rate of 1500 mg/kg organic matter/ hour. c) *E.coli* < 2,000,000 MPN*/ g TS, and incorporation of residual into soil in less than 6 hours. d) Any other USEPA-approved combination that meets Class B requirements for the reduction of pathogens, and vector attraction. e) *E.coli* < 1,000 MPN/ g TS , and Salmonella undetected with a detection limit of < 3 MPN/ 4g TS* **P3:** *E.coli* less than 2,000,000 MPN /g TS , and biological treatment with sludge age ≥ 20 days

* Geometric mean

A comparison of different quantity categories in USA, and Canada is illustrated in Table 2-8.

Table 2-8: Comparison of different biosolids categories (NGSMI, 2003)

Parameter	Category 1	Category 2	Category 3
Terminology			
USEPA	EQ	Class A	Class B
British Columbia	Class A compost	Cass A	Class B
Ontario	No classification		
Quebec	C1, P1	C2, P2	C3, P3
Pathogen Reduction Requirement	< 1,000 MPN FC/ g of TS Density of *Salmonella* < 3 MPN/ 4 g TS	< 1,000 MPN FC/ g TS Density of *Salmonella* < 3 MPN / 4g TS	< 2,000,000 MPN FC/ g Ts

2-3-2- Treatments methods

As mentioned earlier, different classes of biosolids have been defined to protect public health, and environment. To meet the regulatory requirements for each class application of additional treatment is essential.

There are different ways for additional treatments of biosolids; some of which decrease the volume or mass of biosolids like digestion process, while others increase biosolids mass such as adding lime to control pathogens (USEPA, 1999). Figure 2-6 illustrates most of available biosolids treatment processes. Some of these methods will be described below.

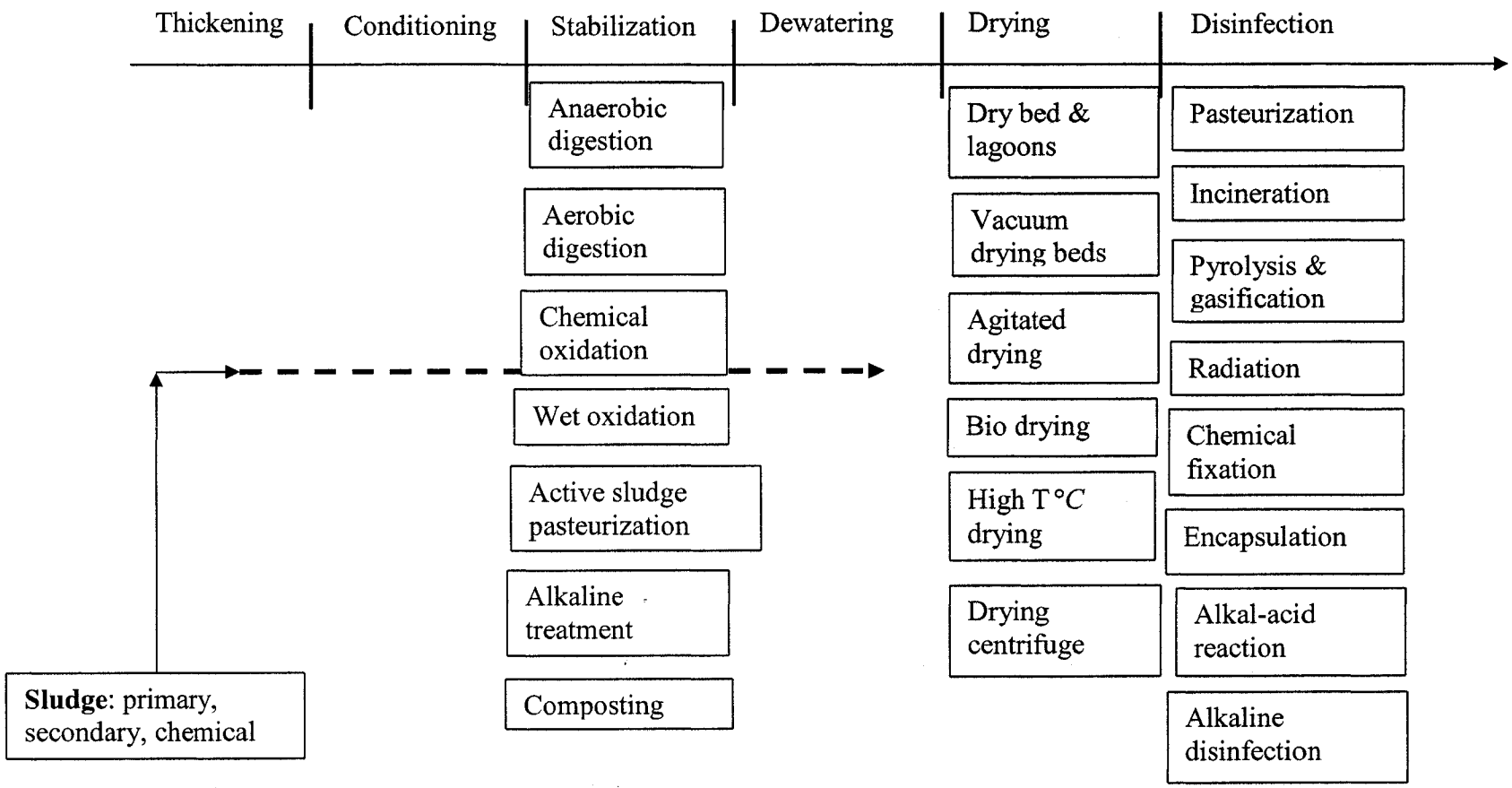


Figure 2-6: Some of the many unit processes available for sludge treatment (Oleszkiewicz, 2002)

2-3-2-1- Processes to significantly reduce pathogens (PSRPs)

These processes are related to Class B biosolids which contain pathogens at levels that are unlikely to be threat for public health. PSRPs can be divided in different clusters.

❖ Aerobic digestion

Aerobic digestion consists of biologically stabilizing biosolids in an open or closed vessel or lagoon using aerobic bacteria with cell residence time between 40 days at $20°C$, and 60 days at $15°C$ to convert the organic solids content to carbon dioxide, water and nitrogen. The high-temperature operation (higher than $55°C$) of aerobic digestion is earning more attention since it produces biosolids with more solid content and less amount of pathogens (Hester et al., 2001; USEPA, 1999).

❖ Anaerobic digestion

Anaerobic digestion encompasses biologically stabilizing biosolids in closed tank to reduce the organic, mass, odor and pathogens content of biosolids. Mean cell residence time for this process is between 15 days at $35-55°C$ and 60 days at $20°C$. Anaerobic digestion is one of the most widely used biosolids stabilization process especially because of methane recovery (USEPA, 1999; Wright, 2001).

❖ Air drying and partially digested sludge

This process comprises of placing biosolids on sand beds, on paved or on unpaved basins for a minimum period of 3 months, and during 2 of the 3 months the ambient average daily temperature above $0°C$. This process can produce a solid content in primary biosolids as high as 45-90% (Hester et al., 2001; USEPA, 1999).

❖ **Alkaline stabilization**

Historically alkaline stabilization has been implemented using either quicklime (CaO) or hydrated lime ($Ca(OH)_2$). Traditional lime stabilization processes are capable of producing biosolids meeting the minimum pathogen reduction requirements found in Part 503. In this process sufficient lime is added so that the pH of the biosolids mixture is raised to 12.0 or above for a period of 2 h (Hester et al., 2001; USEPA, 1999).

2-3-2-2- Processes to further reduce pathogens (PFRPs)

Class A biosolids are subjected to treatment designed to reduce pathogens to below the limits of detection discussed earlier in chapter. In order to meet the standard regulation for Class A, biosolids must have been subjected to methods reducing pathogens as describe in detail below (USEPA, 1999; Wright, 2001).

❖ **Heat drying**

In that drying process biosolids is dried by direct and indirect contact with hot gases to reduce the moisture content to 10% or lower. Either the temperature of the biosolids particles exceeds $80°C$ or the wet bulb temperature of the gas in contact with the biosolids as it leaves the dryer exceeds $80°C$ (Wright, 2001).

❖ **Composting**

Composting is the decomposition of organic matter by microorganisms in controlled environmental conditions, which produces a humus-like martial. During this process, the temperature of biosolids is raised to $40°C$ or higher, and remains at that condition for 5 days (Wright, 2001; USEPA, 1999).

A bulking agent is added to absorb moisture, increases porosity, and to act as a source of carbon. To supply oxygen, and remove exceed heat, aeration, and frequent mixing or turning applies (PDEP, 2006). Treated biosolids by this method has an excellent soil conditioning properties at a pH range of 6.5-8.00 (Hester, 2001; USEPA, 1999).

Windrow, static aerated pile, and within-vessel are three commonly used ways of composting.

- In windrow composting, compost is produced by natural aeration, over long period of time. The sewage sludge and bulking agent mixture are stacked into long piles with 3 to 6 feet (ft) high and 6 to 16 ft wide. The piles are frequently rotated or combined using a front-end loader to make sure steady oxygen supply for the microorganisms, and to decrease the moisture content. In cold weathers, temperature is a crucial factor for pathogen control (BCMAFF, 1996; PDEP, 2006).
- Static aerated piles are application of forced-air rather than mechanical mixing to supply oxygen, and reduce moisture. The sludge-bulking agent mixture is located on top of an aeration system (e.g. perforated piping) with a bed of bulking agent on the top. Then a layer of cured compost will cover the entire pile for insulation, and odor control. Air is sucked through the compost pile by using pumps. Removed air from the compost pile streams into a diffuser or filter pile, which contains the odors given off by the compost pile (PDEP, 2006).

- Within-vessel composting takes place in a reactor with controlled operational condition. Using either the within-vessel, composting method or the static aerated pile composting method, the temperature of the sewage sludge is maintained at 55°C or higher for 3 days (USEPA, 1999). Aeration of material is accomplished by: continuous agitation using aerating machines, which operate in concrete bays (BCMAFF,1996)

In general, within-vessel composting attains the required conditions in approximately 10 days. The static-pile and windrow processes generally require about 3 weeks. Longer composting periods may be necessary to fully stabilize the sludge. If volatile solids remain in the sludge, fecal coliform can later regrow to significant numbers (USEPA, 1999).

❖ Heat treatment

Heat treatment process is applied for both stabilizing and conditioning of biosolids under pressure for a short period of time. In this process, liquid biosolids is heated to a temperature of $180°C$ or higher for 30 minutes (Hester, 2001).

Two processes are using for heat treatment: the porteus and the zimpro (PDEP, 2006).

❖ Thermophilic anaerobic digestion

Minimum temperature must be maintained in the batch thermophilic digesters to qualify biosolids as Class A.The operating condition is based on mean cell residence time 10 consecutive days at $55 - 60°C$ (Wright, 2001).

❖ Beta, and gamma ray radiation

One of the alternatives to treat biosolids for pathogen reduction is radiation process. Two options are available for the radiation of sewage sludge: gamma sources, and electron accelerators.

The most common used industrial gamma source is Co-60; however, Cs-137 is also being used for this purpose. Gamma rays penetrate well in water and sludge; the half-value thickness of Cobalt-60 gamma rays (1.39MeV) is about 28 cm in water in normal liquid sludge, and the half-value thickness of Cesium-137 is 24 cm in water (Wang, 2007).

Beta rays are electrons accelerated by electrical potentials in the velocity of 1 Million Volts. The penetrating power of electron is approximately 3mm/MeV in water. Thus, a thin cross-section of sludge passed through electron beam is required to ensure the contact accelerated electron with its target (Hester, 2001; Wang, 2007).

❖ Pasteurization

The pasteurization process involves heating the sludge to $70°C$, and maintaining that temperature for a minimum of 30 minutes to achieve pathogen reduction (Wright, 2001). Pasteurization can be done using heat exchangers, and tankage or piping to provide a 30-minutes detention time at $70°C$ (Burke, 2000). To meet the standard regulation of USEPA all biosolids must be subjected to EPA-specified time-temperature regime, which means that batch or true plug-flow processing (USEPA, 1999).

Although above mentioned procedures are using vastly; however, each has disadvantage. For example, none of which are able to reduced the heavy metal from the biosolids, and at the same time pathogen reduction effects. They are costly, and their

energy consumption is high. They are not simple, and need high level of professional skill. A simple, cost effective of simultaneous inactivation of various microorganisms is needed.

2-3-2-3- Electrokinetic (EK) technology

The application of electric fields has gained considerable ground for the treatment of a wide variety of polluted matrixes such as soil, sewage sludge and sediment (Acar et al, 1993; Skanavis, 2001). EK technology is based on the application of a low-intensity direct current between the electrodes or an electric potential gradient across electrodes (Ibanez, 2004; Yang et al., 2005; Zhou et al, 2005). As a result of the applied potential gradient, contaminants are transported to positive charge electrode (anode) and negative one (cathode) by electrical field (Elektorowicz, 1995; Wang et al, 2004). Figure 2-7 represents a schematic illustration of EK phenomena.

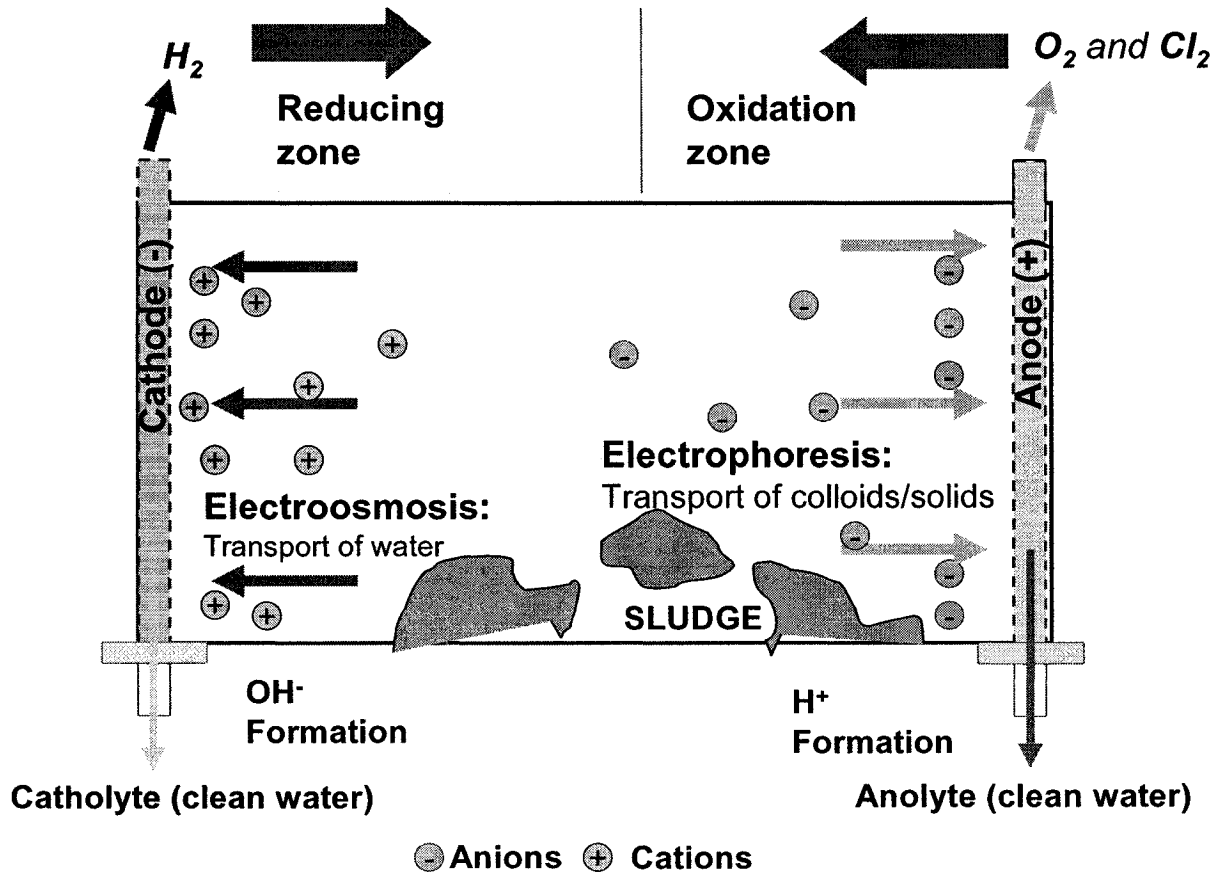


Figure 2-7: Schematic representation of EK phenomena (Elektorowicz, 2006)

2-3-2-3-1- Technology fundamentals

EK is described as the physicochemical transport of charged particles, formation, and transport of fluid in porous media (sludge) under effects of direct current (Alshawabkeh, 2001; Yuana, 2006). Electrokinetic motion happened by shearing the mobile part of the electric double layer (EDL) away from the inner layer (charged surface) (Alshawabkeh, 2001).

Double layer theory describes the distribution of ionic concentrations near electrostatically charged particles (Schramm, 2007) (Figure 2-8).

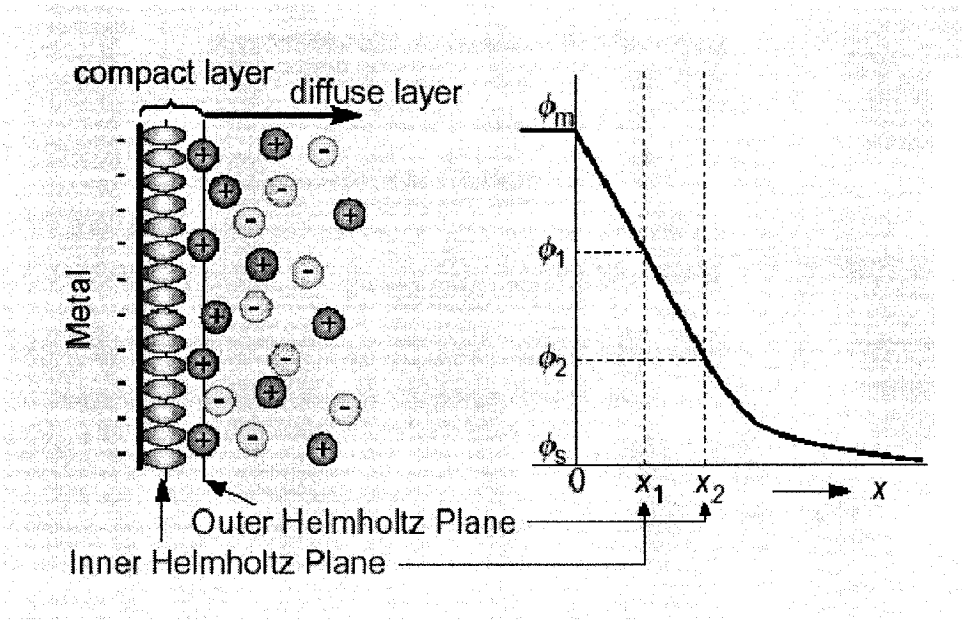


Figure 2-8: Build up an EDL at a solid-fluid interface (NMSU, 2006)

Several EK phenomena exist due to the interaction of electric charges and liquids. These phenomena are associated with the electric double layer and consist of:

- Electroosmosis, sometimes called electroendosmosis, which is migration of bulk fluid persuaded by an immobilized charged surface under direct current (Adams, 2006; Pennathur, 2006)
- Electrophoresis, in which a particle is moved by an electric field relative to the bulk flow (Pennathur, 2006)
- Streaming potential is generated by pressure-driven-flow through the porous media or capillary channel with charged solid surfaces (Mansouri, 2005).

- Sedimentation potential in which the liquid or the solid charged phase moves relative to the other under the influence of hydraulic or gravity force (Keh et al., 2006).

Addition to these phenomena, electrolysis reactions occur at the anode, and cathode (Alshawabkeh, 2001).

"The degree to which each electrokinetic phenomenon occurs depends on the properties of the contaminated matrix, and pore fluid matrix including the degree of saturation, ionic strength of pore fluid, types of ion charge particles present, pH of pore fluid, temperature, porosity, soil composition, and the surface charge of the soil particles" (Leland, 1999).

A combination of various phenomena governs the EK technology has been shown in Figure 2-9. More detail about these phenomena is coming in detail.

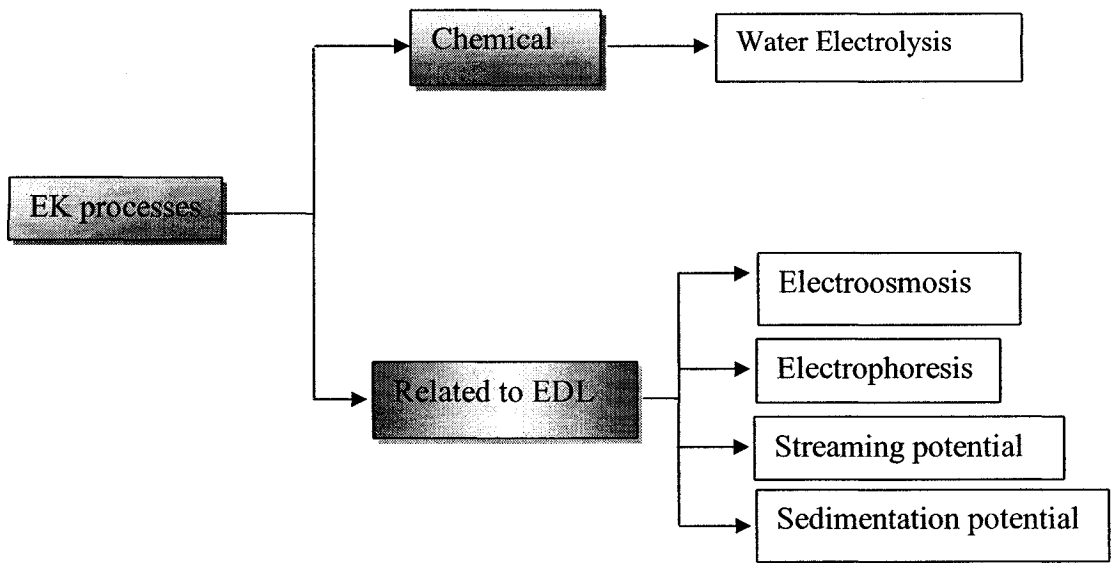


Figure 2-9: Combination of different process, and phenomena involved in EK

❖ Electroosmsois

Migration of water or other liquid medium toward a charged electrode under influence of an electrical potential gradient referred to electroosmosis (Alshawabkheh, 2001; Hakimipour, 2001). This movement occurs because of the viscous drag generated by the mobile counter ions in the electrical double layer. Existence of certain conditions like presence of appropriate minerals, high water content, and low ionic strength of pore fluid causes an important effect of electroosmosis in electrokinetic soil remediation (Alshawabkeh, 2001).

Several theories are described, and evaluated water flow by electroosmosis. One of the most common theoretical descriptions of electroosmsis is Helmholtz-Smoluchowski model. This theory discussed that when electrical field is applied tangentially to a solid-liquid interface, an electrical body force is exerted on the excess counter ions in the diffuse layer of the EDL. The ions will move under the influence of this electrical filed, pulling the liquid with them, and resulting in an electroosmsis flow (Mitchell, 1991).

The rate of water flow is controlled by the balance between the electrical force causing water movement in one direction, and friction between the liquid and the wall in the other (Yeung; 1994). Negative surface charge of material causes electroosmisis to occur from anode to cathode while positive surface charge causes electroosmosis to occur from cathode to anode (Yang et al., 2005). In highly acidic soils the electroosmosis flow will be toward the anode instead of toward cathode (Mitchell, 1991). Electroosmosis flow rate can be given by following equation (Esmaeily, 2002, Mitchell, 1993; Raihan, 2006):

$$q_e = K_e \nabla E A \qquad \text{(Eq.2.1)}$$

Where:

q_e = Electoroosmosis flux (ms^{-1})

K_e = Electroosmosis conductivity ($m^2V^{-1}s^{-1}$)

∇E = Electrical potential gradient (Vm^{-1})

A = Cross sectional area (cm^2).

There is a hypothesis saying that drop of pH due to EK processing will cause a decrease in the coefficient of electroosmotic permeability with a drop in zeta-potential. As a result the electroosmotic flow will start to decrease, and eventually stop at later stages of the process (Acar et al., 1989).

❖ Electrophoresis

The movement of a solid particle through a stationary fluid under the influence of an electric field refers to the electrophoresis. The basic principle behind this phenomenon is the present of charge separation between any surface and the fluid in contact with it (Schramm, 2007). The surface carries an immobilized charge and the electrolyte fluid in contact with the charged surface equalizes the electric charge with an increased density of ions of the opposite charge. This phenomenon plays a significant role in different types of sludge (Raihan, 2006; Esmaeily, 2002).

The importance of electrophoresis will be obvious when using surfactant to form charged particles in electrokinetics remediation of contaminated matrix and also in remediation of slurries (Acar, 1993).

❖ Streaming potential

Driving a liquid through a capillary or porous plug induces a difference of electric potentials called streaming potential. The streaming potential is the opposite electrokinetic phenomenon to electroosmosis. This potential difference is produced due to the movement of mobile ions in EDL, which forms due to the distribution of electric charges close to a charged surface. The observed electrical potential difference is related to the applied hydraulic pressure, and is attributed to the separation of oppositely charged ions in EDL (Chun et al., 2006).

❖ Sedimentation potential (Dorn effect)

Gravitational sedimentation or Dorn effect of charged particles is one of the basic electrokinetic phenomena of colloidal suspensions. It is defined as the electric potential that develops when charged colloidal particles are set in motion with respect to a stationary liquid (Schramm, 2007). This potential is similar to the electrophoresis of charged particles, and is found to correlate with electrophoretic mobility (Ibanez, 2004; Kunkle et al, 1977).

❖ Electrolysis

Passing direct electric current through electrodes submerged in water causes oxidation of water at anode, and produces an acid zone, while reduction at the cathode generates a base zone (Alshawabkeh, 2001). This electrolysis of water describes by the following reactions:

Anode zone: $2H_2O - 4e^- \longrightarrow O_2 \uparrow + 4H^+ \qquad E^0 = -1.299V$ (Eq.2.2)

Cathode zone: $4H_2O + 4e^- \longrightarrow 2H_2 \uparrow + 4OH^- \qquad E^0 = -0.828V$ (Eq.2.3)

The base zone will migrate toward the anode since the ionic mobility of H^+ ions is 1.75 times that of the OH^- ions. This property extremely impacts the electrochemical properties of the contaminated matrix and contaminants remediation (Acar et al., 1993). The factors that affect this mechanism are transport mechanisms such as migration due to electrical gradients, pore fluid advection caused by prevailing electroomotic flow, and diffusion as a result of generated chemical gradient (Esmaeily, 2002; Raihan, 2006).

The rate of electrolysis reactions is related to the total current applied according to Farady's law. This can be calculated by following equation (Alshawabkeh, 2001):

$$J = \frac{I}{z_i F} \qquad \text{(Eq.2.4)}$$

Where:

J = The rate of oxidation or reduction by electrolysis (MT^{-1})

I = The current (A)

z =The charge of ion (for hydrogen z is 1)

F =Farady's constant (96,485 C/mole).

It is necessary to point out that other kind of electrolysis reactions may happen, and reduce the water electrolysis.

2-3-2-3-2- Practical aspect of EK technology

Although scientific base of EK technology for remediation of inorganic species, radionuclide, and heavy metals is documented in the literatures; however, there is a lack of studies on practical aspects of electrokinetic (Schultz, 1997). The number of publications on theoretical formulations and numerical models for EK technology is a

few. There are some studies about the economic modeling and calculation of optimum spacing time and energy requirements of one-dimensional (1-D) filed application; however, no enough experimental data are available on two-dimensional (2-D) or radial applications in soil remediation. Much less work has been done on EK application to sludge (Alshawabkeh, 1999).

Some of the most important elements of practical aspects of EK technology will hereinafter be discussed.

❖ Electrode configuration

Application of electrical current in contaminated matrix can be in the form of 1-D or 2-D. 1-D electric field could be produced through using electrical sheets or non same-polarity electrodes in front of each other while 2-D field is created by square or hexagonal configuration in which one cathode is located in the center, and four (possibility eight) anodes surrounding the cathode (Wise et at., 2000). Electrode layout affects the distribution of electric fields, while decreasing the spacing between electrodes of the same polarity (anode – anode or cathode – cathode) improves electric field distribution, and at the same time might increase the remediation costs. The number of electrodes per unit surface area required for each configuration can be computed by following equation (Alshawabkeh, 1999; Virkutyte, 2004):

$$N = \left[\frac{F_1}{L_E^2}\right]_{1-Dflow} = \left[\frac{F_1}{\pi R_E^2}\right]_{2-Dflow} \qquad \text{(Eq.2.5)}$$

Where:

$N(L^{-2})$ = Number of electrode per unit surface area

$L_E(L)$ = 1-D anode-cathode spacing

$R_E(L)$ = 2-D anode-cathode spacing

F_1 = (dimensionless) shape factor related to electrode configuration.

Table 2-9- presents a comparison of number of electrodes needed per unit surface area for 1-D, and 2-D hexagonal, and 2-D square configurations.

Table 2-9: impacts of configuration on the electrode needed, and size of ineffective areas (Alshawabkeh, 2001)

Config.	Electrode spacing		Number of electids per cell	Area of cell	No. of electrodes per unit area		Ineffective area	
	Opp. charge	Same charge			N	% increase	Aineff	% of Acell
1-D	L_E	L_E	1	L_E^2	$\frac{1}{L_E^2}$	0	$\frac{L_E^2}{2}$	50%
1-D	L_E	$\frac{L_E}{2}$	2	L_E^2	$\frac{2}{L_E^2}$	100%	$\frac{L_E^2}{4}$	25%
1-D	L_E	$\frac{L_E}{3}$	3	L_E^2	$\frac{3}{L_E^2}$	200%	$\frac{L_E^2}{6}$	17%
Square	R_E	$\sqrt{2}R_E$	2	$2R_E^2$	$\frac{1}{R^2}$	0	R_E^2	50%
Hex.	R_E	R_E	3	$\frac{3(\sqrt{3})}{2}R_E^2$	$\sqrt{\frac{4}{3}}R_E^2$	15.5%	$\frac{3R_E^2}{4}$	29%

❖ Electric field distribution

The distribution and intensity of the electric field in contaminated matrix are key parameters in the electrokinetic treatment.

The electric field intensity vector, E [V/m], is defined as (Rittirong, 2007):

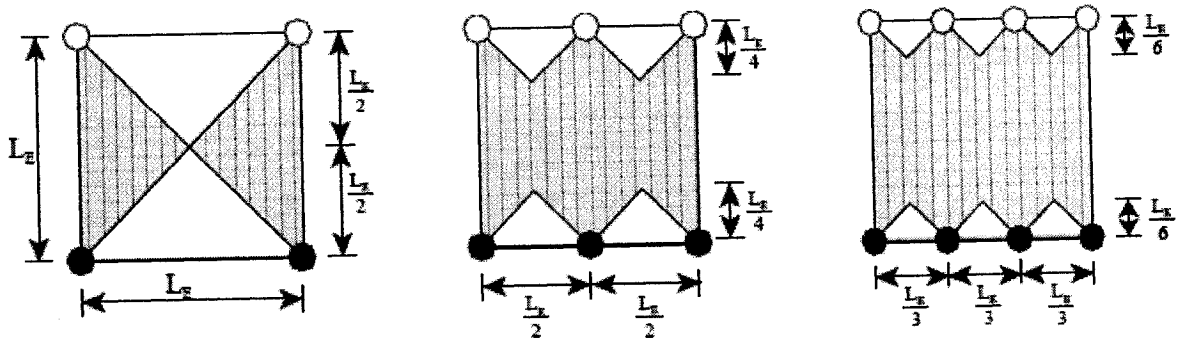
$$E = -\nabla\phi \qquad \text{(Eq.2.6)}$$

Where:

ϕ = The electric potential [V]. Under steady state conditions, the electric field distribution satisfied the following condition:

$$\nabla^2 \phi = 0$$

Considering the electrical field distributions the ineffective area for each cell is in the shape of curvilinear with the base of triangle which its base is the distance between electrodes of the same-polarity. The height of it depends on processing time, electrode spacing, and alignment (Figure 2-10) (Alshawabkeh, 2001).



Area of cell $= L_E^2$

Ineffective Area $= \frac{L_E^2}{2}$

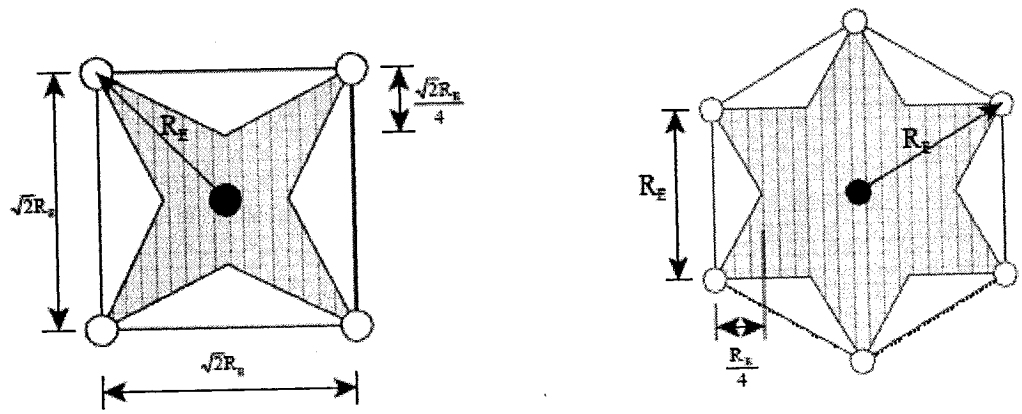
Area of cell $= L_E^2$

Ineffective Area $= \frac{L_E^2}{4}$

Area of cell $= L_E^2$

Ineffective Area $= \frac{L_E^2}{6}$

(a)



Area of cell $= 2R_E^2$

Ineffective Area $= R_E^2$

Area of cell $= \frac{3\sqrt{3R_E^2}}{3}$

Ineffective Area $= \frac{3R_E^2}{4}$

(b)

Area of effective electric field

Area of ineffective electric

Cathode

Anode

Figure 2-10: Approximate evolution of ineffective areas for 1D (a), and 2D (b) electrode configuration (Alshawabkeh, 2001)

2-3-2-3-3-Applicability

EK is a developing technology with application of separating, and extracting radionuclides, heavy metals, organic contaminates from matrixes such as soil, sewage sludge, sediment and groundwater. Contaminants affected by electrokinetic process include (Buehler et al., 1994; Choudhury, 1997; Elektorowicz et al., 2007; 2007a; Esmaeily, 2002; Huang, 2007; Raihan, 2006):

- Heavy metals
- Radioactive species
- Toxic anions
- Dense non-aqueous phase liquids (DNAPLs)
- Cyanides
- Pertroleum hydrocarbons
- Explosives
- Mixed organic/ ionic contaminants
- Nonhalogenated organic pollutants (BETX)
- Polynuclar aromatic hydrocarbons (PAHs)
- *Fecal coliformes*

2-3-2-3-3-1- Application to remove heavy metals

It has been shown that EK technology can be used for removal of heavy metals, radionuclides , and selected organics (Acar et al, 1996; Esmaily, 2002; Kim et al, 2002; Raihan, 2006; Virkutyte, 2002).

An enhanced electrokinetic process for removal of metals Cr, Cu, Fe, Ni, Pb and Zn from industrial wastewater sludge was performed by Yuana et al. (2006). The electrokinetic experiments were conducted under a constant potential gradient (1.25V/cm) with processing fluids of tap water (TW), sodium dodecylsulfate (SDS) and citric acid for 5 days. Results showed that metal removal efficiency of heavy metals for EK-TW, EK-SDS and EK-CA systems are 11.2–60.0%, 37.2–76.5% and 43.4–78.0%, respectively. A highest metal removal performance was found in EK-CA system. The removal priority of investigated metals from sludge by EK process was found as:

Cu > Pb > Ni > Fe > Zn > Cr.

Dewatering, metal removal, organic elimination, and pathogen removal of biosolids using EK technology was studied by Esmaeily (2002). The result of this research work showed an average removal of 84% for zinc, 100% for Cd^{2+}, and Pb^{2+}, 91% for iron.

Acar et al (1994) showed 90% to 95% removal of Cd^{2+} from kaolinite specimen with initial concentration of 99-114 mg/kg.

It has been shown that one of the most influential parameters controlling the transfer of metals from immobile solid phase to a mobile phase is pH (Elektorowicz, 1995; Wang et al, 2004).

Wang et al (2004) observed that migration of heavy metals happened mostly within sewage sludge close to cathode area.

2-3-2-3-3-2- Effects on microorganisms

Studies with subject of effects of EK technology on microorganisms are mainly focused on impacts of this process on remediation of contaminated matrixes. However some research works has been done on the inactivation effects of this technology on living life.

❖ Bioremediation by EK technology

In- situ bioremediation by EK dates back in 1990s, and has been successful for bioremediation of organic pollutant with very low solubility or those with large mass to charge ratio (Lageman, 2001). In many cases, this technique is used to improve biodegradability conditions on in situ bioremediation processes, by promoting the transport of nutrients, such as nitrogen and phosphorous and electron acceptors (Schmidt et al., 2007).

Microbial processes required a source of electron, nutrients and electron acceptor. EK technology is able to transport the necessary oxygen and nutrient in the remediation matrix. Also the passage of electrical current generates some heat which helps to reach the necessary temperature for bioremediation, and facilities the migration of microorganisms to the directed way (Ibanez, 2004).

Alshawabkeh (2001) demonstrated that ranges of DC fields which simulate microbial growth are affected by time of exposure.

The imposed DC electric field is also expected to affect the electrokinetic properties, adhesion and transportation of microorganisms in porous media. As microbes are generally negatively charged, DC fields will cause their transport toward the anode (McLeod et al., 1992); however, Alshwabkeh (2001) observed the tendency of

microorganisms to stick and attach themselves to the electrodes in experiments using diluted sludge samples no matter the polarity of electrodes was.

DeFlaun and Condee (1997) by using a pure bacterial culture in bench-scale soil samples noticed that the rate of transport is related to the effective electrophoresis mobility in contaminated matrix.

It is also important to consider that microorganisms, as a living organisms might show different behavior while transporting in porous media under electric field. This behavior might not be exactly governed by electorophoresis alone (McLeod, 1996). Tendency of microorganisms to create colonies and to attach to the solid particle surface and also other factors which can affect a living object can influence this behavior. Data available in literatures indicate that the presence of heavy metals change the elctrokinetic properties of bacteria (Alshawabkeh, 2001).

❖ Inactivation of microorganisms

The effects of DC on bacterial cells have been studied for several decades. The main concern of these works was focused on the transport of cell, metabolism and viability. It has been shown that exposing bacteria to an electrical current produces environmental stresses on the bacterial cell. Respond to these stresses cause changes in surface properties and cell shape changes. Also this current can affect bacterial activity and growth (Luo et al., 2005).

Present of oxygen and coexisting ions such as chloride ions can be considered as the most important factors responsible for inactivation and lethality effects of DC (Luo et

al., 2005). Also it has been mentioned that pH lower than 2.5 produced in anode area can reduce the content of pathogenic bacteria a thousands times (Wang et al., 2004).

Kalinowski et al. (2004) studied the fungicide effect of DC for treating of two strains of fungi responsible for onychomycosis. They evaluated in vitro effects of low-voltage DC as a fungicide agent on *Trichophyton rubrum*, and *Trichophyton mentagraphytes*. They observed a zoned devoid of fungal growth around cathode, and anode in a current range of 500μ A to 3 mA.

In another research, the response of spores of *Pasteuria penetrans*, the Gram-positive obligate nematode hyperparasite, was studied in a direct current electric field by Afolabi et al (1995). The mobility of the spores and fluorescence measurements were performed in different salt concentrations and at different pH values. A significant electronegative potential at the spore surface of bacteria was observed which was dependent on the pH, salt concentration, and valency of the cation present in the electrolyte medium.

Murtosvkin (1995) showed that motion of bacteria can be caused by electrokinetic phenomena such as electrophoresis and diffusiophoresis. He observed that in contrast to common colloidal particles, electrokinetic phenomena in bacteria can occur without external electric or concentration fields. He mentioned that microorganisms are able to induce such fields by themselves due to exchange processes between cells and the environment, since these processes inevitably accompany the vital activity of any organism. If bacteria carry the surface charge, the diffusion-electric field, induced by bacteria as a result of ion exchange processes, will cause their motion relative to the surrounding liquid.

Drees et al. (2003) conducted a research wok with the main idea of comparing the viability of bacteria, and viruses subjected to direct current. They expose *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa* and bacteriophages MS2, and PRD1 to current ranging from 25-350mA in 5 s pulse in first stage of their experiments. In the second stage, they exposed *E. coli* and MS2 to current 5mA for time period of 20 minutes. Results show that the inactivation rate of *E.coli* was 2.1-4.3 times greater than that of MS2. Also both bacteria and bacteriophages were more resistant to exposure to DC at higher population densities.

Effect of EK on FC bacteria was studies at Concordia University. In their research they use a rectangular EK cell with dimensions of L=22 W=5 D=5.2 under different voltage regimes. Their result showed a negative growth of FC on cells with different voltage of 0.5 to 1.5mV/ cm (Esmaeily et al., 2006).

In another research, Huang et al. (2007) used five different combinations of biosolids; primary, combined primary and secondary, attached growth secondary, waste activated sludge, and anaerobic digested in ten EK cells with total volume of 580mL. The EK were connected to power supply to prepare desired range of low and high voltage gradients (less than 5V/ cm). Tests were running for 6-9 days and EK treated biosolids were evaluated for presence of FC and *Salmonella* spp.. Their results showed no detection of FC and a 9 log reduction of *Salmonella* spp in anode zone of EK cell.

2-3-2-3-4-Enhancement

In general, using a modification technique might result in enhanced EK process. Choosing suitable enhancement conditions depend on the purpose of the process. Some of the procedures applied to enhance the EK will hereafter be listed.

- Catholyte neutralization: this process involves the neutralizing of catholyte pH to increase the extraction of metals.
- Ion-selective membrane: using membrane to enhance transport of metals toward the cathode.
- Chelating or complexing agents: to help solubilization of metals without acidification. This kind of enhancement is suitable for contaminated matrix with reverse electroosmosis phenomenon.
- Enhancement of anolyte pH: to neutralize anode reactions, and to prevent high migration of hydrogen ions. High production of hydrogen ions causes consumption of energy for transportation of this ion, and leads to lower efficiency of system (Alshawabkeh, 2001).
- Other enhancement chemical such as oxidant, and reducer related to the main object of procedure.

2-3-2-3-5- Conclusion

Application of EK in sludge treatment is a comparatively new and emerging technology. This technology can be considered one of the best alternatives for biosolids management.

The major objectives of previous research had focused on the application of the efficiency of EK technology on dewatering and metal removal; however, as this thesis demonstrates, this process is also able to improve the quality of biosolids for pathogen removal to the standard levels (Class A).

The main objective of this research is to apply an enhanced EK technology to inactivate *C. perfringens* spores, an accepted indicator for determining the quality of treatment methods, and Reovirus.

III- APPROACH AND METHODOLOGIES

3-1- Experimental methodology

In order to fulfill the objectives of this research a series of bench scale experiments were set up as is shown in Figure 3-1.

The first stage of this methodology consisted of three steps:

- Design of experimental conditions for each batch EK reactor test according to different factors involved in the experiments according to engineering statistical methods.
- Design of EK reactors, and EK installation set up.
- Initial biosolids sampling from WWTP and preparation for each batch test.

In the second stage, each batch of trials was run according to designed conditions, and samples were taken from specific parts of the reactor for further analyses. In all cases, a set of two control tests were run along with other batch trials. In the control test, the only effecting factor was the duration of the experiment (1 day, and 3 days). The control tests were evaluated microbiologically.

The subsequent stage permitted evaluation of the biological and physicochemical behavior of each batch trial. To do so, a bunch of different measurements were performed: such as amount of pH, ORP, TSS, VSS, chloride, sulfate, nitrogen-ammonium, phosphate, nitrate, nitrate, VFAs, and enumeration of *C. perfringens* spores, and viruses.

The last stage involved two parallel parts. In the first part, a set of experimental conditions were designed for the best reactor considering the voltage gradient, and

exposure time as fixed factors, and each enhancement agent was applied one by one in order to assess the inactivation effect of each one in combination with EK. The second part was based on choosing the seven best set conditions, and running the test without EK. The purpose of this part was to observe the effects of enhancement agents in the absence of EK on inactivation of *C. perfringens* spores.

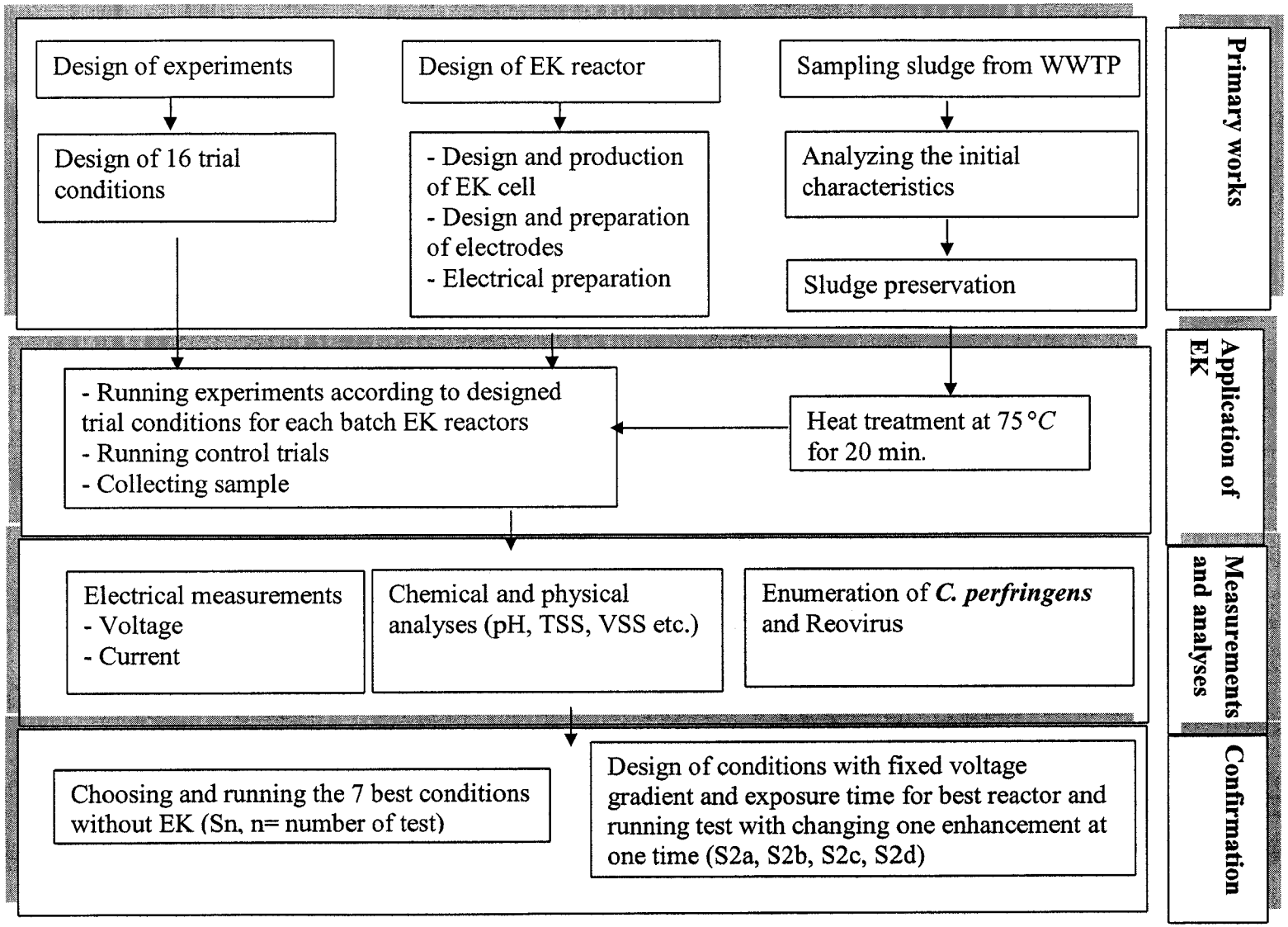


Figure 3-1: Experimental methodology

3-1-1- Design of experiments (DOE)

This section involved choosing factors affecting trials, and design of experimental conditions for each trial.

Choosing important factors

In an EK reactor, two factors are among the most important: voltage gradient, and exposure time. For this research, voltage gradients of lower than 2 V/cm, and exposure time of 1-3 days were selected according to previous works.

Other important factors involved in this work were enhancing agents. As mentioned earlier, considering the literature review (Chapter 2), and research on the effects of using a mixture of oxidant for inactivation of *C. perfringens* spores and viruses (Payment et al., 1993, 1985; Venczel, 1997), a combination of two oxidants: glutaraldehyde and bioxy S/ tetraacetyl ethylene diamine (T.A.E.D) was chosen. These oxidants were prepared by Dr. Fadi Dagher (Atoms Company), and have been known for their effect on biological structure, such proteins, enzymes, and also cell membrane.

Glutaraldehyde is a colorless liquid with a chemical structure of $OHC(CH_2)_3CHO$. It reacts with amino groups, sulfhydryl groups, and possibly with aromatic ring structures. Glutaraldehyde penetrates tissue more slowly, causing extraction of soluble antigens, and modification of the structure (ANSI/AAMI, 2005).

Bioxy S/ (T.A.E.D) activated peroxide provides a means of generating peracetic acid in-situ. This reaction is shown in Figure 3-2, where DAED is dimethyl ethylene diamine. The activity of this compound is not pH related, and can be achieved in neutral pH (Dagher, unpublished report).

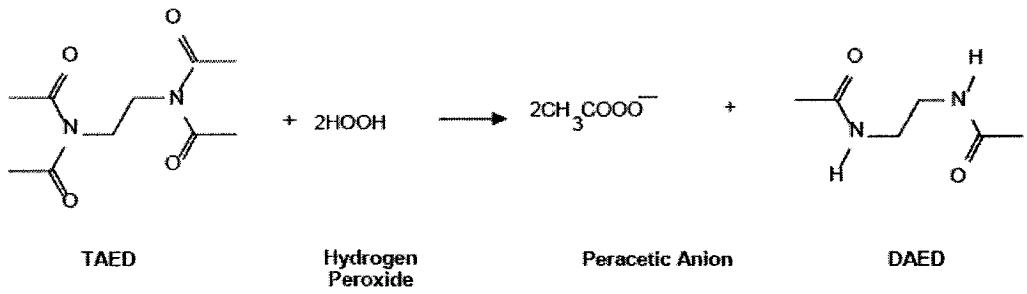


Figure 3-2: TAED reaction with hydrogen peroxide (Dagher, unpublished report)

Research by Elektrowicz (2007), Esmaiely (2002, 2006) and Huang (2007) for inactivation of *Salmonella*, FC, and simultaneous dewatering effect, suggested using an amphoteric enhancer. An amphoteric agent is a compound of ammonium phosphate which contains 30% nitrogen-ammonia, 10% phosphate and 59% sulfate.

Factors involved in this step were as follows (Table 3-1):

- Voltage gradient (at 3 levels)
- Exposure time (at 2 levels)
- Concentration of glutaraldehyde (at 3 levels, including absence of glutaraldehyde- 0 level)
- Concentration of amphoteric (at 3 levels, including absence of amphoteric- 0 level)
- Concentration of Bioxy S/ (T.A.E.D) (at 4 levels, including absence of Bioxy S/ (T.A.E.D)- 0 level).

Table 3-1: Factors involved in the experiments and their level

Factor	Level			
	1	2	3	4
Voltage gradient (V/cm)	0.5	0.7	1.5	-----
Exposure time (day)	1	3	-----	-----
Conc. of glutaraldehyde (% W/v)	0	0.2	0.5	-----
Conc. of amphoteric (% W/v)	0	0.7	1.3	-----
Conc. of Bioxy S/ (T.A.E.D) (% W/v)	0	0.1	0.2	0.4

Design of experiments

A combination of the above mentioned factors and their levels was accomplished by a factorial DOEs based on Taguchi methods, using Qualitek-4 software (Roy, 2001). Taguchi DOE uses two-, three-, and mixed-level fractional factorial designs with emphasis on the effective applications of engineering strategies rather than on advanced statistical techniques (Buyske et al., 2006).

The experiments were designed in two separate phases. In the first phase (phase I), a set of 16 experiments (Table 3-2) were designed, run, and their results assessed.

Table 3-2: Experimental condition for each set of the EK reactor

Factor / EK reactor trial number	Voltage gradient V/cm	Exposure time (d)	Enhancer concentration (%)		
			Glutaraldehyde (v/v)	Amphoteric (w/v)	Bioxy S/ (T.A.E.D) (w/v)
1	0.5	9	0	0	0
2	0.7	1	0.2	0.7	0.1
3	1.5	3	0.5	1.3	0.2
4	0.5	3	0	0	0.4
5	0.5	1	0.5	0	0.2
6	0.7	1	0	1.3	0.4
7	1.5	3	0	0.7	0
8	0.5	3	0.2	0	0.1
9	0.5	3	0	0.7	0.4
10	0.7	3	0.5	0	0.2
11	1.5	1	0.2	0	0.1
12	0.5	1	0	1.3	0
13	0.5	3	0.2	1.3	0.1
14	0.7	3	0	0	0
15	1.5	1	0	0	0.4
16	0.5	1	0.5	0.7	0.2

The second phase (phase II), which was consisted of two parallel parts, was designed based on the results of phase I. These results showed that the reactor 10 was the most successful reactor in the terms of *C. perfringens* spores inactivation.

As shown on Table 3-2, applied condition for the reactor 10 (exposure time 3days, voltage gradient 0.7 V/cm, highest concentration of glutaraldehyde 0.5% v/v, oxidant agent 0.2% w/v, without amphoteric agent) showed that a combination of factors,

and their levels is necessary to achieve a high value of inactivation (4.35 $\log_{10}$ reduction). To assess the degree of importance of each factor individually within the EK reactor, 4 experiments were designed (Table 3-3).

Table 3-3: Experimental condition of the part I phase II for the best batch reactor

Factor / Trial	Voltage gradient V/cm	Exposure time (d)	Enhancer concentration (%)	
			Glutaraldehyde (v/v)	Bioxy S/ (T.A.E.D) (w/v)
S2a	0.7	3	0	0
S2b	0.7	3	0.5	0
S2c	0.7	3	0.5	0.2
S2d	0.7	3	0	0.2

Comparison of Table 3-1 with Table 3-3 showed that the combination of factors in S2a is similar to the trial 14 and S2c to the trial 10, which were already completed. Consequently, the conditions to be tested were S2b and S2d.

In the parallel step, the 7 best trials of the first experimental conditions (Table 3-1) were selected to be run in the absence of EK (Table 3-4). This step was designed to weigh the effect of involving factors (except EK) on inactivation of *C. perfringens.*

Table 3-4: Experimental condition for the part II phase II set

Factor / Trial	Voltage gradient V/cm	Exposure time (d)	Enhancer concentration (%)		
			Glutaraldehyde (v/v)	Amphoteric (w/v)	Bioxy S/ (T.A.E.D) (w/v)
S4	0	3	0	0	0.4
S5	0	1	0	0	0.2
S6	0	1	0	1.3	0.4
S9	0	3	0	0.7	0.4
S10	0	3	0.5	0	0.2
S11	0	1	0.2	0	0.1
S15	0	1	0	0	0.4

3-1-2- Electrokinetic experimental set-up

❖ EK experimental set up

EK experimental set up consisted of the following compartments:

1- EK reactor (designed and made at Concordia University)

2- DC power supply (XantrexxKW 40-25)

3- Collecting containers to collect water leakage through anode and cathode (Nalegene, Fisher Scientific 2006-0008)

4-Deluxe digital Multimeter (Mastercraft)

5- Electrical connections (wires, nuts, and contact washers)

Figure 3-3 displays a schematic diagram of the EK experimental set up.

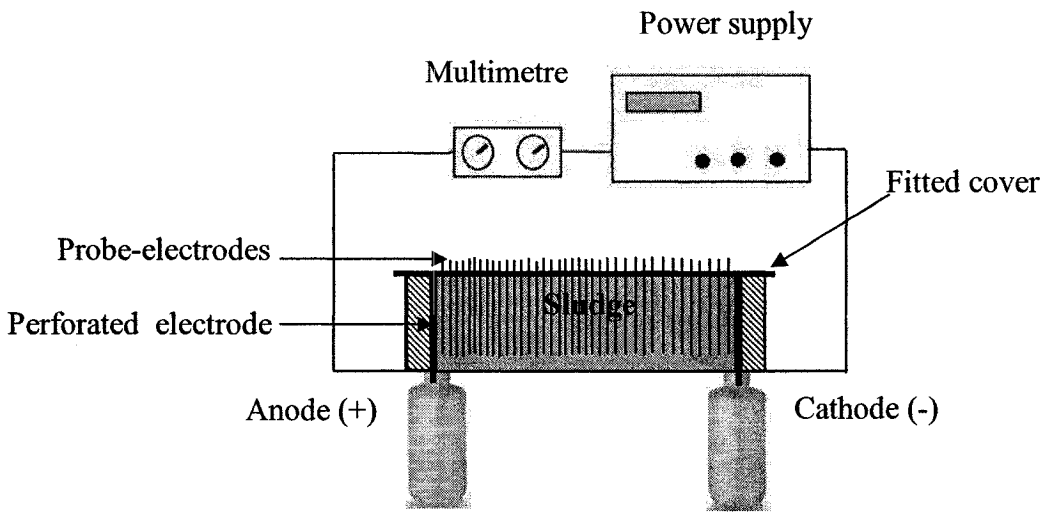


Figure 3-3: Schematic diagram of the EK experimental set up

❖ EK reactor

Experiments conducted in the EK reactors, which consisted of four parts:

1. EK cell:

The EK cell was a Plexiglas rectangular box with internal dimensions of 230-mm length× 50-mm width× 74-mm height, and wall thickness of 6-mm. Plexiglas was chosen as a construction material because it is transparence and therefore suitable for investigating the settling behavior of biosolids during the experiment. The EK cell was equipped at both ends with stainless steel electrodes coated with stainless steel mesh (Figure 3-4)

2. Fitted cover with silver probe-electrodes:

A Plexiglas seal with external dimensions of 263-mm length× 60-mm width was prepared to act as cap for the EK cell. A total of 18 silver probe-electrodes with a diameter of 1mm were attached to this cap (Figure 3-5). These probe-electrodes

penetrated to a depth of 70mm inside the cell to observe continuous changes in biosolids electrical properties during the electrokinetic experiment. The probe-electrodes were located at a distance of 1cm from each other. The distribution of voltage gradient between two electrodes was monitored by direct measurement of the potential between stainless steel electrodes and silver electrodes.

3. Perforated stainless steel (316) electrode coated with stainless steel mesh ($200\,\mu$):

Uniform distribution of electrical field was obtained by using 2 perforated stainless steel electrodes, which acted as an anode and cathode. Each electrode had an external diameter of 10mm, wall thickness of 0.65mm, and length of 102mm. There were 64 holes around the electrode to allow water to go through (Figure 3-6).

4. Inert material to fill the dead areas in the distance behind each electrode and EK cell walls.

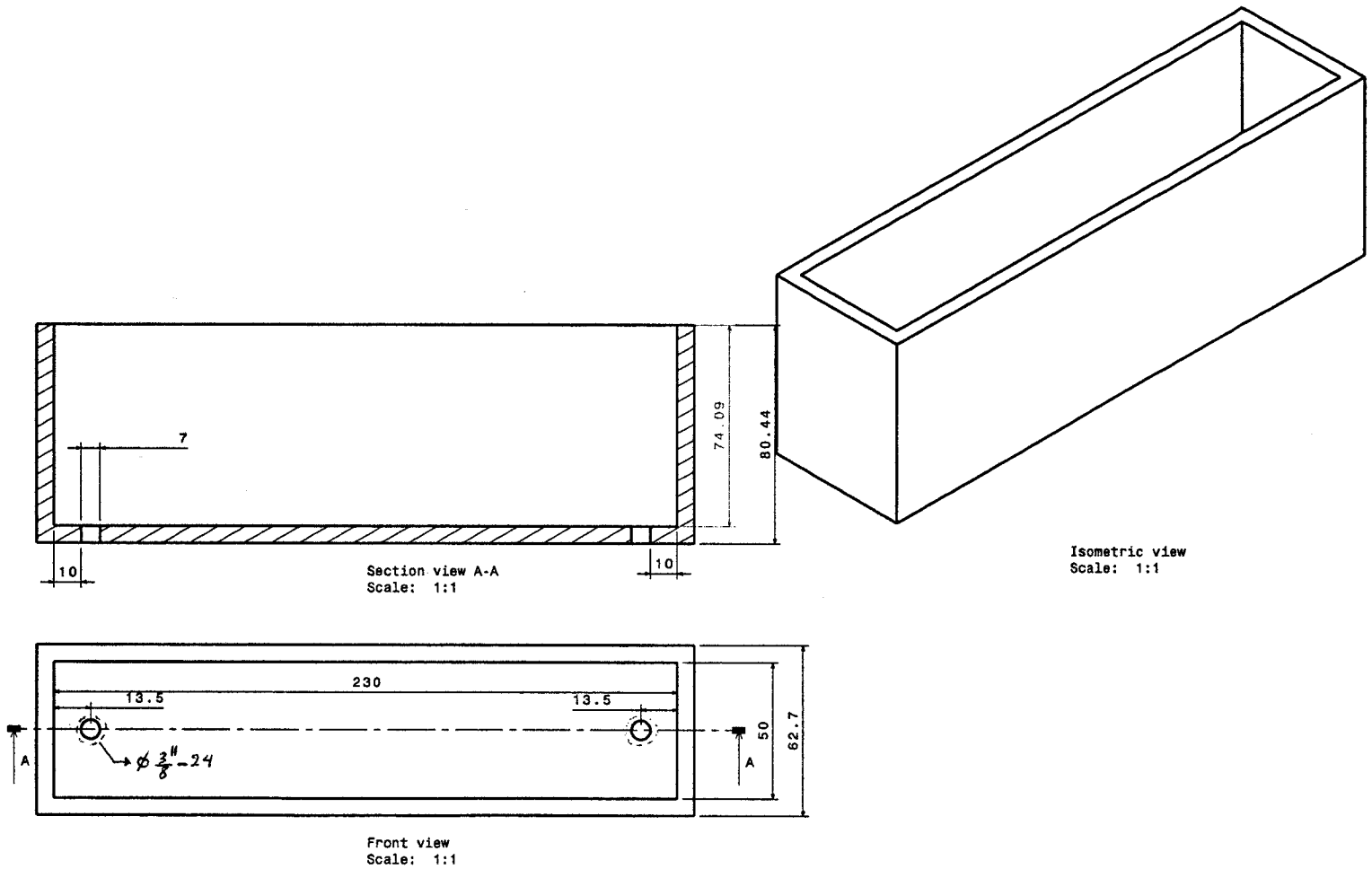


Figure 3-4: Schematic diagram of EK cell

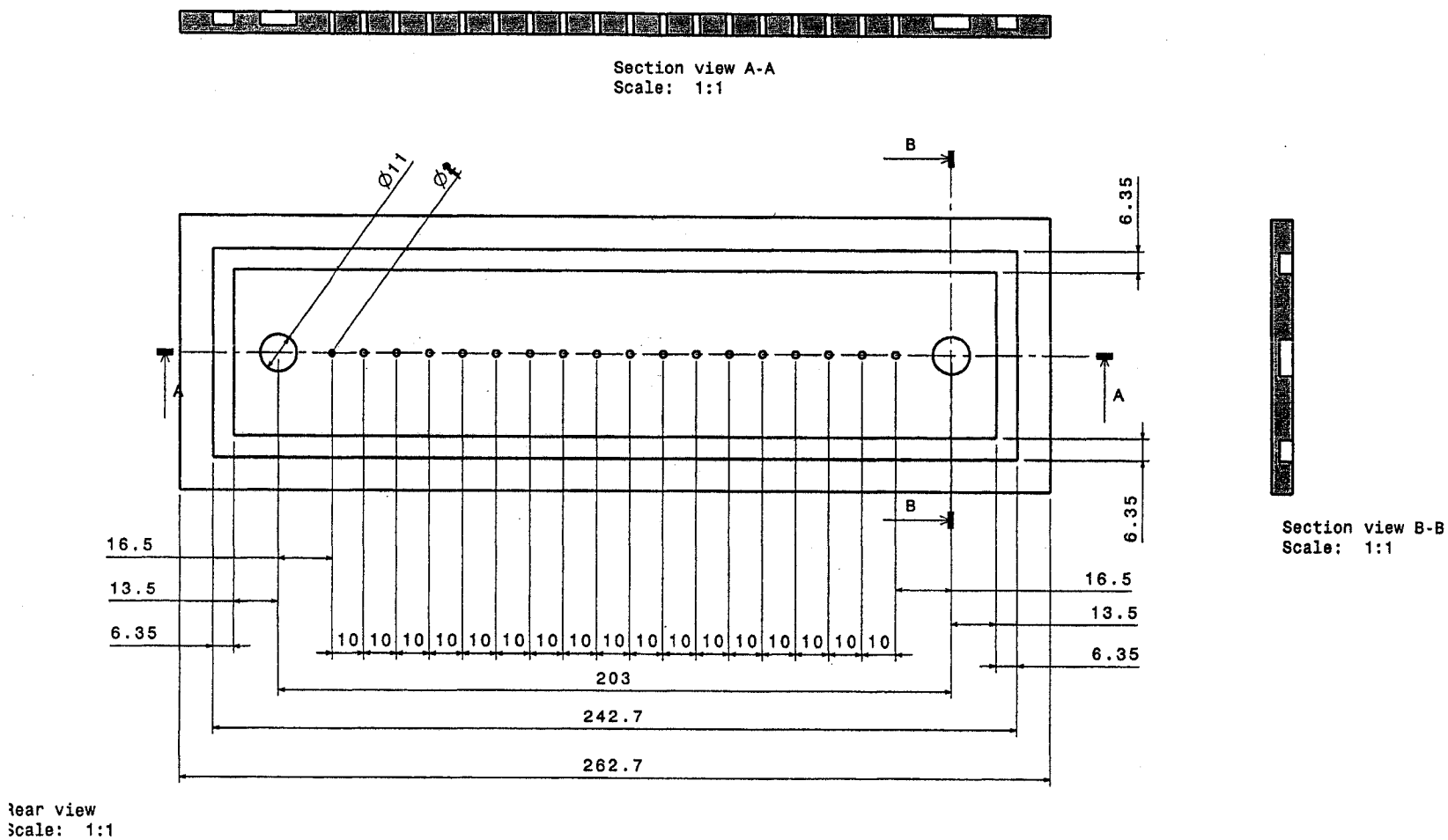


Figure 3-5: Schematic diagram of EK cover with electrode-probes

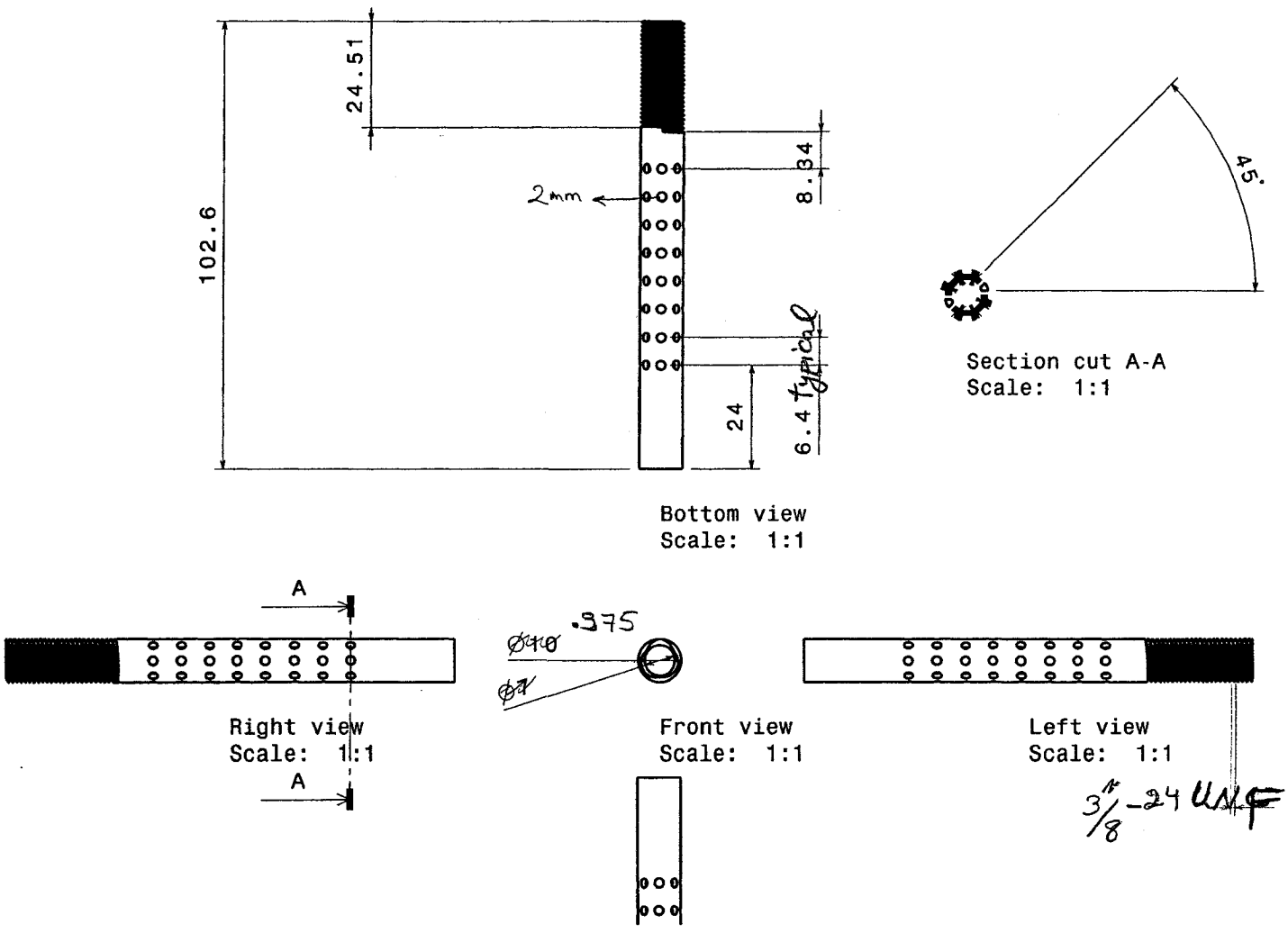


Figure 3-6: Schematic diagram of electrodes

3-1-3- Sampling of biosolids from WWTP

The biosolids were sampled from the Robert O. Pickard Environmental Centre (ROPEC) in Ottawa. This center is one of the largest WWTP facilities in Canada with a surface of 60-hectar area of land. The Pickard Center treats domestic, commercial, and industrial waste water from the Ottawa-Carlton municipality before returning the treated water to the Ottawa River. The facility treats an average capacity of 545 million liters of wastewater per day, with the peak capacity of 1,362 million liters per day. Between 1988 and 1993, the primary treatment facility was upgraded, and a secondary treatment process as well as biosolids processing facilities (anaerobic digestion complex) were added to the system to increase the capacity. These changes nearly doubled the efficiency of the plant. As a result, the Pickard Center now has the ability to remove more than 90 percent of pollutants from received waste water.

Treatment of wastewater in this system is completed through a series of physical, biological, and chemical processes. The preliminary treatment is performed through coarse screening, fine screening, and grit removal. During the primary treatment, settable solids, and floatable material (scum) are removed. In the secondary treatment, dissolved and suspended organic pollutant is removed using naturally occurring bacteria. In this stage phosphorus removal is done by chemical precipitation.

Removed solids from different processes of treatment at the ROPEC undergo an anaerobic digestion process, and produce biosolids. Between 40,000, and 45,000 metric tones of biosolids are generated annually (30 tones of dewatered biosolids per day).

For the purpose of this research, sampling of biosolids was collected after anaerobic digestion as is shown in Figure 3-7. Two 20-liter containers of anaerobically digested sludge were collected. Samples were held at a temperature below $10\,°C$ during transit to the laboratory, and at the laboratory.

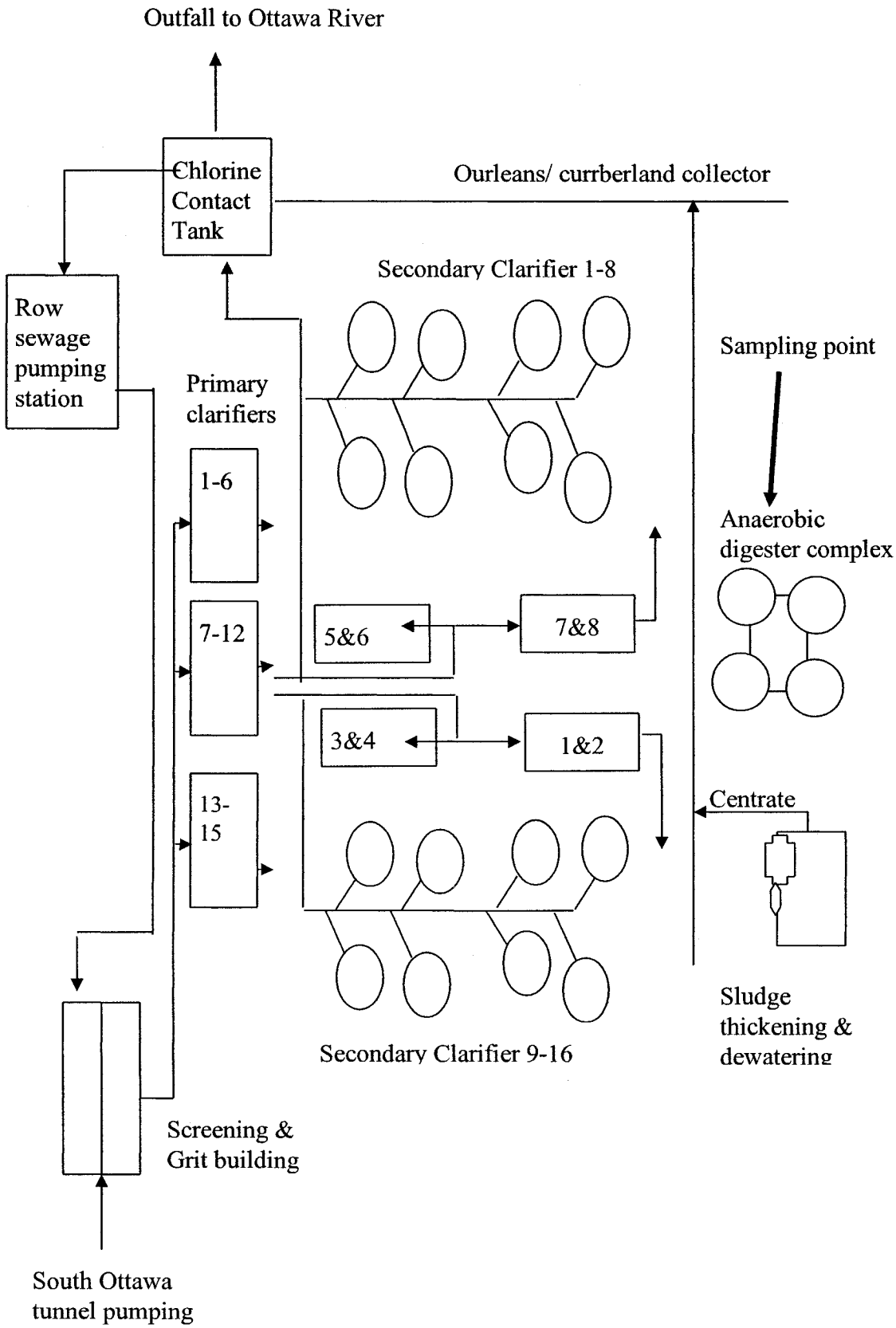


Figure 3-7: Plant outlay of Pickard Center and the sampling point

3-1-3-1- Initial characteristics of collected samples

The anaerobically digested samples were refrigerated and analyzed within 8 h after collection to determine its initial characteristics.

To make the initial analyses, the preserved sample was stirred, and transferred to a 4.5-L container, and was shaken 30 times. The well-mixed sample sludge was used for initial analyses. The obtained values are presented in Table 3-5.

Table 3-5: Initial characteristics of sludge sample

Parameters	Unit	Value
Microbiology		
C. perfringens	CFU /g	1.68×10^{6}
Reovirous	PFU /g	ND*
physicochemical		
Solids	%	2.5
Total Suspended Solids (TSS)	mg /L	28.25
Volatile Suspended Solids (VSS)	%	58
pH	-----	7.63
ORP	mV	-57.30
Nutrients		
Ammonium (NH3-N)	mg /g	30.80
Phosphate (PO_4^{3-})	mg /g	10.65
Inions		
Chloride (Cl^-)	mg /g	7.79
Sulfate (SO_4^{2-})	mg /g	56.64
Nitrate (NO^{3-} -N)	mg /g	ND
Nitrite (NO_2)	mg /g	0.01
Volatile Fatty Acids (VFAs)	mg /g	10.62

*ND= Non Detected

3-2- Laboratory techniques

3-2-1- Equipments and materials

An alphabetic list of materials, and equipments used during the experiments is presented below.

❖ Equipments

- Agate mortar W/PESTLE, 50mm (12-950AA)
- Anaerobic Culture System (VWR, 29446-290)
- Autoclaving biohazardous waste bags (01-815A)

- Autoclaving biohazardous waste bags (01-830D)
- Atomic Absorption spectrophotometer (Perkin Elmer, Analyst 100)
- Analytical Balances (Accumet AR25)
- Buchner funnel (1000 mL)
- Beakers (100, 200, 500, 1000-mL)
- Binder-Free Glass microfiber filters 37mmϕ (Whatman, 0987412B)
- Bunsen burner
- Centrifuge ISE HN-SII (International Equipment Company)
- Centrifuge tube (50 mL)
- Desiccator
- Erlenmeyer flask (50, 100, 250, 500, 1000-mL)
- Electric vacuum pump (Neuberger Knf)
- Fisher stirrer hotplate
- Funnel (7cm)
- Folded filter paper 12.5cmϕ (HACH 892-57)
- Filter paper (Whatman 40, FSSP 9751058)
- Fine pipette Thermo 1mL-5mL (1438685)
- Gooch crucible 40mL (08-195E)
- Graduate cylinder (10, 25, 400, 500, 1000-mL)
- Kimble Screwed test tubes, 25×150mm (14930E)
- Kimble glass vial, 21×70mm (60940D-4)
- Laminar air flow hood-Class II A/B_2 (Forma scientific)
- Micro pipette 2-20 μl (Fisher Brand, 14-900-28)

- Micro pipette 20-200 μl (Fisher Brand, 14-900-26)
- Micro pipette 30-300 μl (Thermo,)
- Muffle furnace (Fisher Scientific)
- Millipore syringe driven filter unit 25mm, 0.2 μm (SLFG 025LS)
- Microscope (Micromaster)
- Pipette (5, 10 mL)
- Petri dishes,100×15mm (08-757-13)
- Portable spectrophotometer (HACH, DR 2800)
- pH meter (Fisher AR25)
- Shaker (Oanadarride Scientific Digital Orbital)
- Self-refilling syringe (Wheaton, 1368950F)
- Volumetric flask (5, 25, 50, 100-mL)
- Water bath (Blue M)

❖ **Materials**

- Acetic acid glacial (A38500)
- Alpha-naphtol or N-(1-naphthyl) ethylene diamine dihydrochloride (ACROS 42399-0250)
- Amino acid reagent (HACH, 1934-32)
- Anaerobic gas pack (B260678)
- BBL Beef extract (B12303)
- BBL Agar (B12304)
- Bacto tryptose (DF0124172)

- Bacto yeast extract (DF01271791)
- BBL dry anaerobic indicator strip (271051)
- Calcium Carbonate (C64-500)
- D- galactose 99+% (ACROS 150611000)
- Enhancers (Biox S/ TAED and Glutaraldehyde from Atom company, Amphoteric,)
- Ethylene glycol (HACH, 2039-53)
- Egg yolk emulsion (Oxoid, SR0047)
- Ethyl alcohol, reagent grade, 95% (A995-4)
- Ferric chloride-sulfuric acid solution (HACH, 2042-53)
- Fluid Thioglycollate (FTG) (Oxoid, CM0173)
- Ferric ion solution (HACH, 22122-42)
- Gelatine bacteriologic (Oxoid, LP0008)
- Glycerol, Enzyme grade (BP229-1)
- Hydrochloridric acid (reagent grade, 4M)
- Hydroxylamine hydrochloride solution (HACH, 818-42)
- High range test N Tube™ AmVer™ Nitrogen Ammonia (HACH 26069-45)
- Lactose (DF0156173)
- Molybdate reagent (HACH, 2236-32)
- Mercuric thiocyanate solution (HACH, 22121-29)
- Microcentrifuge PCR tube (05-402-94)
- NitraVer® 6 nitrate reagent powder pillows (HACH, 21072-49)
- Nitrite, TNT 839 TNTplus™ reagent set (HACH, TNT839)

- Nitric acid (reagent grade, 1% v/v)
- NitriVer® 3 nitrate reagent powder pillows s (HACH, 21071-69)
- Parfilm (13-374-12)
- Perfringes agar base culture media (TSC , and SFP) Oxoid, CM0587)
- Perfringes (TSC) selective supplement B (Oxoid, SR0088)
- Perchloric acid (ACROS)
- Potassium Nitrate (BP 368-5000)
- pH meter buffer solution (pH= 4, 7, 10)
- Phenol red, free acid (Anachemia)
- Protease peptone No. 2 (DF0121175)
- Raffinose (DF0174153)
- Reovirus (Serotype T1L at 1.8×10^{8} PFU/ mL –University of Manitoba)
- Sodium thioglycollate (ACROS 148890500)
- Sodium Phosphate, Dibasic (P002505342)
- Sodium hydride (10N, 30% w/w, SS255B-1)
- Sodium hydroxide standard solution (HACH, 2040-53)
- Sodium hydride (5N, SS274B-1)
- Sulfanilic acid 99% (ACROS 150711000)
- SulfaVer® 4 reagent powder pillows (HACH, 21067-69)
- Sulfuric Acid Standard Solution (HACH, 2038-32)
- Water (reagent-grade)
- Water (Distilled- produced in Concordia laboratory)
- Zinc metal powder, 40 mesh (ACROS, 36726000)

3-2-2-Preparation of batch trials

For each batch trial a volume of 630 mL of well-mixed anaerobically digested biosolids from WWTP was used. Before pouring the biosolids in the reactors, it was incubated for 20 min at 75 °*C* , and cooled immediately in order to activate endogenous *C. perfringens* spores. The procedure of spiking the virus into the biosolids involved adding 0.060 mL of purified T1L Reovirus at 1.8×10^{8} PFU/ mL to 630 mL of biosolids (1.12×10^{7} PFU /gTS), and mixing for 5 min. Batch trial 1 was not spiked with any viruses. Based on each trial condition, appropriate enhancer doses were then added to the heat treated biosolids, and mixed well. After 5 minutes of mixing, biosolids were poured into the reactors. Different specified voltage gradients under variable exposure time were applied (Table 3-2).

3-2-3- Observation and measurement during the experiment

Daily observation of each reactor consisted of:

❖ Effluent collection

The volume of collected catholyte and anolyte were measured on a daily basis to evaluate the effect of various conditions on volume reduction of biosolids. The collected effluent was kept in a refrigerator for further measurements.

❖ Visual observation of the biosolids' behaviour

The movement and settling behaviour of biosolids were also evaluated. For most reactors (except reactors 5, 8, 11 and 15), the movement of liquid was along with the whole body of biosolids; however, in the case of reactors 5, 8 and 11, the liquid

movement was in the surface layer of the biosolids, and for reactor 15, one layer of liquid was located between the top and bottom layers of the biosolids.

❖ **Measuring of electrical parameters**

The voltage gradient was measured along the distance from the cathode at the beginning of each test, successively every six hours. Each reading represents a voltage gradient between the cathode, and a subsequent probe-electrode.

3-2-4- Sampling procedure

After disconnecting the DC power at the end of the experiment, the biosolids was sampled. In each EK cell, samples were taken from 4 sections by dividing a 19.1 cm distance between the electrodes. The firs 4.77cm of biosolids from the anode was assigned as "A", and the next 4.77cm as "MA" (middle area of the anode side). Similarly, the first 4.77cm from the cathode was named "C", and the next 4.77 cm was called "MC" (middle area of cathode side). The dimension of each section was 4.77-cm × 5-cm × 7.4-cm. Two other samples were collected from water effluent from the anode (anolyte), and the cathode (catholyte). Figure 3-8 illustrates the overall sampling scheme.

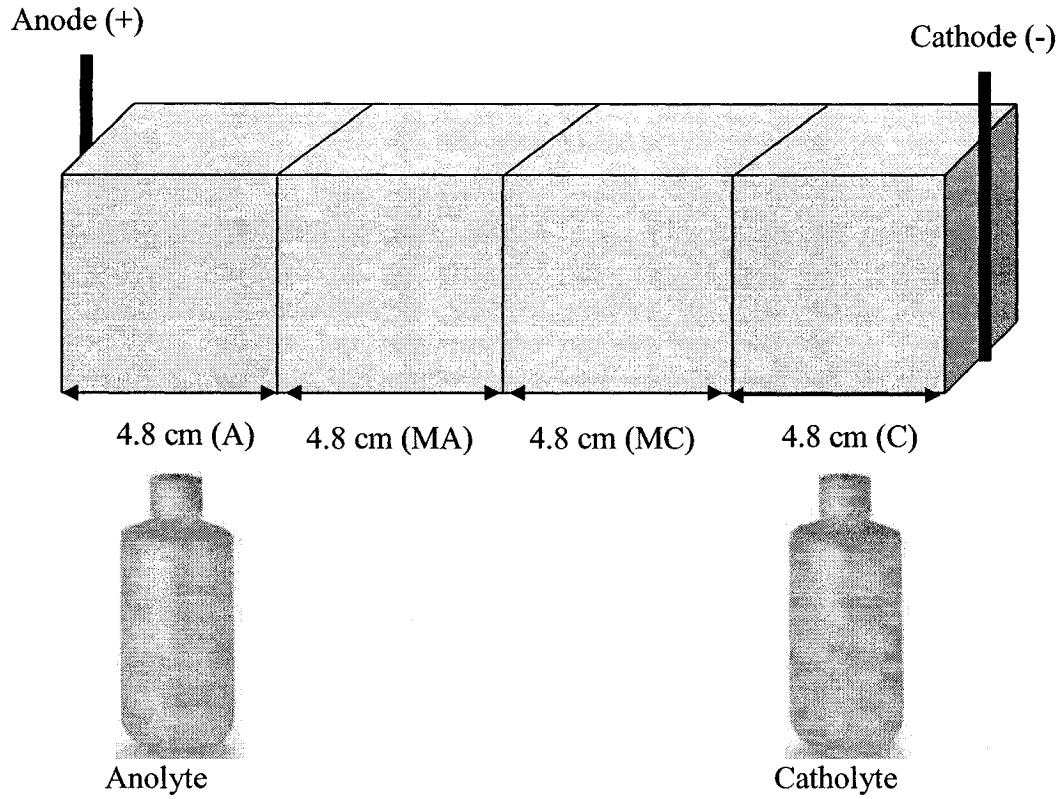


Figure 3-8: Biosolids sampling scheme

A portion of each sample was immediately analyzed for pH, ORP and enumeration of *C. perfringens* spores. The remains of each sample were packed, and labeled individually in 50 mL centrifuge tubes, and refrigerated for further analysis. An amount of 1.2mL of each sample (except for trial 1) was collected in 1.5 microcentrifuge PCR tubes, and sent to the Department of Medical Microbiology (University of Manitoba) under freezing condition for viral tests.

3-2-5- Analytical methods

The collected samples from each run of experiments were subjected to set of analyses such as pH, ORP, TSS, VSS, chloride, sulfate, Nitrogen-ammonium, phosphate, nitrate, nitrate, VFAs, and enumeration of *C. perfringens* spores, and viruses.

3-2-5-1- pH and ORP measurement

The pH and ORP measurement was done by inserting pH probe directly in the collected samples from reactors. The pH meter (Accumet AR25) has been designed in a way, which permits measuring pH and ORP simultaneously.

3-3-2-2- Total suspended solids measurement and total solid percentage

Total suspended solids (TSS) in samples were measured following the standard methods 2540 D (Clesceri, 2000). According to this method, a glass fiber filter disk was put inside of a Gooch crucible with the wrinkle side up. Disk and crucible combination was washed with three successive 20-mL portions of reagent-grade water using vacuum and placed in an oven 105°*C* for 1 h to dry. Since measuring of volatile solids was also desired, the combination was ignited at 550°*C* for 15 min in a muffle furnace, then cooled in a desiccator, and weighted. The cycle of igniting, cooling, desiccating, and weighting continued till a constant weight was obtained or until weight changes was less than 0.5mg of the previous weighting. The combination was kept in the desiccator until needed. After biosolids samples were ready to test, a volume of sample to yield between 2.5 and 200mg dried residue was chosen. The filter and crucible combination was assembled on filtration apparatus, and was wet with a small amount of reagent-grade water. Then, a measured volume of well-mixed sample was poured onto the seated glass-fiber filter. After removing the whole sample water, filter was washed with three successive 10-mL volumes of reagent-grade water, and suction continued to remove extra water. The filter and crucible combination was removed from vacuum apparatus, and

dried for 1 h at 150°C in an oven, cooled in a desiccator, and weighted. The cycle of drying, cooling, desiccating, and weighting was repeated until getting a weight change less than 0.5mg of the previous weight.

The mass of TSS per liter of sample was calculated according to following equation:

$$\text{mg TSS/L} = \frac{(A-B)\times 1000}{D} \qquad \text{(Eq. 3.1)}$$

Where:

A= mass of filter, and crucible combination+ dried residue, [mg]

B= mass of filter, and crucible combination [mg]

D= sample volume, [mL]

Percentage of total solids calculated following equation

$$\%\ \text{total solids} = \frac{(A-B)\times 100}{(C-B)} \qquad \text{(Eq. 3.2)}$$

Where:

A= mass of filter and crucible combination+ dried residue, [mg]

B= mass of filter and crucible combination [mg]

C= mass of wet sample + filter and crucible combination [mg]

3-3-2-3- Volatile suspended solids measurement

The procedure to determine the amount of volatile suspended solids (VSS) in the samples was done following the standard method 2540 E (Clesceri, 2000). For this purpose the samples obtained after the TSS tests were igniteted in a muffle furnace at a temperature of 550°C for 15 to 20 min. then cooled in a desiccator to balance the

temperature, and weighted as soon as it had cooled. The cycle of igniting, cooling, desiccating, and weighting was repeated until weight change was less than 0.5mg of the pervious weight.

The mass per liter of VSS content was then calculated as follows:

$$\text{mg VSS /L} = \frac{(A-B)\times 1000}{D} \qquad \text{(Eq. 3.3)}$$

Where:

A= (mass of residue + filter, and crucible combination) before ignition, [mg]

B= (weight of residue + filter, and crucible combination) after ignition, [mg].

3-3-2-4-Nitrogen-Ammonia (NH_3-N) measurement

Measuring of nitrogen-ammonia was performed according to HACH method 10031 in 655nm. The method is called "Salicylicate Method", and is based on combination of ammonia compounds with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green-colored solution (HACH, 2007). The detection rate is 0.4 to 50mg/L of NH_3-N.

The procedure consisted of adding 0.1 mL of filtrated sample to one AmVer dilute reagent test N tube for high range ammonia nitrogen. Then, it continued with adding one ammonia salicylate reagent powder pillow to the sample in the vial, and

shaking during a reaction period of 20 min. The concentration was expressed as mg/L of NH_3-N.

For the samples with high acidity or high alkalinity pH was adjusted using either sodium hydroxide standard solution 5N or 1:5 dilution of perchloric acid.

For all measurement using HACH methods a 10-fold dilution of sample in reagent-grade water was prepared, centrifuged at 3500 rpm for 15 min., and then filtered using HACH folded filter paper 12.5cmϕ.

3-3-2-5- Nitrite (NO_2) measurement

Measuring of nitrite was based on HACH method 10207 so called "Diazotization Method". The experimental range for this method is 0.015 to 0.6mg/L of NO_2^--N. The method is based on reaction of nitrite in the sample with a primary aromatic amine in acidic solution to form a diazonium salt. These couples with an aromatic compound to form a colored complex that is directly proportional to the amount of nitrite present (HACH, 2007).

To measure the nitrite in the sample 2 mL of filtrated sample poured in barcode vial, and wait for 10 min to read the concentration at 515 nm.

3-3-2-6- Nitrate (NO_3^--N) measurement

HACH method 8192 was used to measure nitrate in range 0.01 to 0.5 mg/L of NO_3^--N. The method is called "Cadmium Reduction Method". In this reaction cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium

with sulfanilic acid to form an intermediate diazonium salt. The salt couples with chromotropic acid to form a pink-colored product (HACH, 2007).

The procedure was consisted of adding one NitraVer®6 reagent powder pillow to 10 mL filtrated sample, shaking the sample vigorously for 3 min., and keeping it without further shaking for 2 min. the second reagent (NitriVer®3) powder pillow was added to sample along with 30sec shaking time , and followed by a 15 min reaction time. The concentration of nitrate was read at 507nm as mg/L of NO_3^--N.

3-3-2-7- Phosphate (PO_4^{3-}) measurement

HACH method 8178 was used to measure phosphate in the samples. The method is called "Amino Acid Method", and its measuring range is 0.23 to 30 mg/L of PO_4^{3-}. In this method, ammonium molybdate, in a highly acidic solution, reacts with orthophosphate to form molybdophosphoric acid. This complex is then reduced by the amino acid reagent to yield an intensely colored molybdenum blue compound (HACH, 2007).

To accomplish the analysis, 1mL of molybdate reagent was added to 25mL filtrated sample. After shaking, an amount if 1mL of amino acid reagent solution was added to mixture. A reaction time of 10 min was necessary before reading the concentration of phosphate in the sample at 530 nm.

3-3-2-8- Sulfate (SO_4^{2-}) measurement

Sulfate measurement analyses were done according to HACH method 8051 which has an application range of 2 to70 SO_4^{2-} mg/L. The reaction of sulfate ions in the sample

with barium in the SulfaVer® 4, and forming of a precipitate of barium sulfate is base of this method. The amount of turbidity formed is proportional to the sulfate concentration (HACH, 2007).

HACH square sample cell was filled with 10mL filtrated sample, and then one SulfaVer® 4 reagent powder pillow was added to sample, and swirled vigorously to dissolve the powder. After this step the cell kept without moving for 5 minutes reaction time. The concentration was determined with HACH spectrophotometer at 450nm, and expressed as mg/L of $SO4^{2-}$.

3-3-2-9- Chloride (Cl^-) measurement

The chloride analysis was carried out based on HACH method 8113 also called "Mercuric Thiocyanate Method" with application range of 0.1 to 25.0 mg/L Cl^-. This method is based on the reaction of chloride in the sample with mercuric thiocyanate to form mercuric chloride, and liberate thiocyanate ion. Thiocyanate ions react with the ferric ions to form an orange ferric thiocyanate complex. The amount of this complex is proportional to the chloride concentration (HACH, 2007).

To measure chloride, a 10 mL of clear sample was transferred to a HACH sample cell. Another sample cell was filled with 10 mL of deionized water as a blank. Then, an amount of 0.8mL of mercuric thiocyante solution was added to each sample cell, and mixed well; subsequently, 0.4mL of ferric ion solution was added. The mixture was swirled, and kept without disturbing for a two-minute reaction time. The concentration of chloride in sample was read by a HACH spectrophotometer DR2800 at 455nm.

3-3-2-10- Volatile Fatty Acids (VFAs) measurement

The analysis of volatile acids was done according to HACH method 8196 at 495 nm. This method also called "Esterification Method" with the detective range of 27 to 2800 mg/L. The method is based on esterification of the carboxylic acids present in the sample, and subsequent determination of the esters by the ferric hydroxamate reaction. All volatile acids present are reported as their equivalent mg/L as acetic acid (HACH, 2007).

In this procedure 1.5 mL of ethylene glycol and 0.2 mL of 19.2N sulfuric acid standard solution were added to 0.5 mL of filtrated sample. The mixture was boiled for 3 min., and then it was cooled till 25 $°C$ by running tap water. An amount of 0.5 mL of hydroxylamine hydrochloride solution, 2 mL of 4.5N sodium hydroxide, 10 mL ferric chloride sulfuric acid solution, and 10 mL of deionized water were added to sample respectively. After 3 min reaction time the concentration was read.

3-3-2-11- Electrical parameters

Current passing through the circuit, and voltage between cathode, and each probe-electrode was measured directly using a digital multimeter. Changes of resistances were calculated using Ohm's law by following equation (Esmaeily, 2002; Raihan, 2006):

$$\text{Ohm's Law: } R = \frac{V}{I} \qquad \text{(Eq. 3.4)}$$

Where:

R= Resistance (Ohm)

V= Voltage difference (V)

I= Electrical current (A)

3-2-2-12- Procedure for enumeration of Clostridium perfringens spores

The procedure for enumeration of *C. perfringens* spores was consisted of three steps. The first step was "presumptive phase" in which selective media were devised for isolating *C. perfringens* from biosolids contaminated with other bacteria. Many of these media contain sulfite, and some other iron salts. Although sulfite is inhibitory for other bacteria, it is only slightly so for the clostridia. Most of clostridia reduce sulfite to sulfide, giving rise to black colonies or black zones around colonies in media containing iron. The most common solid media for the presumptive enumeration of *C. perfringens* include SPS (sulfite-polymyxin-sulfaziazine), TSN (tryptose-sulfate-neomycin), SFP (shahidi-ferguson perfringens), TSC (tryptos-sulfite-cycloserine) and OPSP (oleandomycin-polymyxin-sufadiazine perfringens). TSC was found to be the most satisfactory for the purpose of this research.

The second step was "confirmed phase" in which according to physiological characteristics of *C. perfringens,* the presence of *C. perfringens* was confirmed in several microbiological steps.

In the third step, *C. perfringens* was isolated for growth and spore production. Figure 3-9 shows three steps enumeration procedure.

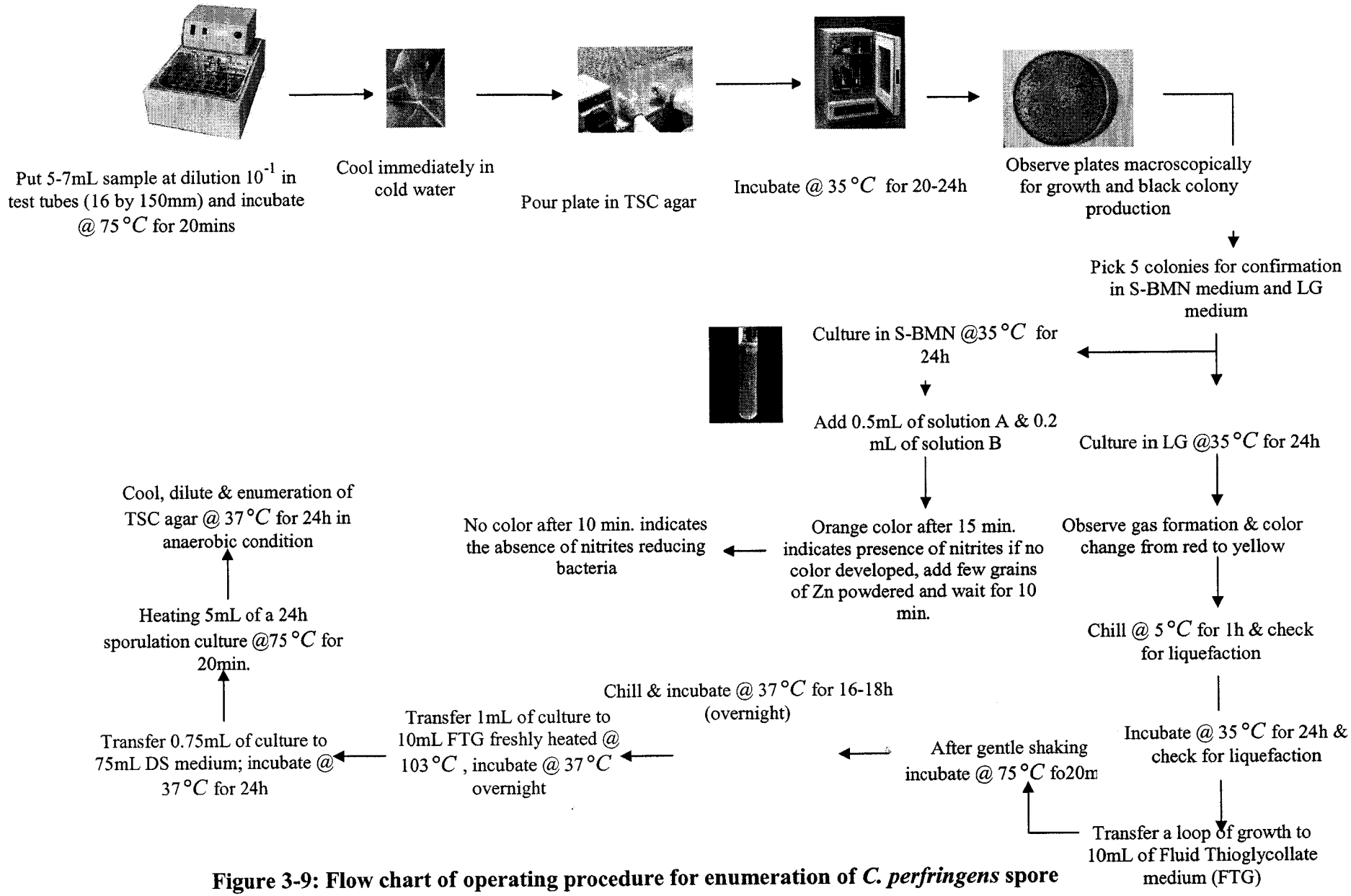


Figure 3-9: Flow chart of operating procedure for enumeration of *C. perfringens* spore

❖ Presumptive phase

In this step, heat-resistant spores were produced through the removal of vegetative cells due to heat treatment. For enumeration of *C. perfringens* spores, 5-7 mL samples of 10-fold dilution at 0.01M phosphate buffer (pH 7.0) in test tubes were incubated in a water bath at 75 °*C* for 20 min., and were cooled immediately in cold water. Then, higher dilutions for heat-shocked sample were prepared. TSC culture media with "perfringens TSC selective supplement B" was used to separate *C. perfringens* from other probable existing bacteria. An amount of 1mL of each dilution was added to TSC using "pour plate" method. The plates were incubated in anaerobic conditions using incubate jar for 20-24 hours at 35 °*C* . Incubation for up to 24 hours prevents from including other clostridia in the count. Some other clostridia require at least 48 hours of incubation at 35-38 °*C* in TSC agar to obtain appreciable growth. After incubation, the plates were observed macroscopically for growth, and black colony production.

All *C. perfringens* colonies produce apparent halos (luminous radiance, crown of light). Plates showing estimated 20-200 black colonies were selected. Black colonies were counted, and number of *C. perfringens* spp. / g solids were calculated (Figure 3-10, Appendix B). Furthermore, 5 colonies were picked for confirmation in supplement buffered nitrate-mobility (S-BNM) medium, and lactose gelatin (LG) medium.

❖ Confirmed phase

As described earlier, *C. perfringens* are non-mobile, sulfide, and nitrite producing (or sulfite, and nitrate reducing), and gelatin liquefying bacteria. According to these characteristics, a confirmation process was designed for this stage.

After presumptive phase, simultaneously five presumptive *C. perfringens* colonies from each enumeration agar per sludge sample were stab-inoculated into S-BNM medium, and into LG medium. Table 3-6, and 3-7 illustrate the compound of S-BNM, and LG media respectively.

Table 3-6: Composition of supplement buffered nitrate-mobility (S-BNM) medium

Composition	Amount g/L
Beef extract	3.0
Peptone	5.0
KNO3	5.0
Na_2HPO_4	2.5
Agar	3.0
D- galactose	5.0
Glycerol	5.0

Table 3-7: Composition of lactose gelatine (LG) medium

Composition	Amount g/L
Tryptose	10.0
Yeast extract	10.0
Lactose	10.0
Na_2HPO_4	5.0
Phenol red	0.05
Gelatin	120

Nitrate-Motility Test

The S-BMN cultures were tested for presence of nitrite by adding 0.5 mL of solution A, and 0.2 mL of solution B to test tube. Orange color that develops within 15 minutes indicated presence of nitrites. If no color developed, a few grains of powdered zinc (Zn) metal was added, and let stand for 10 minutes. No color change after addition of Zn indicated that organism is incapable of reducing nitrates.

Solution A:

To make solution A, an amount of 8 g of sulfanilic acid was dissolved in 1L of 5N CH_3COOH.

Solution B:

Solution B was made by dissolving 5 g of alpha-naphtol or N-(1-naphthyl) ethylene diamine dihydrochloride in 1 L of 5N CH_3COOH .

Lactose-gelatin test

The colonies were examined in LG medium for gas production and color change. The color change from red to yellow indicated that lactose fermented with production of acid. After incubation period of 24 hours, culture tubes were chilled for 1 hour at 5 °*C* , and checked for gelatin liquefaction. If medium was solidified, additional 24 hours re-incubation at 35 °*C* was performed, and test for gelatin liquefaction was repeated (Figure B-1, Appendix B).

Non-motile, Gram-positive bacilli that produced black colonies in TSC agar, reduced nitrates to nitrites, produced acid, and gas from lactose, and liquefied gelatin within 48 hours were identified as *C. perfringens*.

❖ Isolation, growth and spore production phase

Once confirmed as *C. perfringens*, a loop of growth from the lactose-gelatin medium was transferred into 10 mL of fluid thioglycollate medium (FTG), and dispersed by gentle shaking. The medium was heated at 75 °*C* for 20 minutes, cooled, and incubated at 37 °*C* for 16-18 hours. The plastic caps of the culture tubes were kept on but not too tight to permit evolved gas to escape. One milliliter portion of this culture was

transferred to 10 mL of deoxygenated (freshly heated to 103 °C for 10 min to repel oxygen, and cooled to 37 °C) FTG medium, and incubated overnight. The culture was added at 1% concentration of Duncan-Strong (DS) sporulation medium (0.75 mL FTG culture to 75 mL DS medium), and incubated at 37 °C for 24 h. Gram staining, and light microscopy were carried out on dilution of these suspensions to ascertain the proportion of vegetative bacteria versus spores. Table 3-8 sets the DS compound of sporulation medium.

Table 3-8: DS sporulation medium compound

Composition	Amount g/L
Protease peptone	15.0 g
Yesat extract	4.0 g
Sodium thioglycollate	1.0 g
Soluble starch or Raffinose	4.0 g
Sodium phosphate (dibasic hepta hydrate)	10.0 g

No special precautions were followed to maintain anaerobic conditions other than inclusion of sodium thioglycollate, and stationary incubation of the culture. Heat-resistant spore levels were determined after incubation in the sporulation medium by heating 5 mL of a 24 h-sporulating culture for 20 minutes at 75 °C in a capped tube, followed by cooling, dilution, and enumeration of TSC agar. The plates were counted after 24 hours of incubation at 35-37 °C in an anaerobic jar (HPA, 2005a; HPA, 2005; ICR, 1996).

Calculation of spore density

Number of spores of *C. perfringens* in sample was calculated on basis of percentage ofcolonies tested that were confirmed as *C. perfringens.* For example, if geometric mean plate count of 10^{-4} dilution was 105 and 6 of 10 colonies tested were

confirmed as *C. perfringens*, number of *C. perfringens* spores or CFU per g solids was $105 \times \frac{6}{10} \times 10{,}000 = 630{,}000$.

The efficiency of inactivation was calculated as $Log \frac{N_t}{N_0}$, where N_0 is the initial concentration of the microorganism at zero time in control sample, and N_t is the geometric mean of concentrations after each inactivation trial.

3-3-2-13- Virus counting tests

The procedure for virus counting was performed in Department of Medical Microbiology, and Infectious Disease, University of Manitoba. The performed procedure was as follows:

Briefly, serial 10-fold dilutions of viral lysate stockes were made in gel saline, and their titers were determined in duplicate on L929 cell monolayers in six-well cluster dishes. After viral attachment (1h in room temperature), cells were overlaid with 3 mL per well of a 50:50 (vol/vol) mixture of 2% Bacto agar, and completed 2× medium 199. Cells were incubated at $37\,°C$. Monolayer were fed a second time with 2 mL of completed agar-199 per well at 3 days after inoculation. A final overlay with medium 199 which contained 1% agar and 0.04% neutral red was performed 6 days after inoculation. Plaques were counted 18 to 24 h after neutral red overlay (Coombs et al. 1994).

IV- RESULTS AND DISCUSSION

To evaluate the effect of EK and other factors, several parameters were measured. Due to the effect of the amphoteric agent on initial concentration of nitrogen-ammonia, phosphate and sulfate, the results for these elements will be presented in three groups: the reactors without amphoteric agent, the reactors with 0.7% w/v of amphoteric agent and the reactors with 1.3% w/v amphoteric agent. For the rest of, in order to a make comprehensive comparison, the results for each set of trials will be shown in three groups according to voltage gradients (0.5 V/ cm, 0.7 V/ cm and 1.5 V/cm). All results presented here are the geometric mean of three replicate experiments.

4-1- Effects on the physiochemical properties

4-1-1- Effluent removal in the EK reactors

The amount of effluent was measured every 24 hours during running the experiments, according to collected volume of anolyte and catholyte. Table 4-1 shows the volume of leakage collected for each trial (Figure 4-1). In the case of the reactor 1, only the total collected volume, and 3 days running of experiments have been shown; however, the duration of this trial was 9 days.

Table 4-1: Volume of anolyte and catholyte for each batch test

EK reactor trail number	Anolyte volume (mL)			Catholyte volume (mL)			Total (mL)
	24 h	48 h	72 h	24 h	48 h	72 h	
1*	15	12	11	150	80	70	325
2	215	NA	NA	150	NA	NA	365
3	210	60	10	150	10	5	445
4	3	2	0.5	110	105	60	280.5
5	7	NA	NA	150	NA	NA	157
6	250	NA	NA	160	NA	NA	410
7	200	50	20	110	30	10	420
8	3	0.5	0.5	100	60	40	204
9	105	11	9	180	30	20	359
10	7	3	1	130	70	35	246
11	46	NA	NA	235	NA	NA	281
12	270	NA	NA	135	NA	NA	405
13	180	20	30	132	23	20	405
14	10	7	3	100	107	58	285
15	10	NA	NA	200	NA	NA	210
16	82	NA	NA	42	NA	NA	124

* : The results of three days running test were shown.
NA: Not applicable. The reactors with 1 day exposure time.

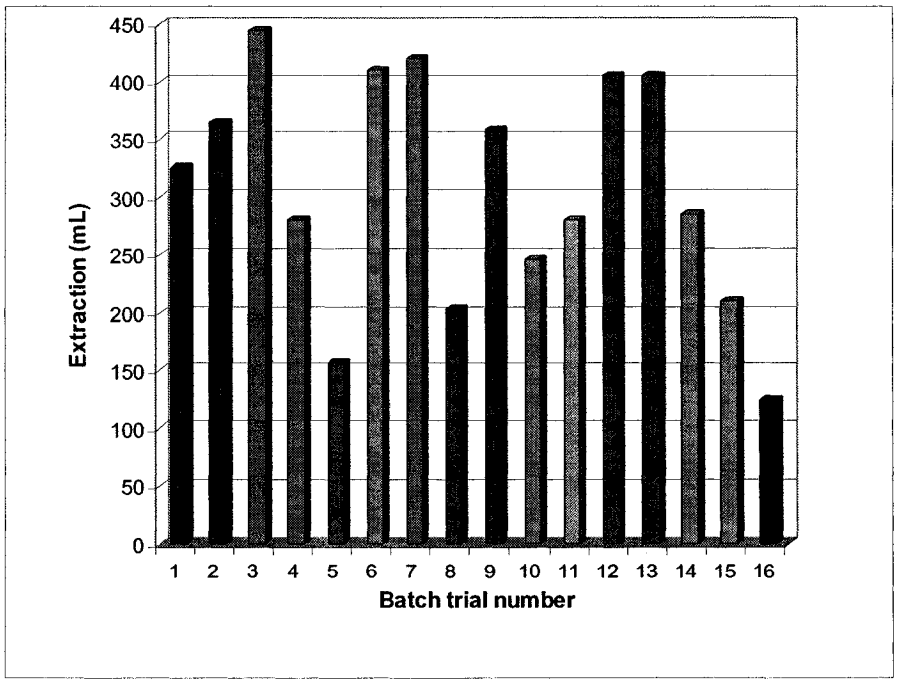


Figure 4-1: Total drainage in batch trials

The results show the efficiency of the enhanced EK system to dewater will increase by adding amphoteric agent, increasing voltage gradient and exposure time. For example, the reactor 3 with 1.3% w/v concentration of amphoteric, 3 day exposure time and voltage gradient of 1.5 V/cm had the highest volume of leakage.

Removal of water in EK reactors can be explained as follows: the water in the biosolids contains cations that, under the effect of a continuous electrical current, are attracted by the negative pole, the cathode. Through viscous action, the movement of the cations through the water in the sludge carries water molecules towards the cathode, where the water is finally drained out of the sludge.

It was also observed that the reactors with the amphoteric agent, namely 2, 3, 6, 7, 12, 13 and 16, had higher volumes of leakage compared to the reactors without the amphoteric agent. This fact is due to the reaction of the amphoteric agent with colloid particles in biosolids, the subsequent formation of bigger colloids and, finally, the removal of free water from the biosolids.

Also, it was noted that the reactors with the amphoteric agent had higher leakage in the anode area compared to the cathode area. This water removal was proportional to the amount of added amphoteric agent. These results indicate the effect of the amphoteric agent on electroosmosis. As mentioned in earlier chapters, the direction of the electroosmotic flow is decided by the nature of the charges on the surface of solid particle. Application of the amphoteric agent causes a net excess amount of cations, which drag more water molecules toward the anode area.

4-1-2- Effects on TSS, VSS and TS%

The TSS, VSS and TS% were measured following the procedures explained in section 3-3-2-2- and 3-3-2-3. Figure 4-2 demonstrates geometric mean of total solids in all batch reactors and in initial sample without any treatment assigned as "Ini".

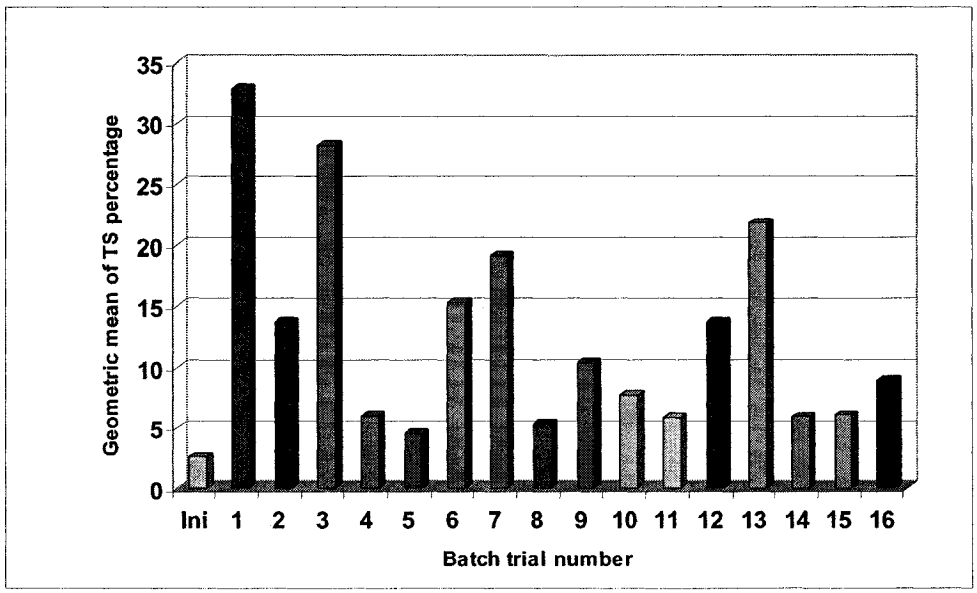


Figure 4-2: Geometric mean of solid content distribution in all batch reactors and in initial sample

The data presented in Figure 4-2 show that:

- In all the reactors, percentage of TS after treatment reached to higher amount than initial value (2.6%) showing that EK had an effective impact of dewatering of biosolids due to effect of electrophoresis, electroosmosis and sedimentation potential.
- Exposure time has significant effect on homogenous increasing of the total solids in EK reactor. As it can see with surge of time until 9 days (the reactor 1) the percentage of TS increased to 33%.

- The result of TS percentage of the reactors 3, 6, 12 and 13 shows that adding 1.3 % w/v of the amphoteric agent helps in achieving higher percentage of TS comparing to condition without the amphoteric enhancer.
- High gradient of EK field is also another factor to increase the percentage of TS in EK reactor. Furthermore, it seems that there is a synergistic effect of amphoteric agent, exposure time and high gradient of voltage on this increase. The reactors 3 and 7 display the importance of this synergistic effect on percentage of TS.
- Oxidant agents did not show any effects on increase of percentage of TS as the lowest percent of TS was monitored in reactor with oxidants (reactor 5).

❖ TSS, VSS and TS% in the reactors with the voltage gradient of 0.5 V/cm

TSS, VSS and TS% from the experiments with a voltage gradient of 0.5 V/cm is shown in Table 4-2. Figure 4-3 illustrates the distribution of these parameters between the electrodes. The highest geometric mean of TS% for these group of trials belongs to the reactor 1 (9 days exposure time) with 33%, and the lowest is related to the reactor 5 with 4%. The standard deviation of TS% for the reactor 1 was 7 and for the reactor 5, this value was 2.46.

The results show that at low voltage gradient of 0.5 V/cm, exposure time plays the important role in increasing of the amount of TSS in the EK reactors.

Table 4-2: TSS, VSS and TS% for the reactors with voltage gradient of 0.5 V/cm

EK reactor trail number	Code	TSS (g/L)	VSS (g/L)	TS %
1	C	82.3	21.2	29.5
	MC	146.3	69.0	35.6
	MA	73.4	24.3	26.3
	A	250.3	81.2	42.3
				M*= 32.9
4	C	8.3	6.3	3.9
	MC	10.4	5.1	4.6
	MA	20.1	11.3	7.6
	A	32.1	14.2	9.8
				M= 6.1
5	C	9.4	5.6	3.4
	MC	8.8	4.5	3.2
	MA	12.4	6.3	4.5
	A	24.0	12.5	8.5
				M= 4.2
8	C	8.5	5.6	3.1
	MC	10.2	4.3	3.6
	MA	21.0	10.3	7.6
	A	26.1	13.6	9.5
				M= 5.3
9	C	15.4	8.6	5.6
	MC	28.1	15.3	10.2
	MA	45.5	21.8	16.5
	A	35.0	21.2	12.6
				M= 10.4
12	C	23.7	14.3	8.6
	MC	36.3	23.1	13.2
	MA	40.0	25.2	14.3
	A	60.0	36.0	21.5
				M= 13.6
13	C	45.1	25.0	16.2
	MC	39.9	24.0	14.5
	MA	72.3	48.0	21.2
	A	259.5	191.0	45.1
				M=21.7
16	C	14.0	8.60	5.1
	MC	21.5	13.3	7.8
	MA	22.8	15.0	8.3
	A	52.9	31.4	19.2
				M= 8.9
* Geometric mean= M				

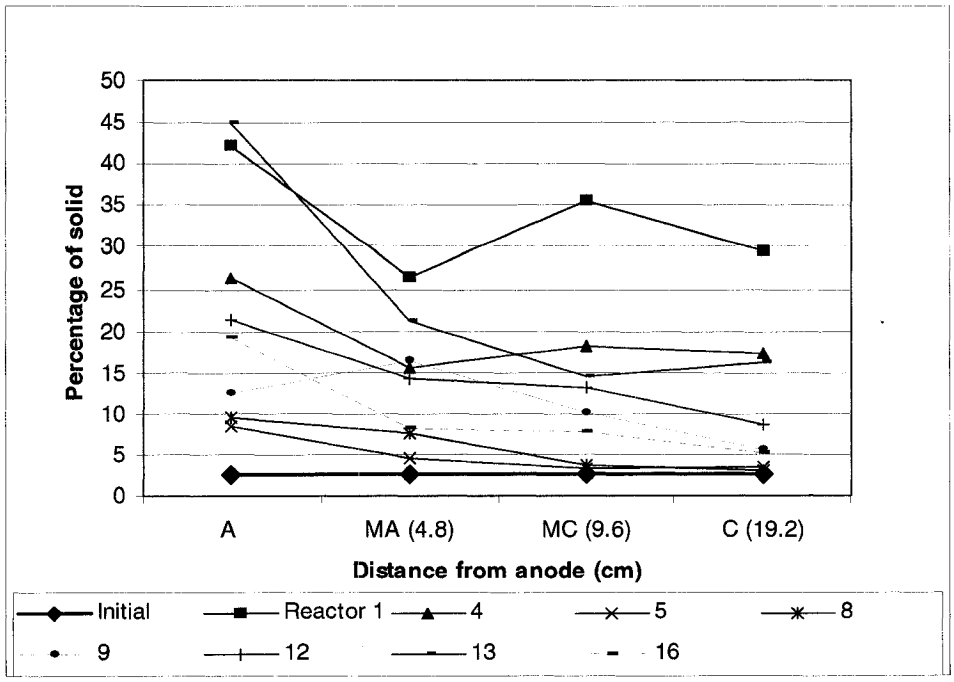


Figure 4-3: Percentage of solids in the EK reactors with voltage gradient of 0.5 V/cm

❖ TSS, VSS and TS% in the reactors with the voltage gradient of 0.7 V/cm

Table 4-3 presents the TSS, VSS and TS% in the experiments with voltage gradient of 0.7 V/cm. Their corresponding graphs have been shown in Figure 4-4. The reactor 6 showed the highest percentage of total solid with 15% and the reactor 14 presented 6% which was the lowest value for this group.

The standard deviation showed the value of 5.71 for the reactor 6 and 3.20 for reactor 14.

Table 4-3: TSS, VSS and TS% for the reactors with voltage gradient of 0.7 V/cm

EK reactor trail number	Code	TSS (g/L)	VSS (g/L)	TS %
2	C	13.9	8.9	15.6
	MC	21.9	14.6	9.5
	MA	24.6	16.8	12.3
	A	65.8	32.1	20.3
				M*= 13.8
6	C	32.0	18.0	11.6
	MC	32.0	18.5	11.5
	MA	74.9	29.0	17.4
	A	65.0	40.0	23.5
				M= 15.28
10	C	10.5	6.6	3.8
	MC	26.14	16.2	9.5
	MA	24.0	13.5	8.6
	A	32.0	19.0	11.6
				M= 7.74
14	C	9.7	5.7	3.5
	MC	11.5	6.7	4.2
	MA	22.56	14.21	8.2
	A	28.06	17.7	10.2
				M= 5.92
* Geometric mean= M				

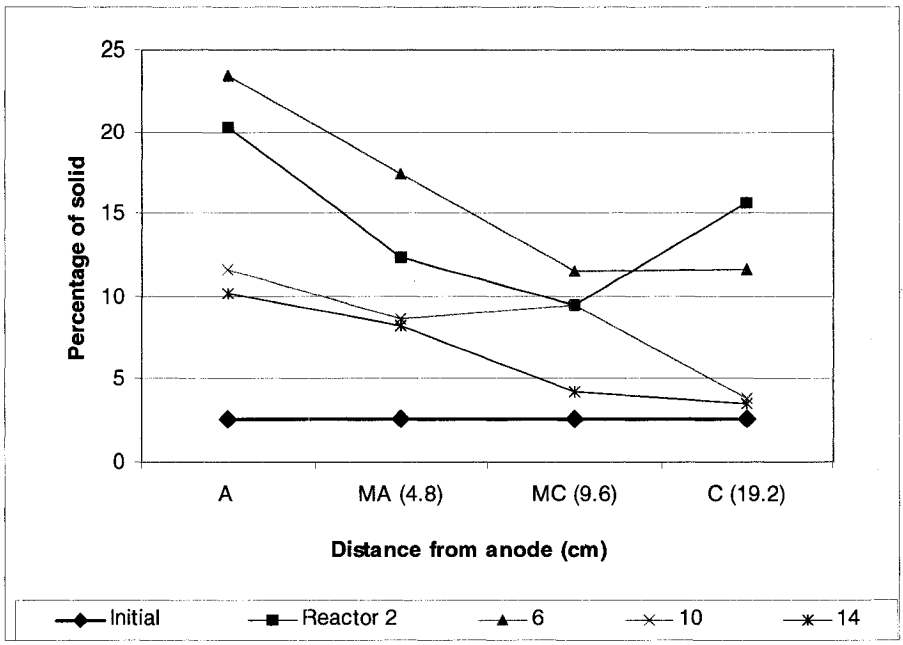


Figure 4-4: Percentage of solids in the EK reactors with voltage gradient of 0.7 V/cm

❖ TSS,VSS and TS% in the reactors with the voltage gradient of 1.5 V/cm

Table 4-4 and Figure 4-5 demonstrate TSS, VSS and TS% in the experiments with voltage gradient of 1.5 V/cm. An amount of 28% of TS was the highest value observed in this group which was related to the reactor 3. The reactor 11 had the lowest mean percentage of TS with value of 6%.

The standard deviation showed the value of 18.97 for the reactor 3 and 2.72 for reactor 11.

Table 4-4: TSS, VSS and TS% for the reactors with voltage gradient of 1.5 V/cm

EK reactor trail number	Code	TSS (g/L)	VSS (g/L)	TS %
3	C	34.0	20.9	12.3
	MC	60.0	27.5	21.7
	MA	125.0	78.0	45.3
	A	143.9	85.0	52.3
				M*= 28.2
7	C	20.3	10.3	17.4
	MC	50.4	23.1	18.3
	MA	43.1	21.3	15.6
	A	74.1	19.8	26.3
				M= 19
11	C	8.80	3.0	3.2
	MC	14.6	5.0	5.3
	MA	21.0	6.6	7.5
	A	26.0	9.14	9.5
				M=5.9
15	C	11.5	3.61	4.2
	MC	12.4	3.7	4.5
	MA	21.8	6.52	7.9
	A	25.3	7.25	9.1
				M=6.07
* Geometric mean= M				

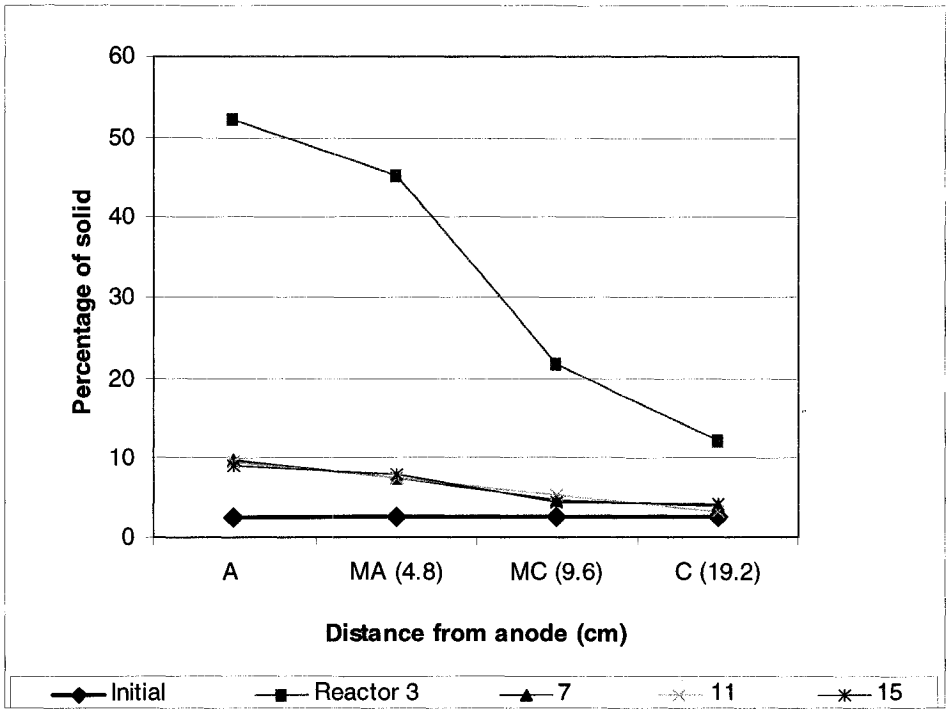


Figure 4-5: Percentage of solids in the EK reactors with gradient voltage of 1.5 V/cm

The evaluation of TS% in all reactors implies that lower voltage gradient plays an important role in dewatering process; in this case the majority of reactors achieved a high TS% content.

In summery, the effect of the amphoteric agent and low voltage gradient are determining factors for dewatering process.

4-1-3- Effects on pH

pH value of biosolids was measured at the beginning and at the end of each batch tests. The initial pH was 7.38. It was observed that the pH values in the cathode area were higher than pH in the anode area because of formation of OH^- in cathode and H^+ in anode due to electrokinetic reactions.

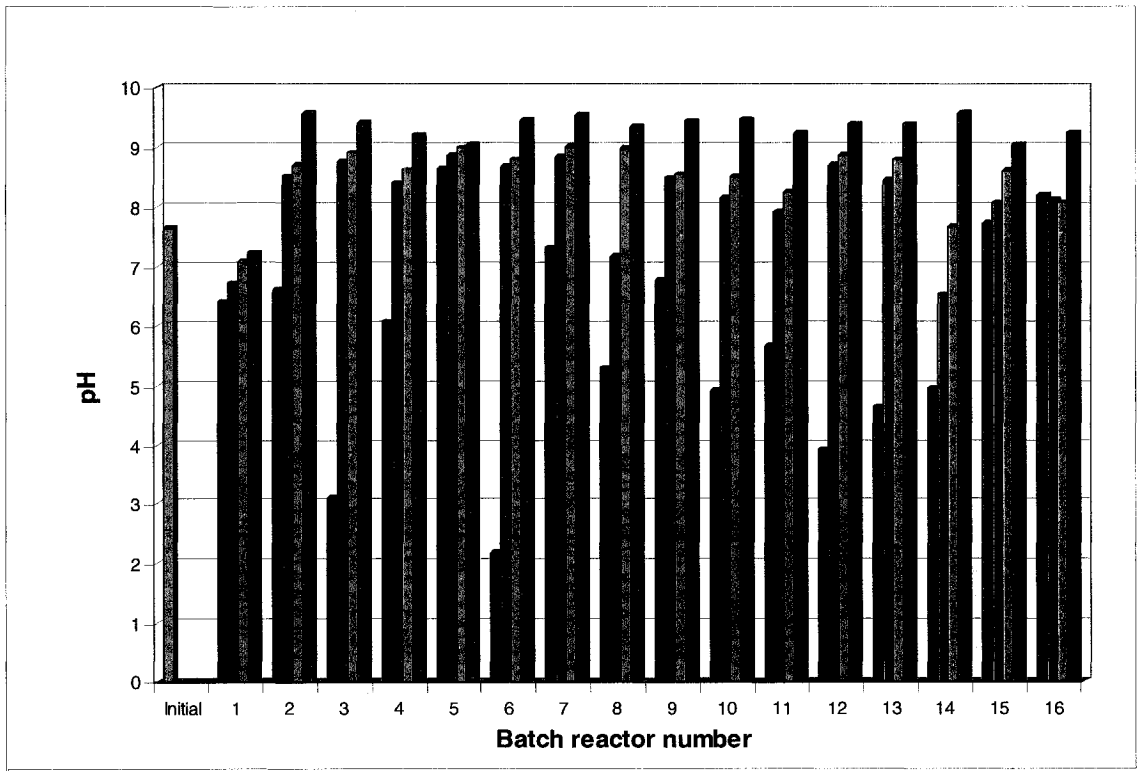


Figure 4-6: pH distribution profiles in different parts of each batch reactors

Figure 4-6 shows the pH distribution profiles in different parts of each batch reactor. The most important observation of these results will be listed as follow:

- In all cases the highest and lowest pH value was related to cathode and anode area of each reactor, respectively. This fact is the result of OH^- and H^+ formation at the cathode and anode, respectively due to electrolysis of water.
- The highest pH value of 9.56 was belonged to reactor 14 (voltage gradient 0.7,with no enhancement agent and exposure time of 3 days), and the lowest value of 2.17 was related to reactor 6 (voltage gradient 0.7, exposure time of 1 day, 1.3% w/v of the amphoteric agent, and 0.4%w/v).

- The highest pH gradient (7.37 pH/ cm) between cathode and anode belongs to reactor 6 with pH value of 9.54 in cathode and 2.17 in anode when high amount of amphoteric, and oxidant were applied.
- The lowest pH gradient (0.39 pH/ cm) between cathode and anode was related to reactor 5 with pH value of 9.03 in cathode and 8.64 in anode. This reactor had the 0.5% (v/v) of glutaraldehyde agent and 0.2% (w/v) of Bioxy S/TEAD.
- Combination of the amphoteric agent with high concentration of Bioxy S/TEAD helps to reach lower pH at anode (reactor 6).
- The reactor 14 had the same experimental condition as reactor 1 except voltage gradient and exposure time. The comparison of pH values between these two reactors suggests that higher voltage gradient express higher pH gradient.
- Exposure time seems do not have a direct impact on pH gradient.
- The Reactors 3, 6, 12 showed a pH lower than 4 at the anode area.
- In the reactor 1, the pH lower than 7.5 was observed in entire reactor.
- Except for the reactor 1, all other reactors showed a pH higher than 9 in the cathode area.

❖ pH in the reactors with the voltage gradient of 0.5 V/cm

Table A-1 (Appendix A) shows the results of pH in batch reactors with voltage gradient of 0.5V/cm. Also Figure 4-7 illustrates the pH distributions for these batch reactors.

The highest and lowest value of pH in EK treated biosolids were found in the reactor 9 in cathode (pH 9.44) and the reactor12 in anode (pH 3.92), respectively.

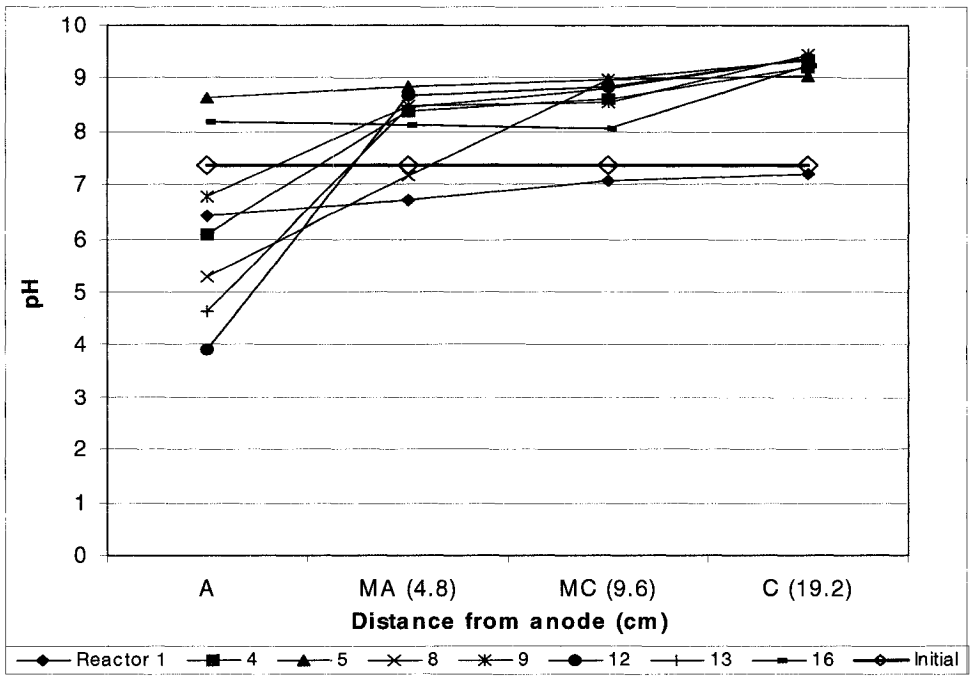


Figure 4-7: pH distribution in the EK reactors with voltage gradient of 0.5 V/cm

❖ pH in the reactors with the voltage gradient of 0.7 V/cm

The results of pH in batch reactors with voltage gradient of 0.7 V/cm have been shown in Table A-2 (Appendix A). Figure 4-8 is the graphs for pH distributions for these batch reactors. In this group the maximum pH belongs to the reactors 2 and 14 in the cathode area with value of 9.56 and the lowest pH belongs to the reactor 6 at the anode area with value of 2.17.

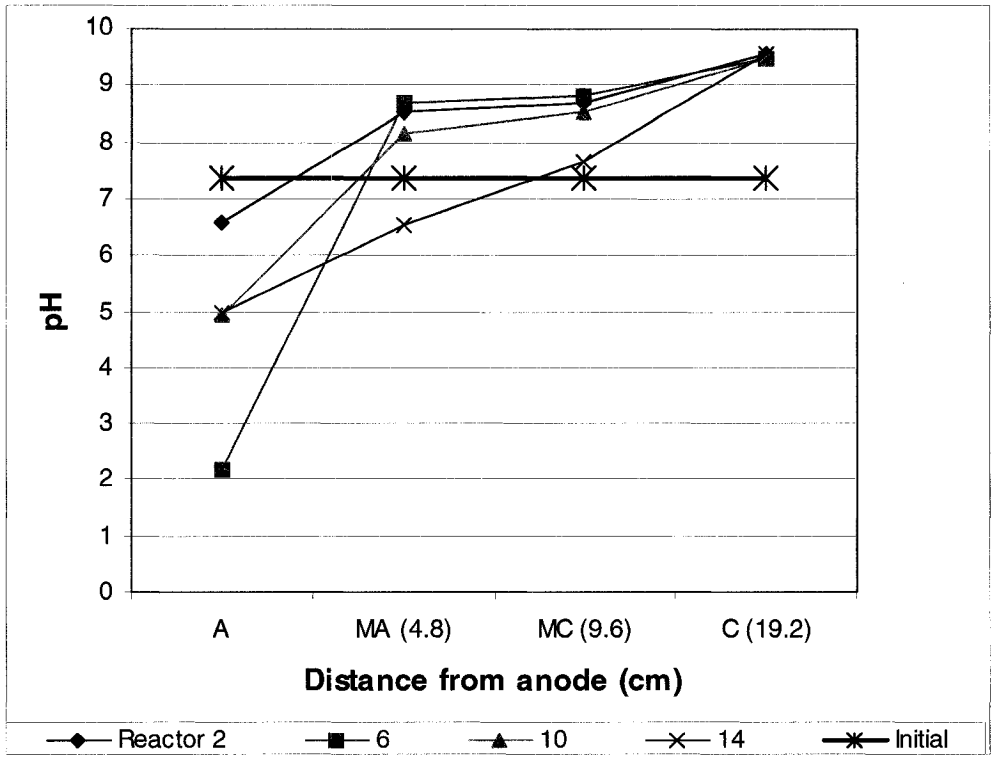


Figure 4-8: pH distribution in the EK reactors with voltage gradient of 0.7 V/cm

❖ pH in the reactors with the voltage gradient of 1.5 V/cm

Table A-3 (Appendix A) has the results of pH in batch reactors with voltage gradient of 1.5 V/cm. In addition, Figure 4-9 illustrates the pH distribution for these batch reactors. In this group, the cathode area of the reactor 7 has the highest value of pH 9.54, and the anode area of the reactor 3 has lowest pH value of 3.10.

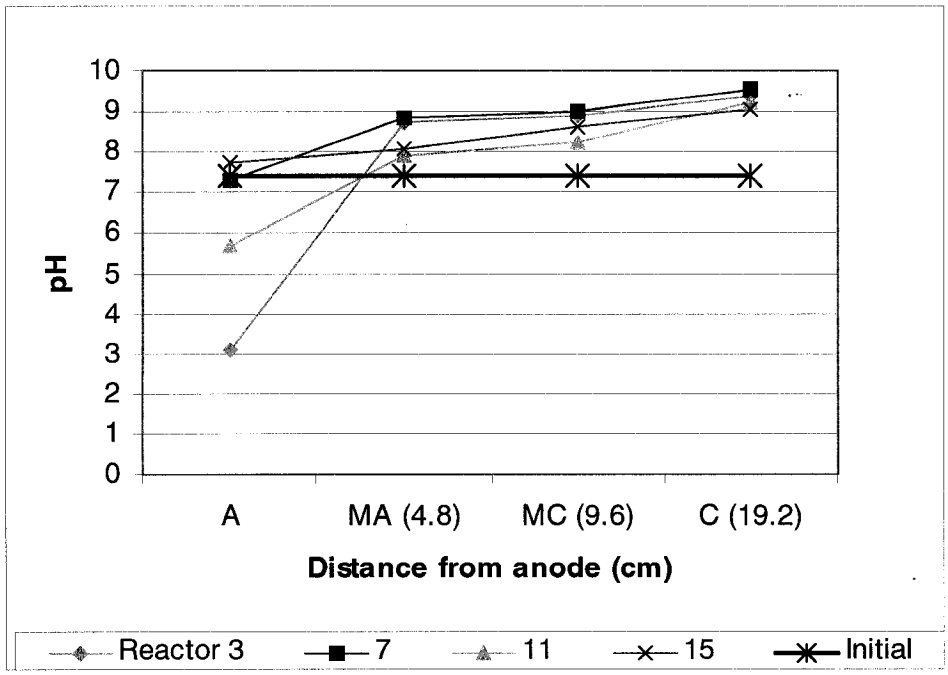


Figure 4-9: pH distribution in the EK reactors with gradient voltage of 1.5 V/cm

Overall result of pH shows that the content of the amphoteric enhancer is a determining factor for higher pH gradient between the anode and cathode.

4-1-4- Effects on ORP

The value of ORP was measured after each treatment with respect to an Ag/AgCl reference electrode. The initial ORP value for untreated biosolids was -57.30 mV. Figure 4-10 presents the ORP distribution profiles for each reactor between the anode and cathode.

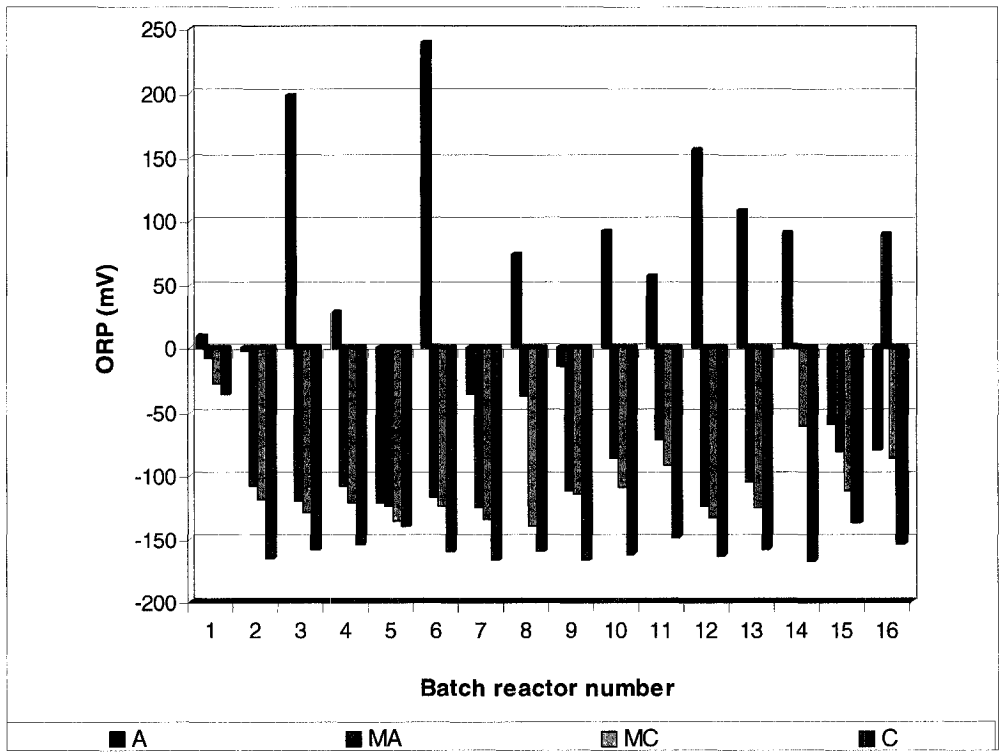


Figure 4-10: ORP distribution profiles in different parts of each batch reactor

The obtained results have the following conclusions:

- In all batch reactors the results of ORP is dependent on pH gradient. Considering this fact that unlike the pH electrode, which responds only to hydrogen activity, an ORP electrode responds to all ions that have oxidizing and reducing activity, the obtained results can imply a balance between distributions of hydrogen ions and oxidizing activity.
- The anode area in the reactor 6 showed the highest oxidation zone (240 mV) among the other reactors; however, this value is lower than minimum targeted ORP value of 650 mV for disinfection systems. This phenomenon could be

due to masking effect of EK oxidation and reduction zone on ORP gradient produced by activity of oxidant agents.

- The reactors 2, 3, 6, 7, 8, 9, 10, 14 and 16 showed a reducer zone higher than 150mV in the cathode area.
- The evolution of ORP result in the anode area of the reactors 3, 6, 8, 10 and 16 showed a tendency toward oxidation area which could be considered due to effects of combination of two oxidant agents in these reactors.

❖ ORP in the reactors with the voltage gradient of 0.5 V/cm

Table A-1 (Appendix A) has the results of pH in the batch reactors with voltage gradient of 0.5 V/cm. In addition, Figure 4-11 illustrates the ORP distribution for these batch reactors. The results of ORP show that the treated biosolids in the reactor 12 had the highest value (ORP 155.80 mV, in the anode) and biosolids in the reactor 9 showed the lowest value (ORP -166.7mV, in the cathode).

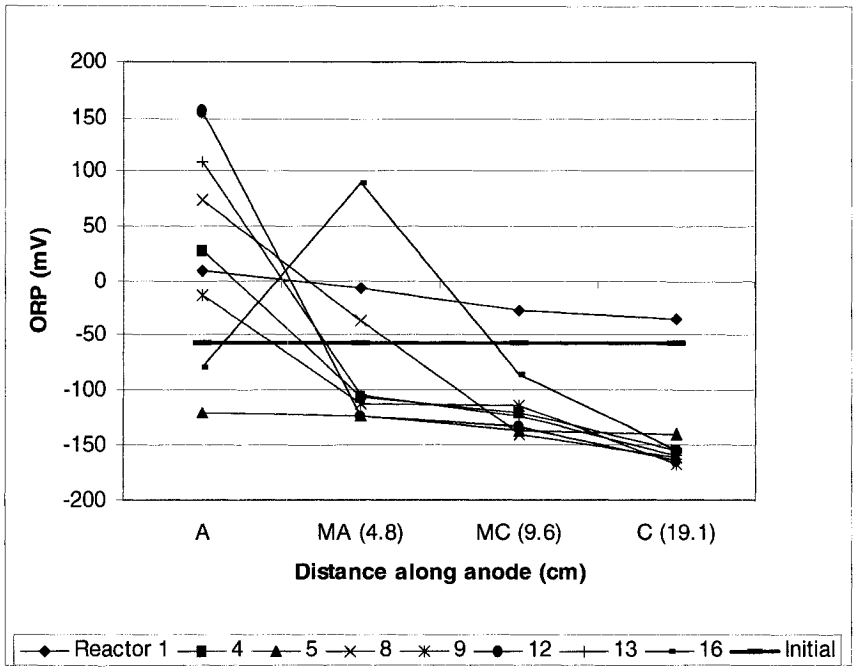


Figure 4-11: ORP distribution in the EK reactors with voltage gradient of 0.5 V/cm

❖ ORP in the reactors with voltage gradient of 0.7 V/cm

Table A-2 (Appendix A) has the results of pH in the batch reactors with voltage gradient of 0.7 V/cm. ORP values show the highest for the reactor 6 at the anode with value of 239.40mV and the lowest for the reactors 2 and 14 (-167.30mV). Figure 4-12 shows the ORP distribution for this group of reactors.

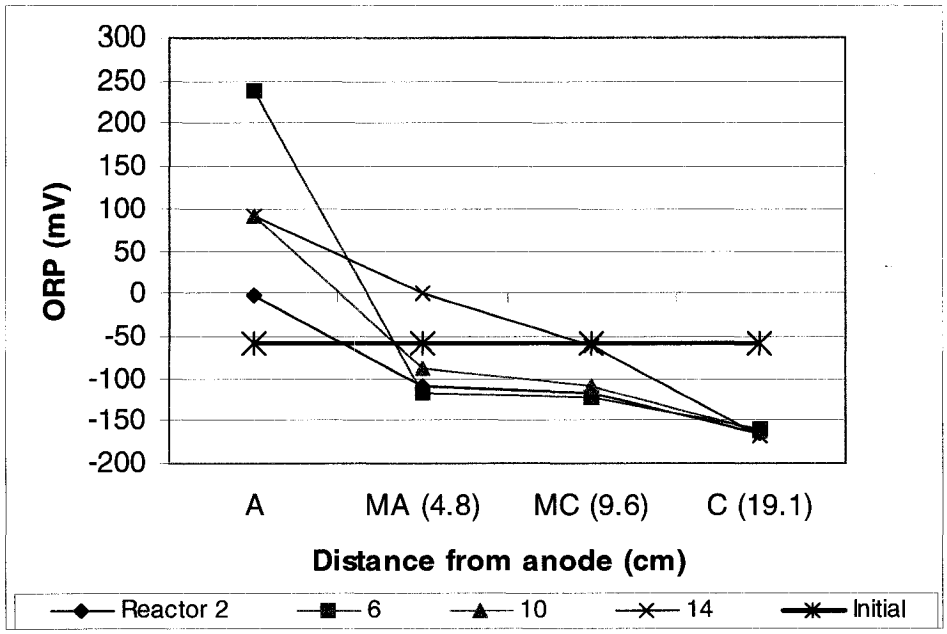


Figure 4-12: ORP distribution in the EK reactors with voltage gradient of 0.7 V/cm

❖ ORP in the reactors with the voltage gradient of 1.5 V/cm

Table A-3 (Appendix A) and Figure 4-13 have the results of pH in the batch reactors with voltage gradient of 1.5 V/cm. The results for ORP show the highest value of 197.80 mV for the reactor 3 in the anode area and the lowest ORP of -166.80 mV for the reactor 7.

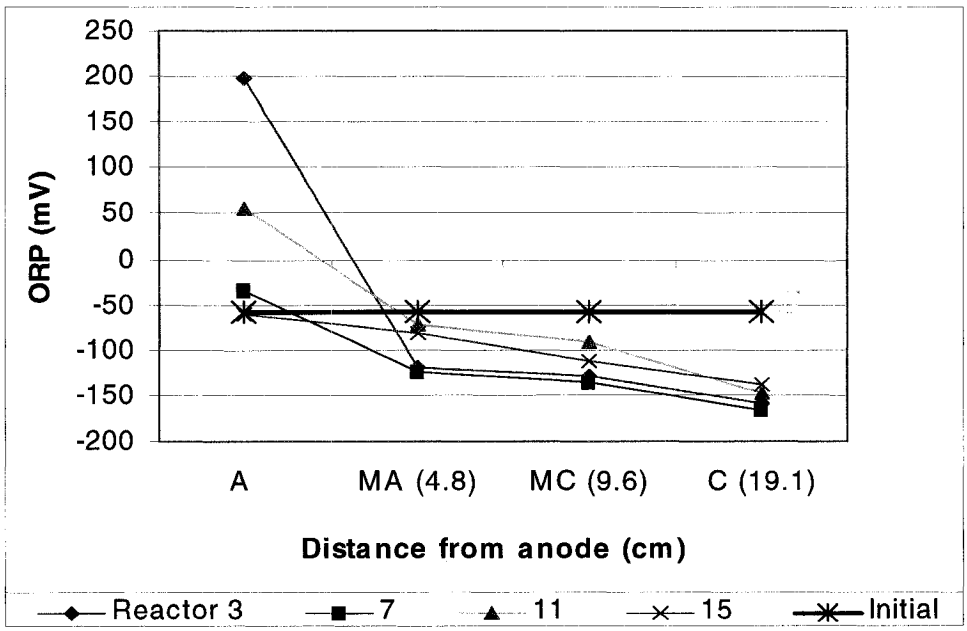


Figure 4-13: ORP distribution in the EK reactors with voltage gradient of 1.5 V/cm

Total evolution of ORP results in all reactors show that the higher voltage can generate higher oxidation state. The ORP gradient between the anode and cathode increases with addition of the amphoteric enhancer. The effect of oxidant enhancers (Glutaraldehyde and Bioxy S/ TAED) on ORP attenuates in a low concentration of these agents.

4-1-5- Effects on nitrogen-ammonia (NH_3-N)

The evaluation of results in Figure 4-14 shows that:

- Obviously the reactors with the amphoteric agent, namely 3, 6, 12, 13, and 16, showed the highest amount of the nitrogen-ammonia.
- Although the reactors 5, 8 and 14 did not have the amphotreric agent; however, a high amount of nitrogen-ammonia was observed in these reactors. In the case of the reactors 5 and 8, this phenomenon could be related to

chemical activity of glutaraldehyde, its affinity to react with the ammonia and consequently releasing it at the acidic zones.

- The anode area of the reactor 12 showed the highest concentration of the nitrogen-ammonia comparing to the other reactors with the same amount of the amphoteric agent.
- The highest concentration of oxidant agents decreases the concentration of the nitrogen-ammonia (the reactor 3).

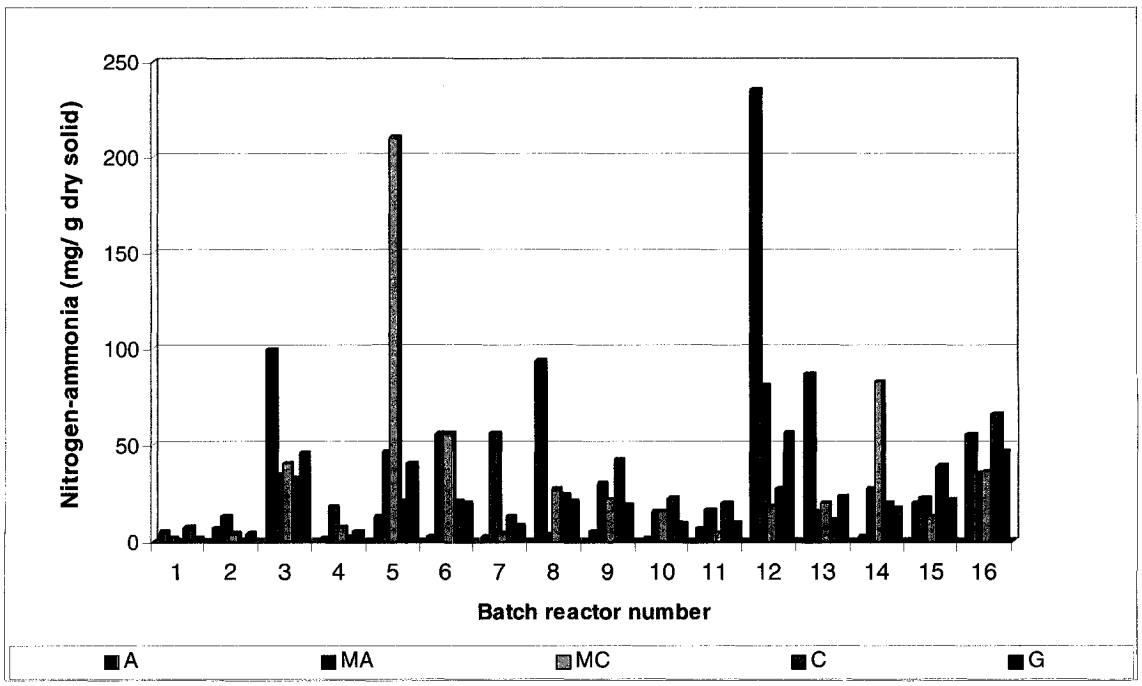


Figure 4-14: Nitrogen-ammonia concentration profiles in different parts of each batch reactor and their geometric mean

Considering the effect of the amphoteric agent on the initial concentration of the nitrogen-ammonia (Chapter 3), the results in this section divided in three groups as: the

reactors without amphoteric agent, the reactors with 0.7 % (w/v) amphotreic agent, and the reactors with 1.3 % (w/v) amphoteric agent. Each group will hereafter be discussed. .

❖ Nitrogen-ammonia in the reactors without the amphoteric agent

Data of the nitrogen-ammonia values related to this group has been shown in Table A- 4, Table A-5, TableA-6 (Appendix A), Figure 4-15 and Figure 4-16.

- The initial concentration of nitrogen-ammonia in this group was 30.80 mg/ g dry solid.

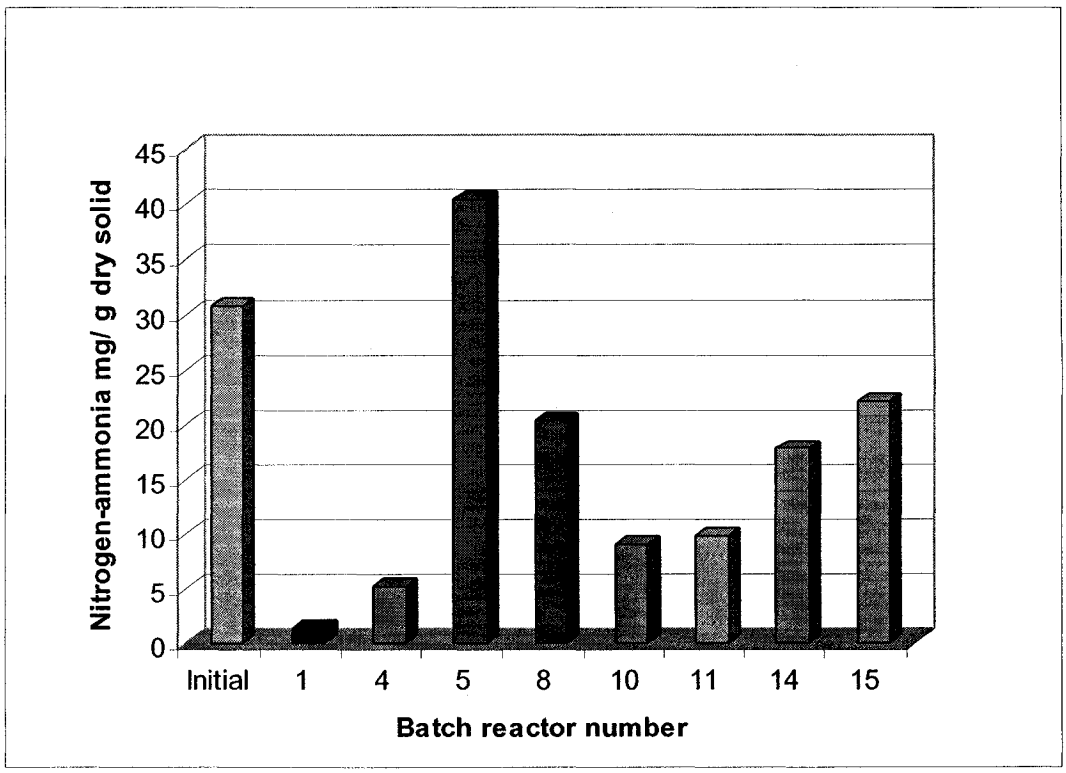


Figure 4-15: Average nitrogen-ammonia content in the reactors without the amphoteric agent

- The highest nitrogen-ammonia reduction (95%) was observed in the reactor 1 and the lowest reduction (28 %) was found in the reactor 15.
- In the case of the reactor 1, exposure time and the removal of nitrogen-ammonia through drainage system could be considered as a major factor in high reduction value (amount of nitrogen-ammonia in the catholyte was1190 mg/ L and in the anolyte was 170 mg/L)
- The reactor 5 showed an increase of 30% at concentration of the nitrogen-ammonia in entire reactor. It could be considered as affinity of glutaraldehyde to react with amino acids content of living cell and as a result, formation of nitrogen-ammonia from other forms nitrogen.

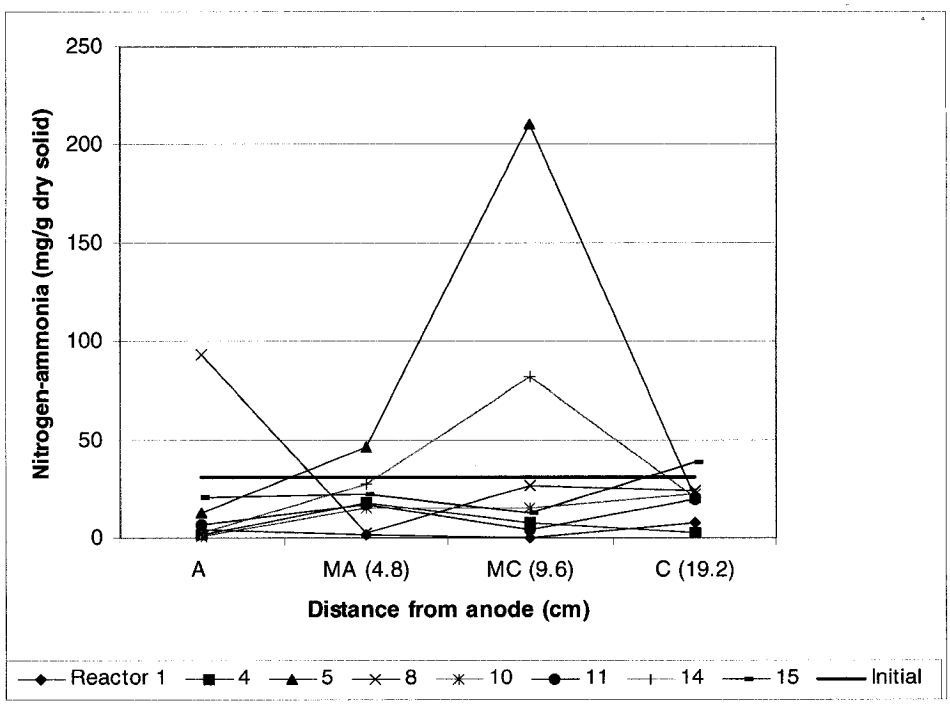


Figure 4-16: Nitrogen- ammonia changes in the EK reactors without the amphoteric agent

❖ Nitrogen-ammonia in the reactors with concentration of 0.7% w/v the amphoteric agent

Data of the nitrogen-ammonia related to this group has been set in the Table A-4, Table A-5, Table A-6 (Appendix A), Figure 4-17 and Figure 4-18.

- Initial amount of the nitrogen-ammonia in this group was 165 mg/ g dry solid which was a sum of nitrogen-ammonia content in the initial sample from WWTP and the amount added to reactor through the amphoteric agent.

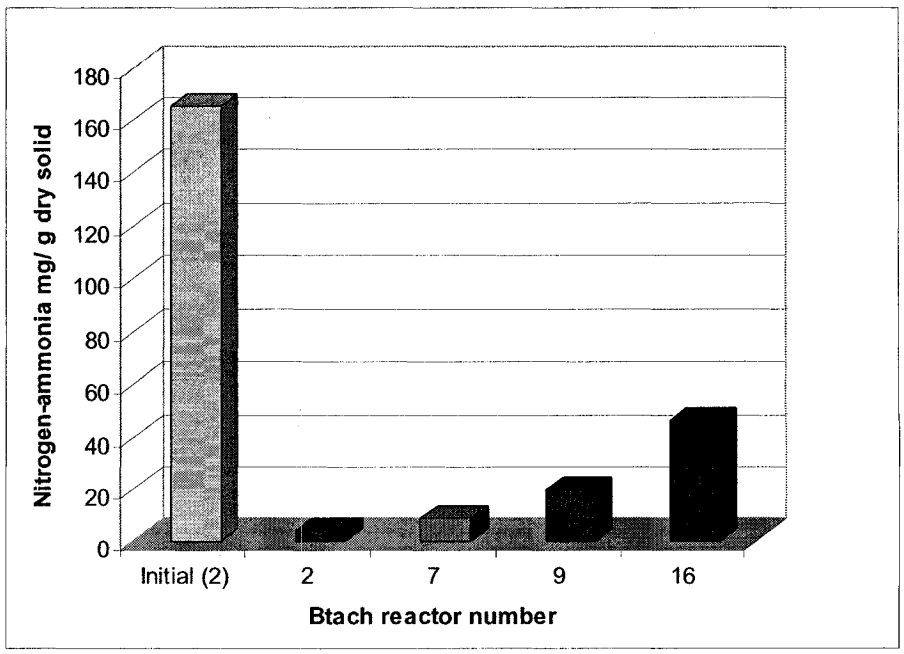


Figure 4-17: Average nitrogen-ammonia content in the reactors with 0.7 % the amphoteric agent

- The reactor 2 with 97% reduction owns the highest reduction of the nitrogen-ammonia, while the reactor 16 with 72% reduction shows the lowest reduction value.
- Increase in concentration of Bioxy S/ TAED causes an increase in reduction of nitrogen-ammonia.

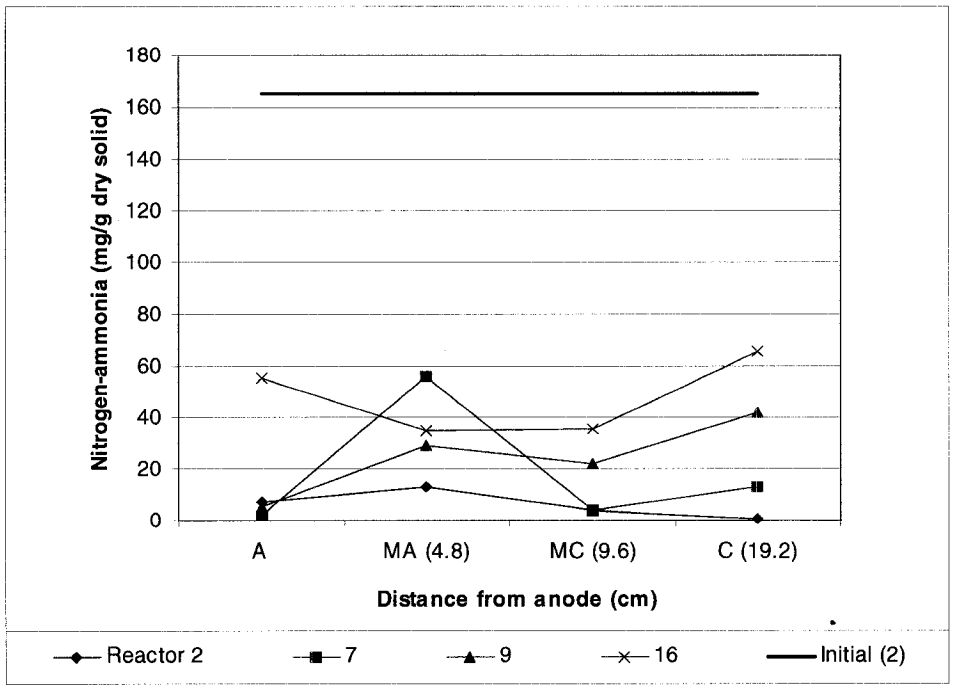


Figure 4-18: Nitrogen- ammonia concentration in the reactors with 0.7 % the amphoteric agent

❖ Nitrogen-ammonia in the reactors with concentration of 1.3 % w/v the amphoteric agents

Data of nitrogen-ammonia values related to this group has been set in Table A-4, Table A-5, Table A-6 (Appendix A), Figure 4-19 and Figure 4-20.

- The initial concentration of the nitrogen –ammonia was 284.2 mg/ g dry solid.
- In this group the reactor 6 displayed 93 % reduction. This amount of reduction could be considered due to electroosmosis and the removal of this compound through drainage system. A high amount of the nitrogen-ammonia was measured in the catholyte (12,000 mg /L) and the anolyte (1600 mg /L).

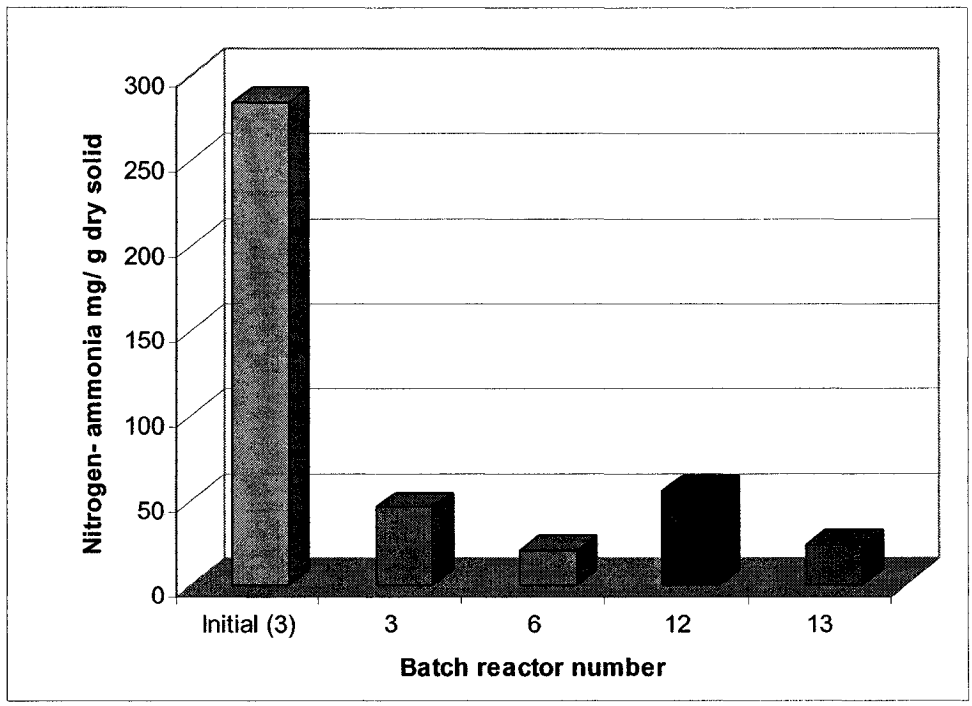


Figure 4-19: Average nitrogen-ammonia content in the reactors with 1.3 % the amphoteric agent

- The reactor 12 had 80% reduction, which was the lowest reduction of nitrogen-ammonia in this group.
- As with pervious group, an increase in the amount of Bioxy S/ TAED causes high reduction in the nitrogen-ammonia concentration.

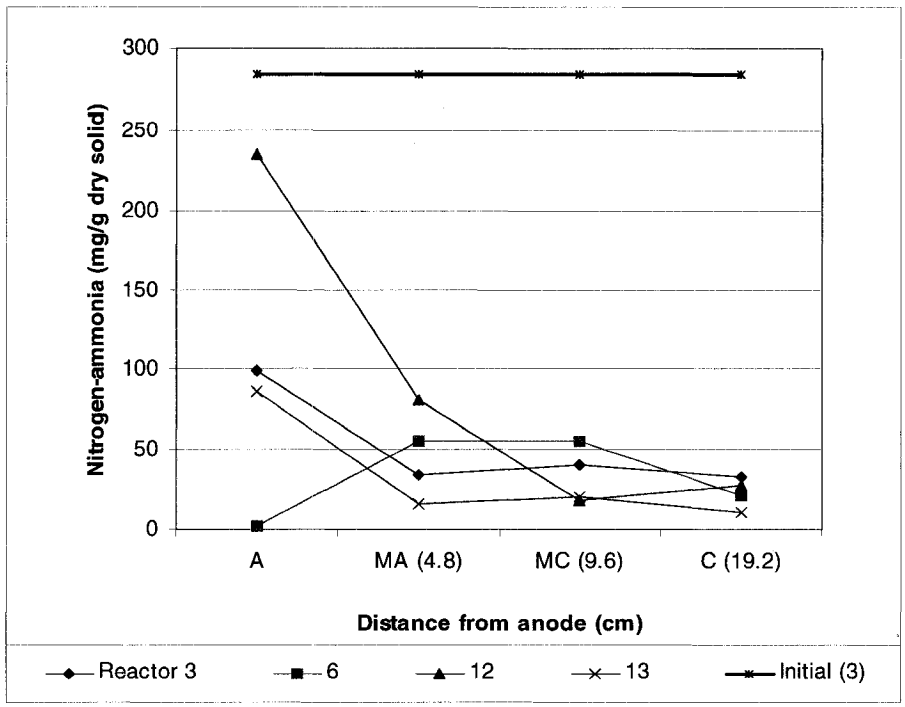


Figure 4-20: Nitrogen- ammonia concentration in the reactors with 1.3 % the amphoteric agent

The overall evaluation of the results for nitrogen-ammonia showed that the nitrogen-ammonia content in the reactors increases with increasing the amphoteric enhancer and decreasing the voltage gradient.

4-1-6- Effects on nitrite (NO_2)

The nitrite distribution profiles in each batch reactor have been illustrated in Figure 4-21. In this group:

- The measured amount of the nitrite was lower than 0.1(mg/ g dry solid) almost in all reactors.

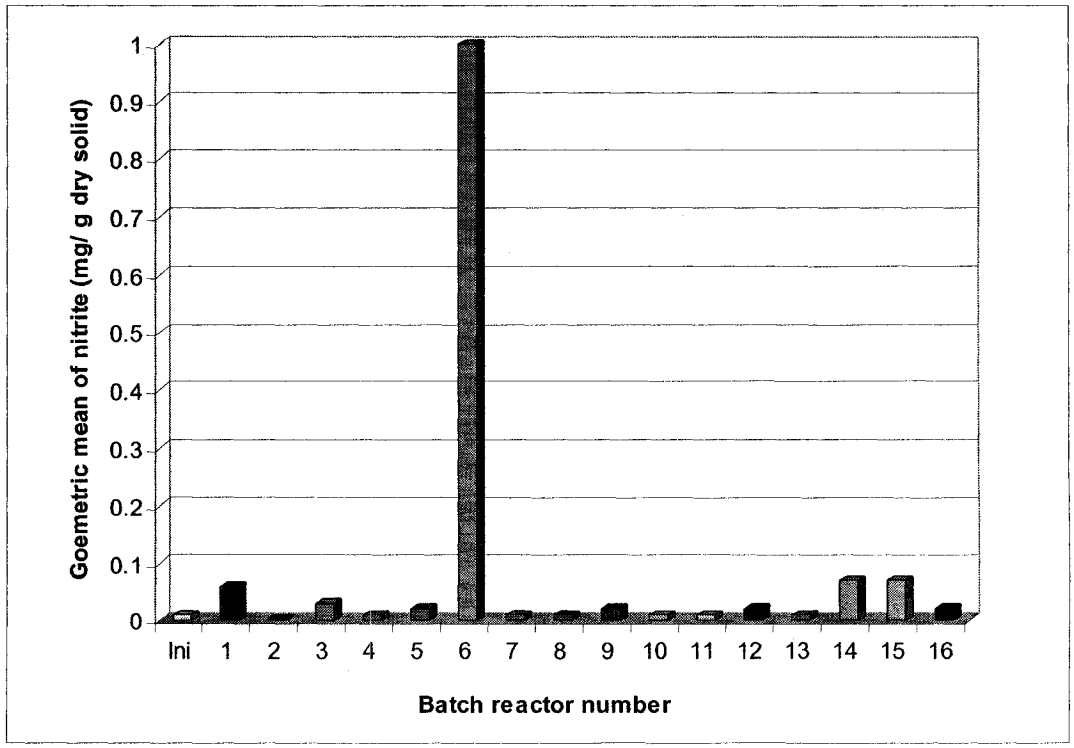


Figure 4-21: Average nitrite content in the reactors

- The average values of the nitrate concentration in the reactors 1, 3, 5, 6, 9, 12, 14, 15 and 16 show an increase. This phenomenon is related to this fact that under anaerobic condition, nitrate and sulfate reducing bacteria (*C. perfringens*) may use nitrate as an alternative electron acceptor and reduce it

to nitrite. Also increase of total solids caused an increase in percentage of nitrite in the reactors.

- The other factor that can be considered as an important factor on changes in concentration of nitrite is the affinity of ammonium to react with oxygen and production of nitrite.
- Evaluation of data obtained from effluents show the high amount of nitrite in these liquids (Table 4-5). It could be concluded that the removal of this compound took place through drainage system of the reactor and EK process helping this removal.

Table 4-5: Concentration of nitrogen-ammonia, nitrite and nitrate in drainage

EK reactor trail number	Code	Unit	Nitrogen-Ammonia	Nitrite	Nitrate
1	Catholyte	mg/ L	1190	0.1	0.01
	Anolyte	mg/ L	170	0.51	<0.01
2	Catholyte	mg/ L	3100	<0.01	<0.01
	Anolyte	mg/ L	630	<0.01	<0.01
3	Catholyte	mg/ L	1830	120	120
	Anolyte	mg/ L	11140	10000	300
4	Catholyte	mg/ L	1090	1.70	0.1
	Anolyte	mg/ L	1430	0.44	0.1
5	Catholyte	mg/ L	1300	0.11	0.1
	Anolyte	mg/ L	NL*	NL	NL
6	Catholyte	mg/ L	12,000	0.22	<0.01
	Anolyte	mg/ L	1660	0.66	<0.01
7	Catholyte	mg/ L	3130	4300	140
	Anolyte	mg/ L	5050	400	21
8	Catholyte	mg/ L	1410	0.1	0.3
	Anolyte	mg/ L	860	0.8	0.9
9	Catholyte	mg/ L	1540	0.23	0.8
	Anolyte	mg/ L	2100	1.00	0.2
10	Catholyte	mg/ L	510	0.06	0.30
	Anolyte	mg/ L	270	0.01	0.40
11	Catholyte	mg/ L	35	40	60
	Anolyte	mg/ L	42.60	60	1450
12	Catholyte	mg/ L	4900	<0.01	<0.01
	Anolyte	mg/ L	1400	<0.01	0.1
13	Catholyte	mg/ L	3600	0.1	0.1
	Anolyte	mg/ L	1500	0.01	0.2
14	Catholyte	mg/ L	1970	0.2	30
	Anolyte	mg/ L	590	0.01	<0.01
15	Catholyte	mg/ L	150	400	200
	Anolyte	mg/ L	191	3000	130
16	Catholyte	mg/ L	1800	0.1	0.3
	Anolyte	mg/ L	1400	0.35	0.4

❖ Nitrite in the reactors with the voltage gradient of 0.5 V/cm

Data of resulting from nitrite analysis has been presented in Table A-4 (Appendix A) and Figure 4-22. The highest value of nitrite in EK treated biosolids belongs to the reactor 12 (the anode area with amount of 0.31 mg/ g dry solid).

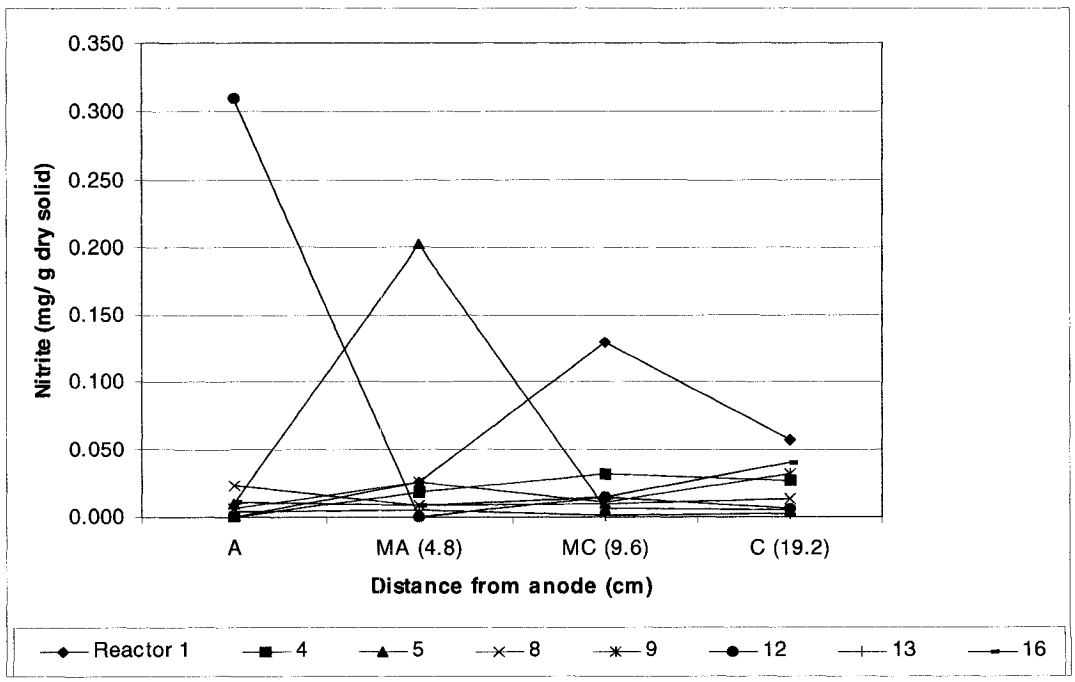


Figure 4-22: Nitrite concentration in the reactors with gradient voltage of 0.5 V/cm

❖ Nitrite in the reactors with the voltage gradient of 0.7 V/cm

Table A-5 (Appendix A) and Figure 4-23 show the data obtained from analysis of biosolids for nitrite in this group of the reactors. The results indicate that the highest value of nitrite in EK treated biosolids was related to the reactor 10 (with an amount of 0.44 mg/ g dry solid in the middle anode area). The lowest values of nitrite in EK treated biosolids was observed in the reactor 14.

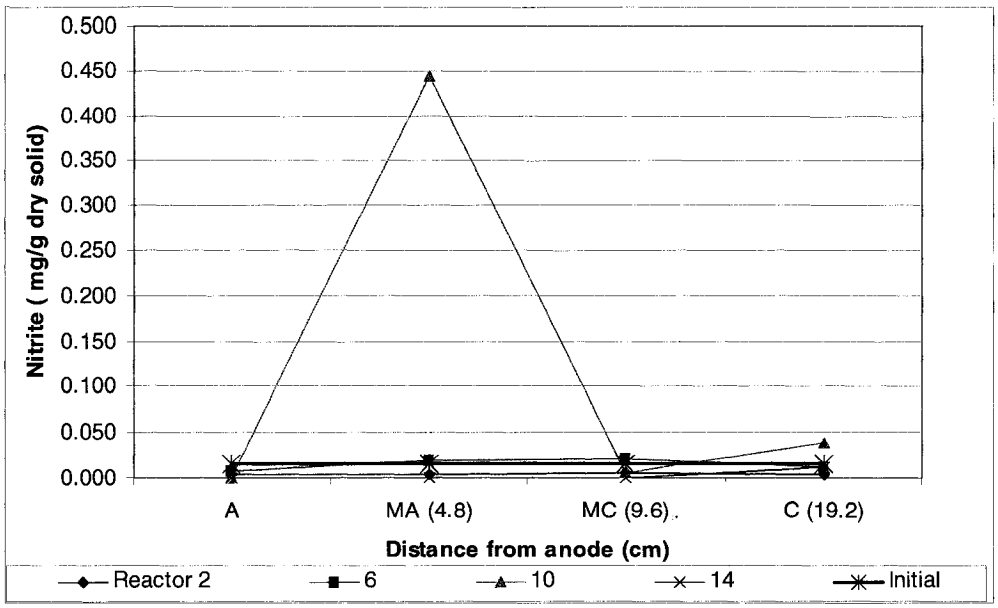


Figure 4-23: Nitrite concentration in the EK reactors with gradient voltage of 0.7 V/cm

❖ Nitrite in the reactors with voltage gradient of 1.5 V/cm

The data obtained from analysis of EK treated biosolids for nitrite have been illustrated in Table A-6 (Appendix A) and Figure 4-24 .The highest value of the nitrite was corresponded to the reactor 15 (with amount of 1.29 mg/ g dry solid in the middle anode area). The reactor 7 showed the lowest value of this compound.

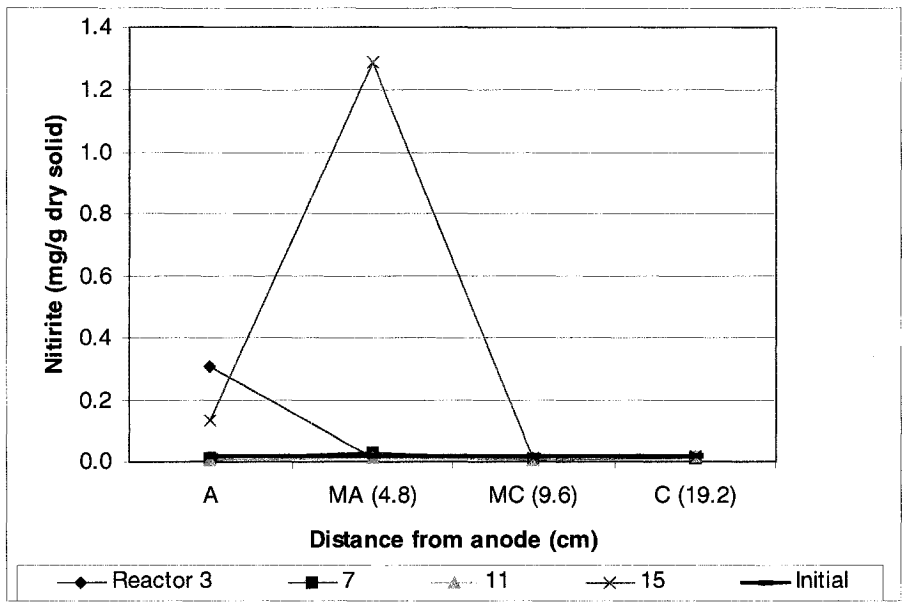


Figure 4-24: Nitrite concentration in the reactors with voltage gradient of 1.5 V/cm

The total assessment of the nitrite concentration in the all conditions shows that the amount of nitrite increases with increasing voltage gradient. A strong DC electrical field encourages stronger electrokinetic process, including oxidation of ammonia to nitrite and nitrate.

4-1-7- Effects on nitrate (NO_3-N)

The evaluation of the results in Table A- 4, Table A-5, Table A-6 (Appendix A) and Figure 4-25 indicates that:

- Amount of nitrite in all reactors was rather low.
- The geometric mean of the nitrate shows an increase in average value of nitrate in treated biosolids for the reactors 4, 5, 7, 8 and 16. As mentioned earlier, this increase can be considered as a reaction of oxygen, produced from

oxidant agents, with nitrogen-ammonia in the biosolids and subsequently, production of nitrate.

- Existence of various strains of bacteria (*C. perfringens*) in the biosolids which are able to use nitrate as an alternative electron acceptor in cell reactions might be one of the major factors of changes in content of nitrate in the reactors.

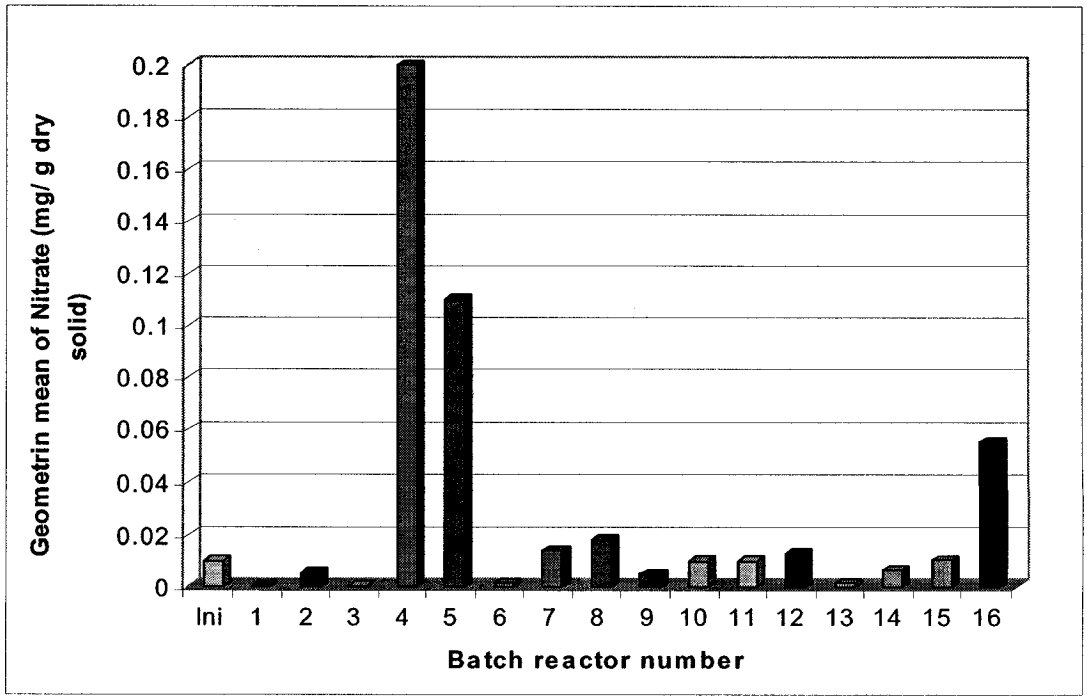


Figure 4-25: Average nitrate content in the reactors

❖ Nitrate in the reactors with the voltage gradient of 0.5 V/cm

Data resulted from nitrate analysis has been shown in Table A- 4 (Appendix A) and Figure 4-26. The highest value of nitrate in EK treated biosolids belongs to the reactor 5 (the middle anode area with amount of 436.5 mg/ g dry solid).

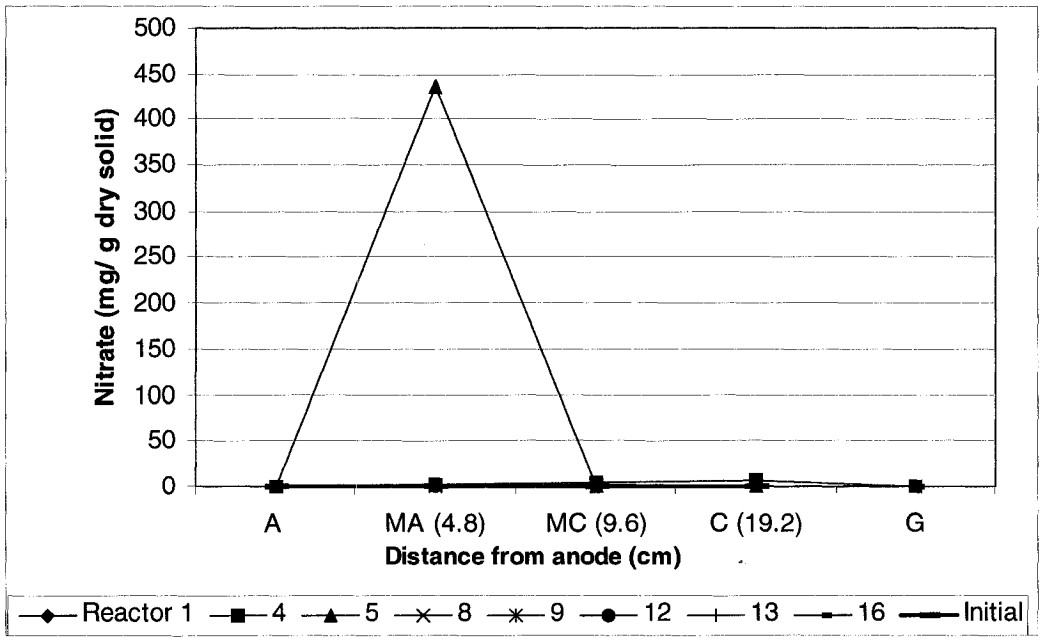


Figure 4-26: Nitrate concentration in the reactors with gradient voltage of 0.5 V/cm

❖ Nitrate in the reactors with the voltage gradient of 0.7 V/cm

Table A-5 (Appendix A) and Figure 4-27 represent the data obtained from analysis of biosolids for nitrate in this group. Results show that the highest value of nitrate in EK treated biosolids were related to the reactor the reactor 10 (with amount of 0.019 mg/ g dry solid in middle anode area). The lowest value was observed in the reactor 14 (middle cathode).

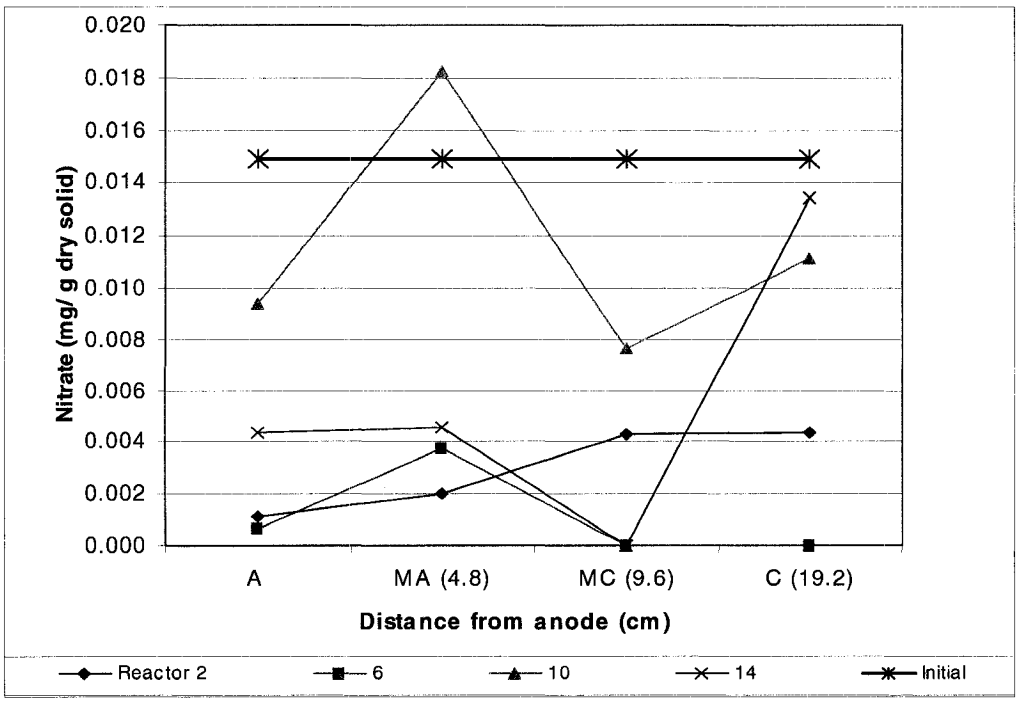


Figure 4-27: Nitrate concentration in the reactors with gradient voltage of 0.7 V/cm

❖ Nitrate in the reactors with the voltage gradient of 1.5 V/cm

The data obtained from analyasis of EK treated biolsolids for nitrate of this group has been illustrated in Table A-6 (Appendix A) and Figure 4-28.The highest value of nitrate in EK treated biosolids was correspond to the reactor 15 (with amount of 0.04 mg/ g dry solid in the cathode area). The reactor 7 showed the lowest value of these compounds.

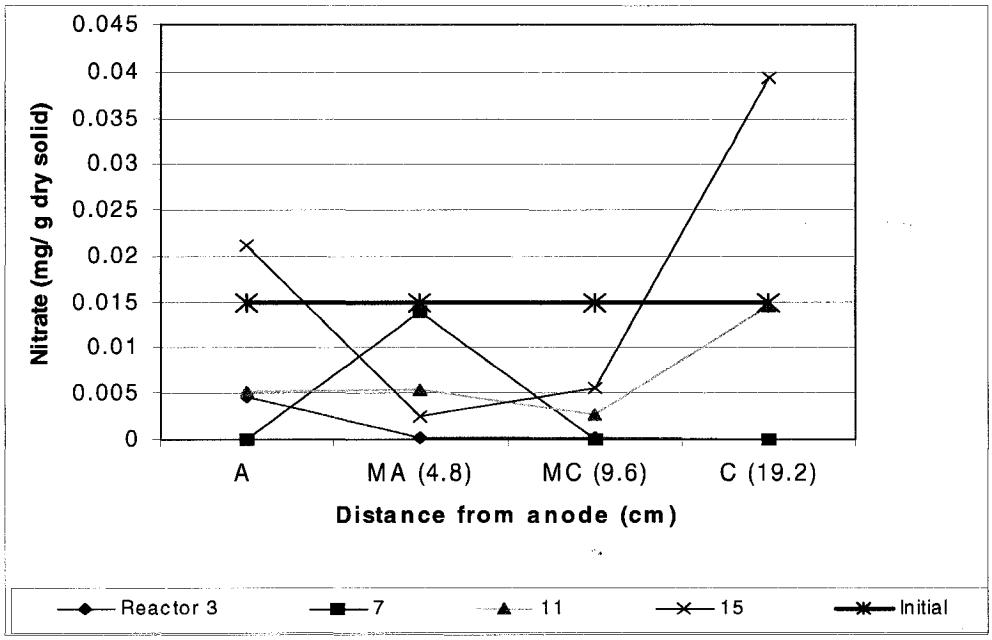


Figure 4-28: Nitrate concentration in the reactors with voltage gradient of 1.5 V/cm

Same with nitrite, the overall evaluation of the nitrate concentration in the all conditions shows the effect of increasing voltage gradient on surge of nitrate.

4-1-8- Effects on phosphate (PO_4^{3-})

As mentioned at the beginning of this chapter due to effect of the amphoteric agent on initial concentration of phosphate, the results of this element is represented in three groups as follows.

❖ Phosphate in the reactors without the amphoteric agent

Figure 4- 29 shows the value of average amount of phosphate in this group. Considering the initial value of phosphate (2.65 mg/ g dry solid), the results show an increase of phosphate in most of the treated biosloids. This phenomenon can be described that: the phosphates in the biosolids adsorbs to the colloid part of sludge, specifically at the solid-aqueous solution interface. The bonds between phosphate molecules and

colloids particles are very strong which do not allow phosphate to remove through leakage system. Also in the parallel reaction, phosphate molecules react with iron and forms "iron phosphate" which is insoluble in water and sediments in the body of biosolids.

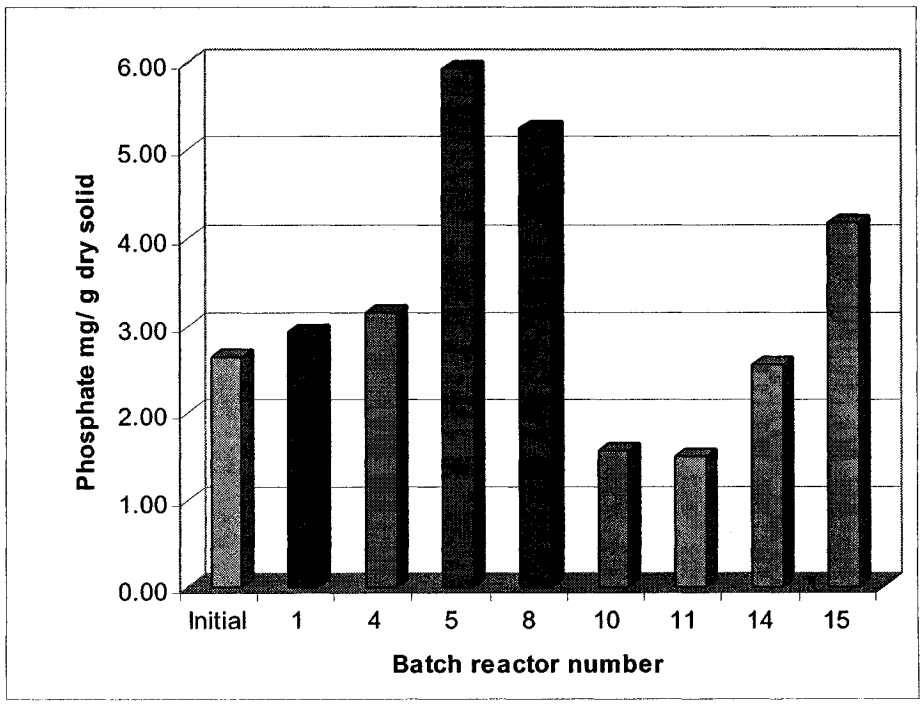


Figure 4-29: Average phosphate content in the reactors without the amphoteric agent

The results in Figure 4-30 indicate that the highest value of phosphate in EK treated biosolids was observed in the anode area of the reactor 5 with amount of 50 mg/g dry solid. The lowest value was related to the reactors 1(in the middle cathode area 0.42 mg/g dry solid). The highest reduction of phosphate was observed in the reactor 11 with amount of 44%.

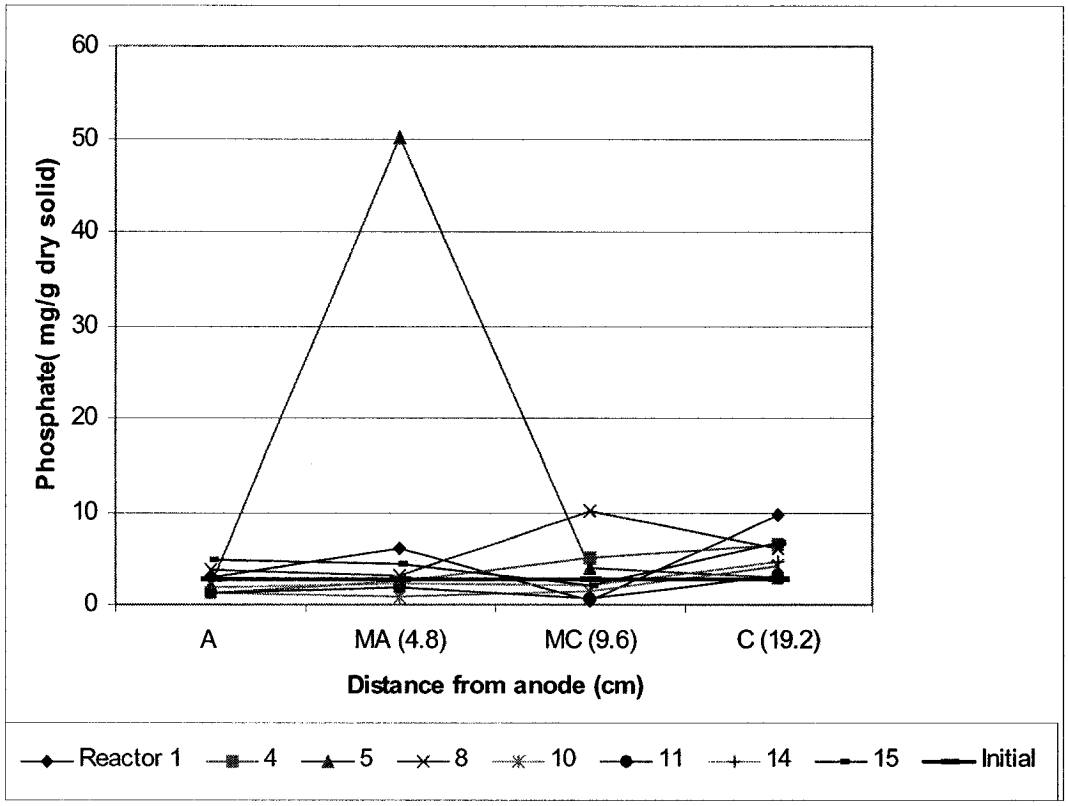


Figure 4-30: Phosphate concentration in the reactors without the amphoteric agent

❖ Phosphate in the rectors with concentration of 0.7% w/v the amphoteric agent

Table A-7, Table A-8 and Table A-9 (Appendix A) shows the phosphate concentration in tests with in this group. Figure 4-31 and Figure 4-32 are graphs related to these data. The results show that:

- The reactor 7 with 95% removal was the best reactor in this case.
- The reactor 16 showed an increase in the concentration of phosphate which as explained in previous section in related to accumulation of phosphate in the colloid particles of biosolids.

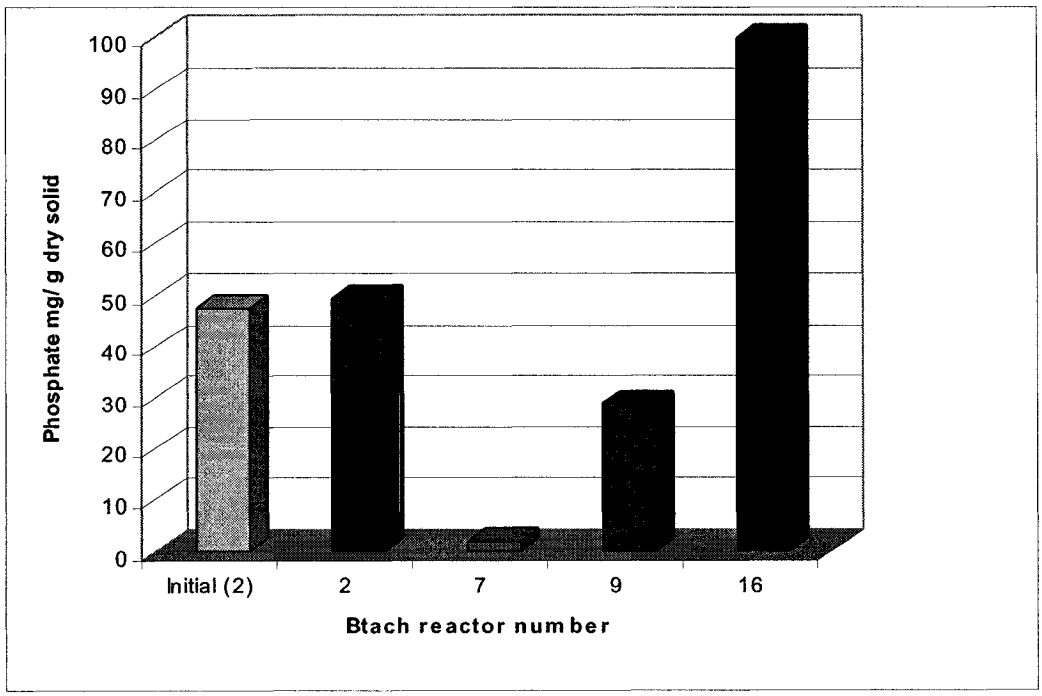


Figure 4-31: Average phosphate content in the reactors with 0.7 % (w/v) the amphoteric agent

Also it was observed that:

The reactor 2 in the middle anode area with amount of 270 mg/ g dry solid has the highest value of phosphate. The lowest value was related to the reactors 7 (in the anode area with amount of 0.03 mg/ g). In this group the initial concentration of phosphate was measured at the level of 47.5 mg/ g dry solid.

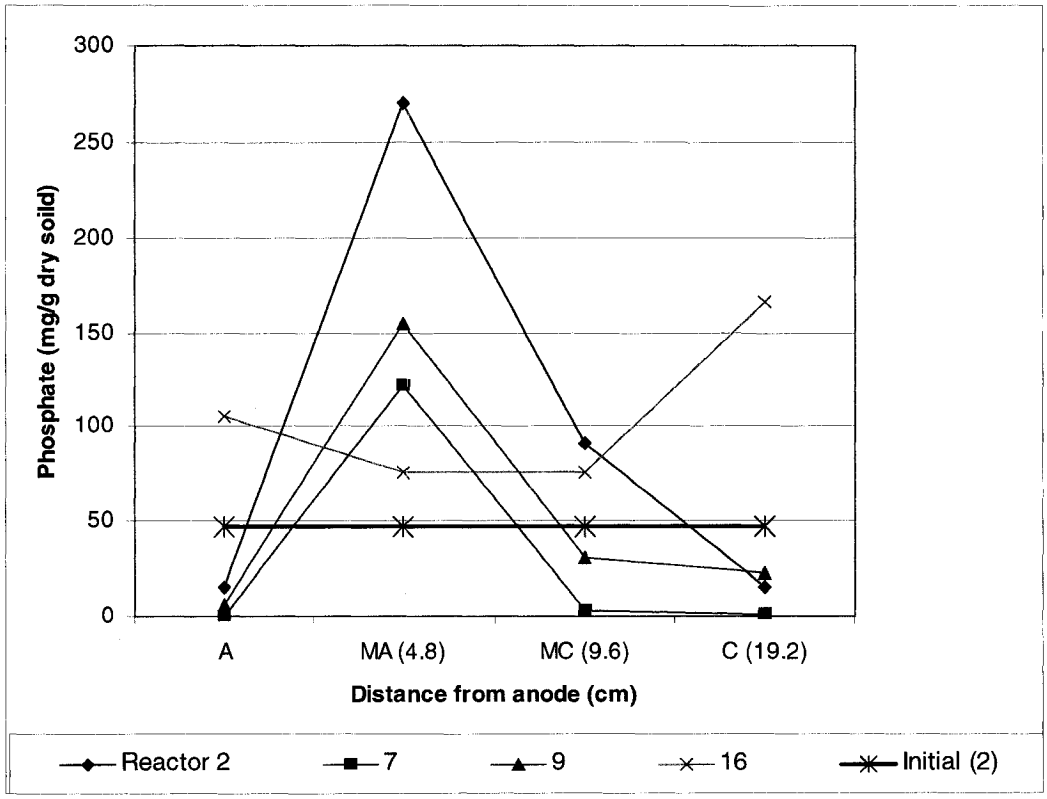


Figure 4-32: Phosphate concentration in the reactors with 0.7 % (w/v) the amphoteric agent

❖ Phosphate in the rectors with concentration of 1.3% w/v the amphoteric agents

Values of phosphate from the experiments in this group have been shown in Figure 4-33 and Figure 4-34. Data shows that the reactor 3 presents the highest value of phosphate 1511 mg/ g dry solid. The reactor 6 in the cathode area shows the lowest value of phosphate with amount of 0.14 mg/ g dry solid.

- The initial phosphate content was 98 mg/ g dry solid for this group.
- The reactor 6 had the highest removal (84%).

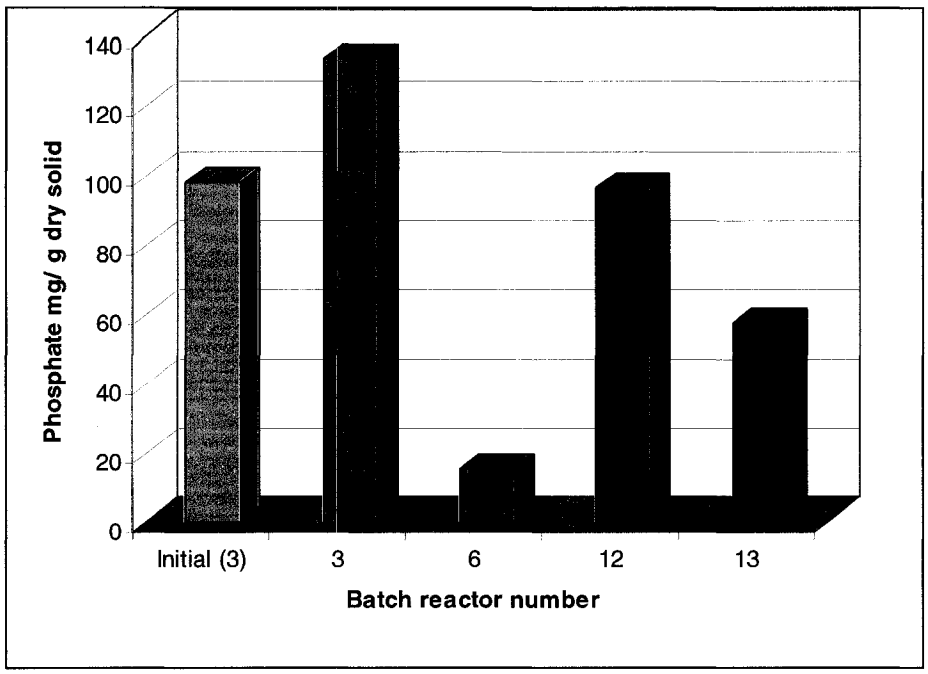


Figure 4-33: Average phosphate content in the reactors with 1.3 % (w/v) the amphoteric agent

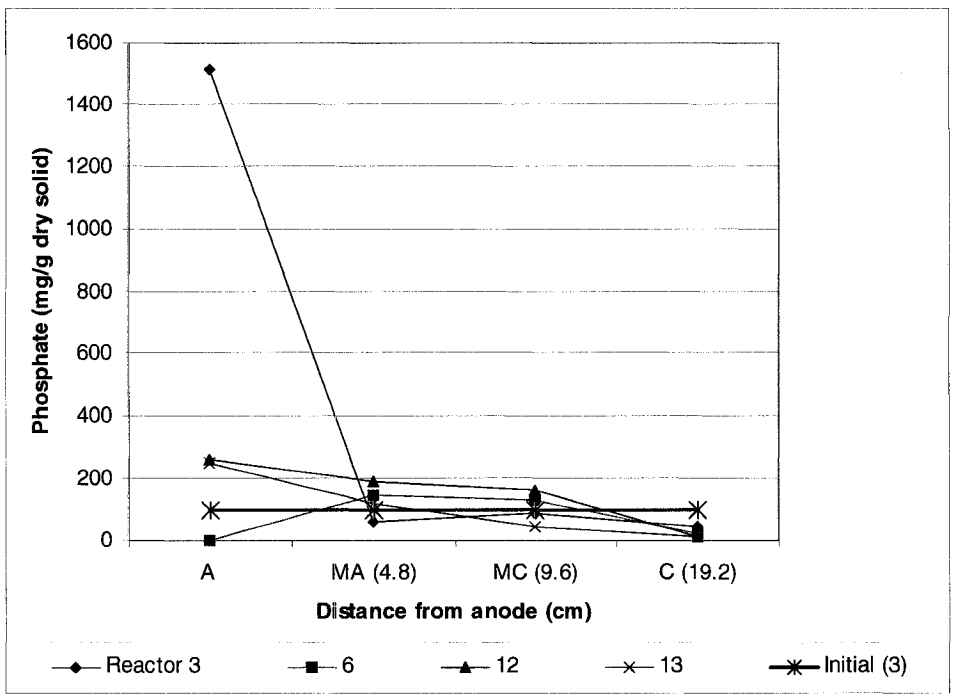


Figure 4-34: Phosphate concentration in the reactors with 1.3 % (w/v) the amphoteric agent

The overall assessment of phosphate concentration shows an increase with increasing of the amphoteric content of the reactor. Furthermore, it was noticed that the distribution of phosphate content follows the distribution of TSS in the reactors.

4-1-9- Effect on Sulfate (SO_4^{2-})

As with nitrogen-ammonia and phosphate the results related to sulfate analysis presents in three groups based on the concentration of the amphoteric agent.

❖ Sulfate in the reactors without the amphoteric agent

The initial value of sulfate for this group was 56.64 mg/ g dry solid (Figure 4-35).

- The sulfate concentration in the group without amphoteric agent was reduced as much as 97%, 95% and 95% in reactors 11, 4 and 5, respectively, while this reduction in reactor 8 was less than 1%.

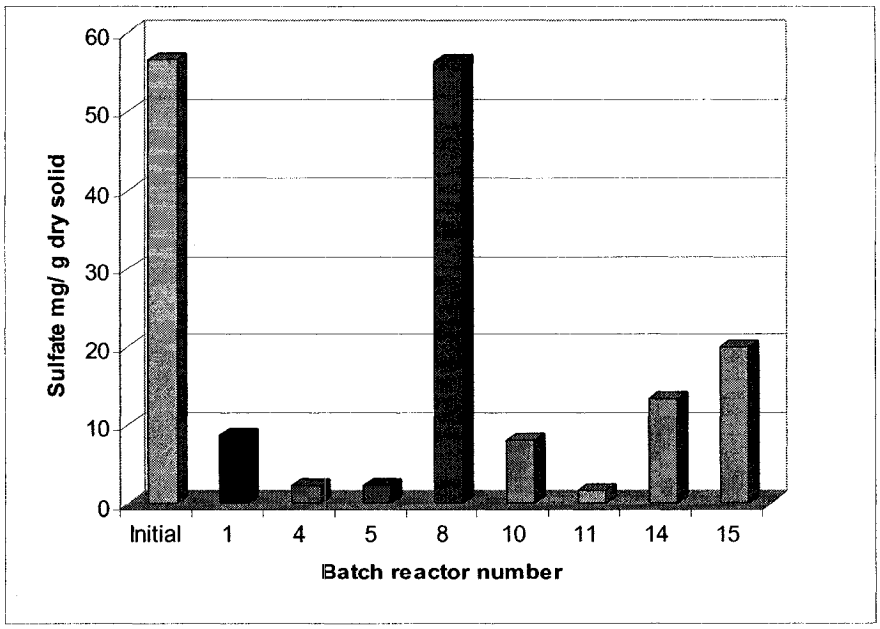


Figure 4-35: Average sulfate content in the reactors without the amphoteric agent

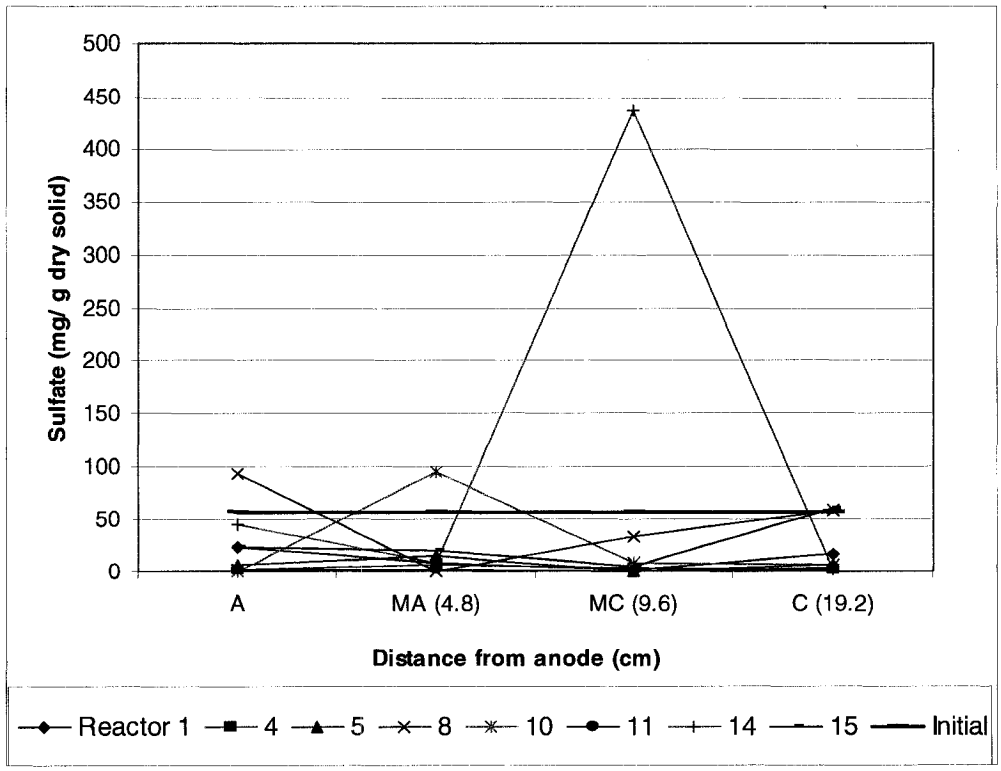


Figure 4-36: Sulfate concentration in the reactors without the amphoteric agent

❖ Sulfate in the reactors with concentration of 0.7% w/v the amphoteric agents

- The initial concentration of sulfate in this group was 221 mg/ g dry solids.
- The reactor 2, 7 and 9 reach the reduction of 99 %, 98%, and 97%, respectively (Figure 4-37). The reactor 16 with 68 % reduction showed the lowest value. These results give the impression that higher voltage and longer exposure directed results to better removal.

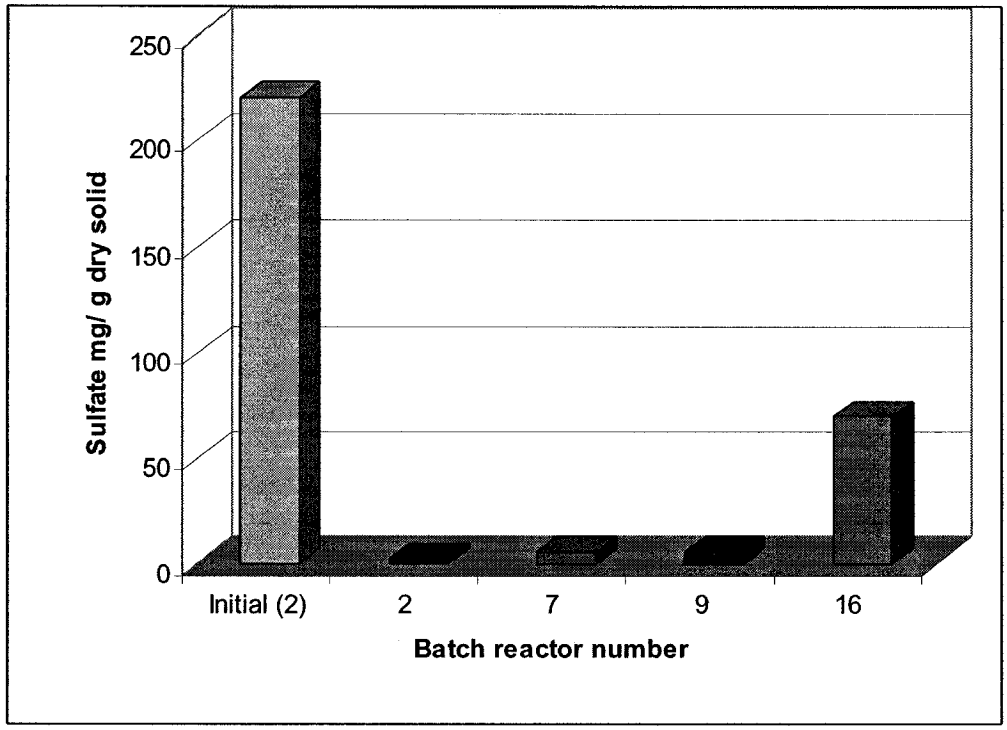


Figure 4-37: Average sulfate content in the reactors with 0.7 % (w/v) the amphoteric agent

The results show the highest value (400 mg/ g dry solids) of sulfate in the cathode area of the reactor 16. The lowest value (3.88 mg/ g dry solids) was observed in the middle anode area of the reactor 2 (Figure 3-38).

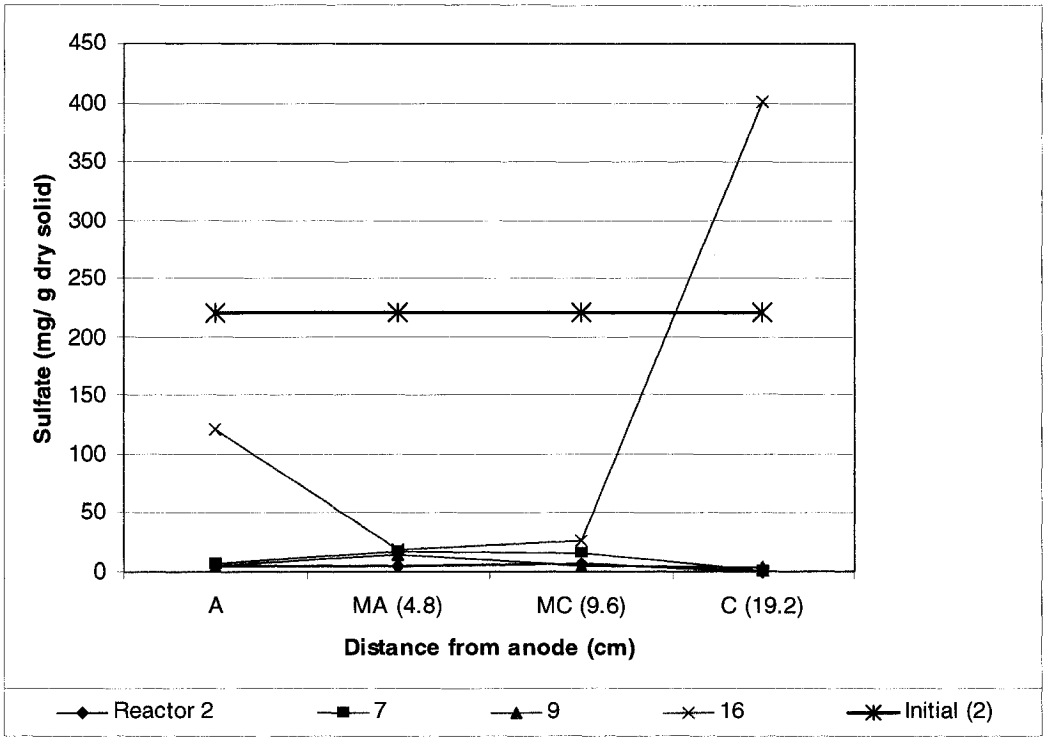


Figure 4-38: Sulfate concentration in the reactors with 0.7% the amphoteric agent

❖ Sulfate in the rectors with concentration of 1.3% w/v amphoteric agents

The initial amount of sulfate in this group was 360 mg/ g dry solid (Figure 4-38).

In this set of the reactors, the reactor 6 showed the highest reduction of sulfate (97%). The lowest reduction was related to the reactor 12 with value of 60.28%. In this case seems that higher voltage gradient had the better effect on sulfate removal.

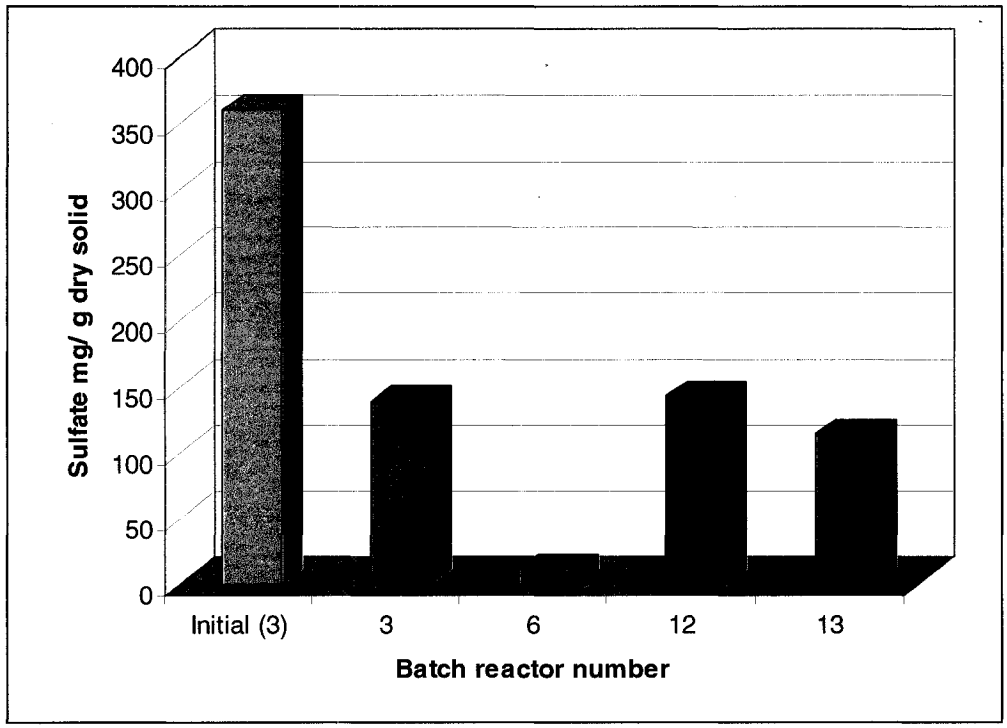


Figure 4-39: Average sulfate content in the reactors with 1.3 % (w/v) the amphoteric agent

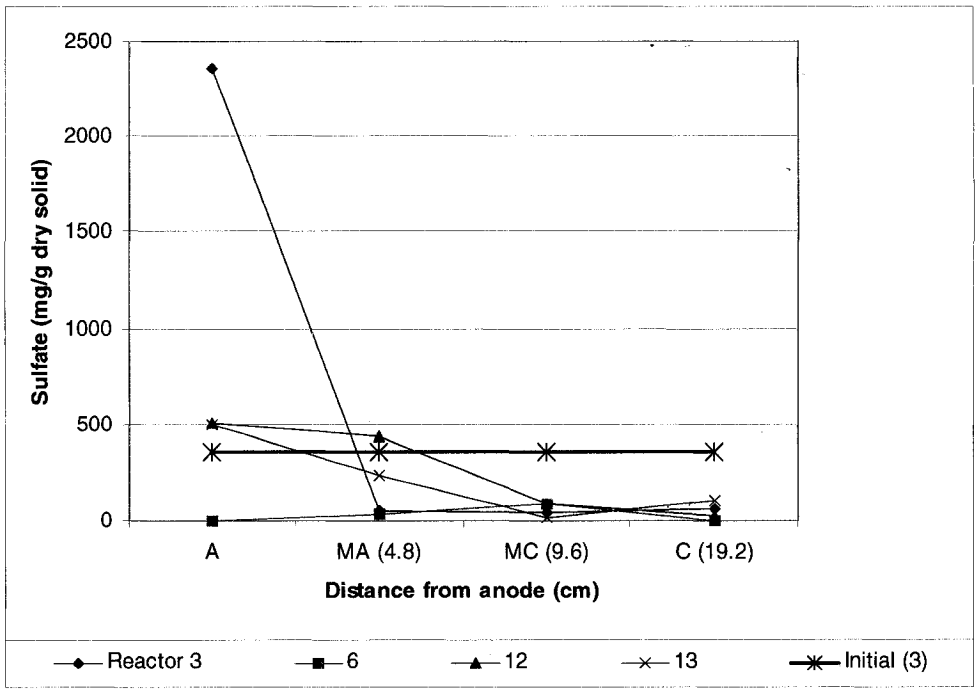


Figure 4-40: Sulfate concentration in the reactors with 1.3% the amphoteric agent

The results in Figure 4-40 show that the highest concentration of sulfate (2357mg /g dry solids) was measured in the reactor 3 in the anode area. And the lowest value (2.37 mg /g dry solids) was related to the anode area of the reactor 6.

The total evaluations of the result of sulfate in all reactors show that: the sulphate content increases with addition of amphoteric enhancer, but electrokinetic condition created by each of the electrical field permits subsequent reduction of sulfate. This effect is related to the impact of high voltage gradient on ionic transport of sulfate toward the anode area, formation of sulphuric acid and then transport of it toward the cathode area due to electroosmosis.

4-1-10- Effects on chloride (Cl^-)

Table A- 7, Table A- 8 and Table A-9 and Figure 4-41 show the results from chloride analysis. The evaluation of results shows that:

- The initial value of chloride was 7.79 mg/ g dry solid.
- Reactor 13 with 91% reduction showed the highest reduction of chloride, while reactor 5 had the lowest reduction (0.39%), implies positive effect of exposure time on chloride reduction.
- The value of chloride in the reactor 14 showed an increase of 3% comparing to initial value. This fact could be considered as a subsequence of increasing TS percentage and also insufficient removal of chloride through electrophoresis process and drainage system.

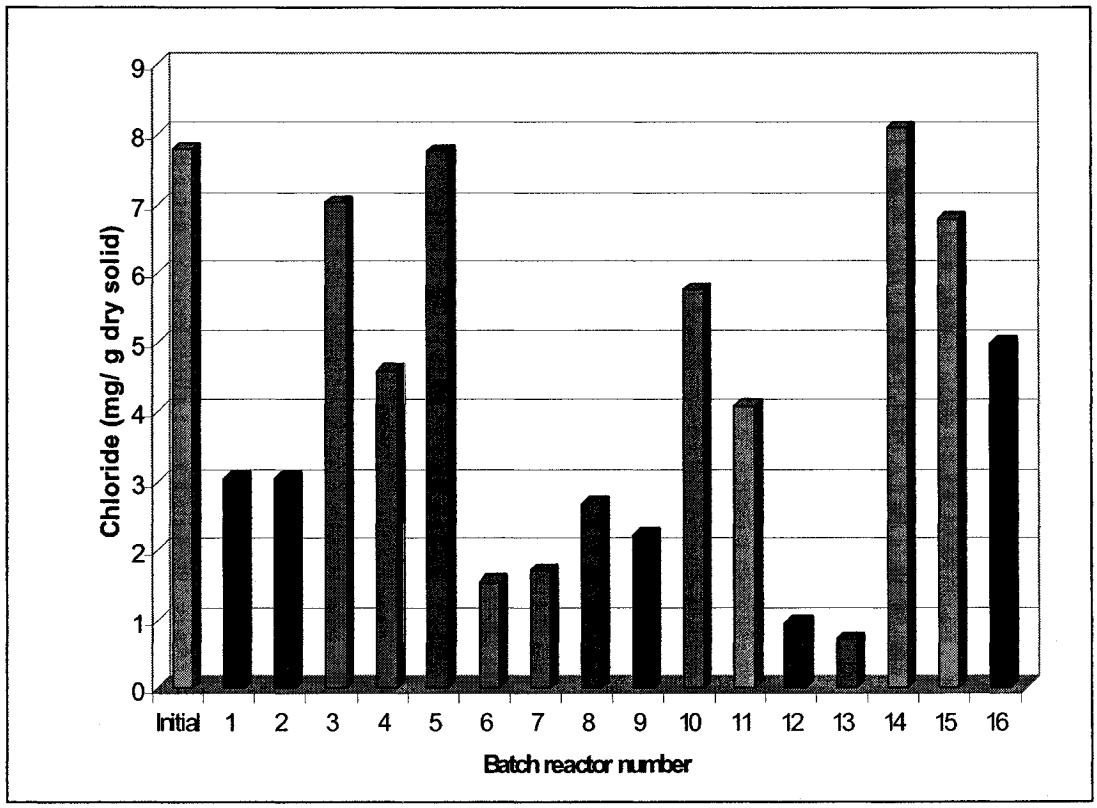


Figure 4-41: Comparison of chloride content of each reactor with initial value

❖ Chloride in the reactors with the voltage gradient of 0.5 V/cm

The results of chloride analysis from the experiments with voltage gradient of 0.5 V/cm has been shown in Table A-7 (Appendix A) and Figure 4-42. Data shows that the reactor 5 presented the highest value of chloride in the middle anode area with amounts of 74 mg/ g dry solid. The reactor 13 in anode zone shows the lowest value of chloride with amount of 0.12 mg/ g dry solid.

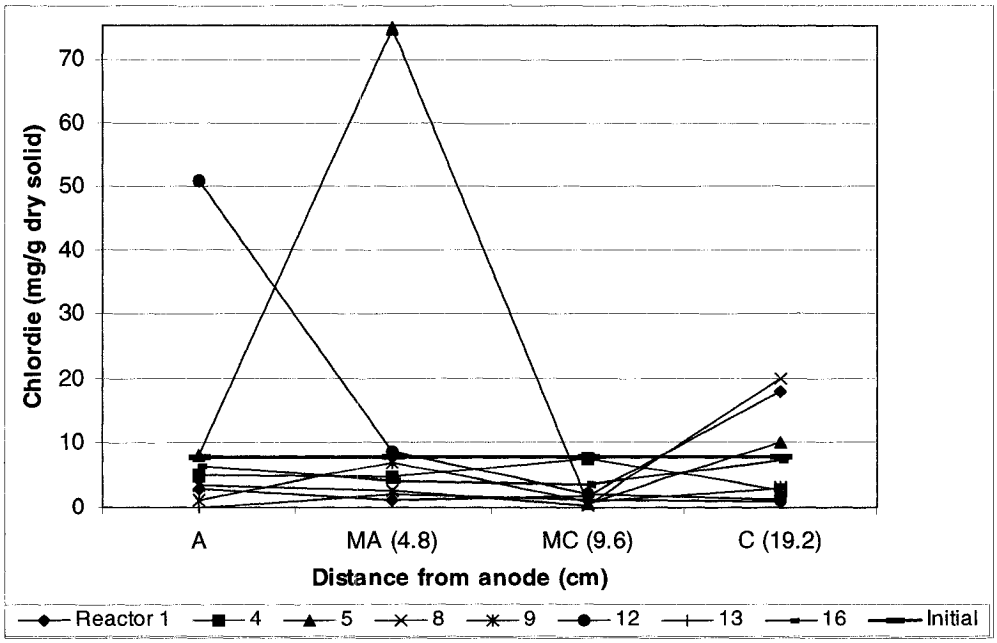


Figure 4-42: Chloride concentration in the reactors with voltage gradient of 0.5 V/ cm

❖ Chloride in the reactors with the voltage gradient of 0.7 V/cm

Table A-8 (Appendix A) and Figure 4-43 show the results of chloride analysis in this group of reactors. The reactor 10 presents the highest value of chloride in the cathode area with amounts of 27 mg/ g dry solid. The reactor 6 in the anode area shows the lowest value of chloride with amount of 0.42 mg/ g dry solid.

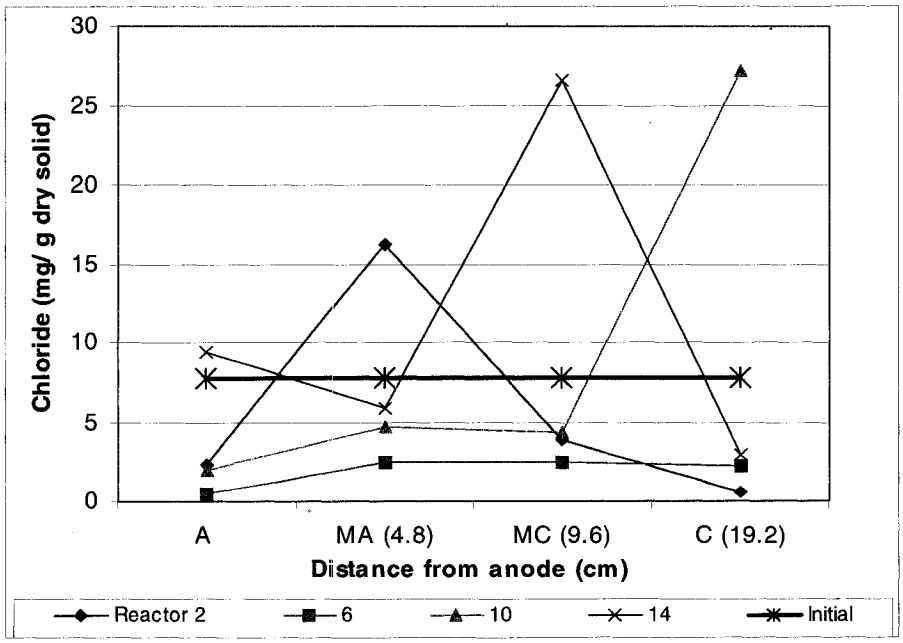


Figure 4-43: Chloride concentration in the reactors with the voltage gradient of 0.7 V/ cm

❖ Chloride in the reactors with the voltage gradient of 1.5 V/cm

The results of analysis of this group in Table A-9 (Appendix A) and Figure 4-44 show the reactor 3 has the highest value of chloride in the anode area with amounts of 172 mg/ g dry solid. The reactor 7 in the cathode area shows the lowest value of chloride with amount of 0.78 mg/ g dry solid.

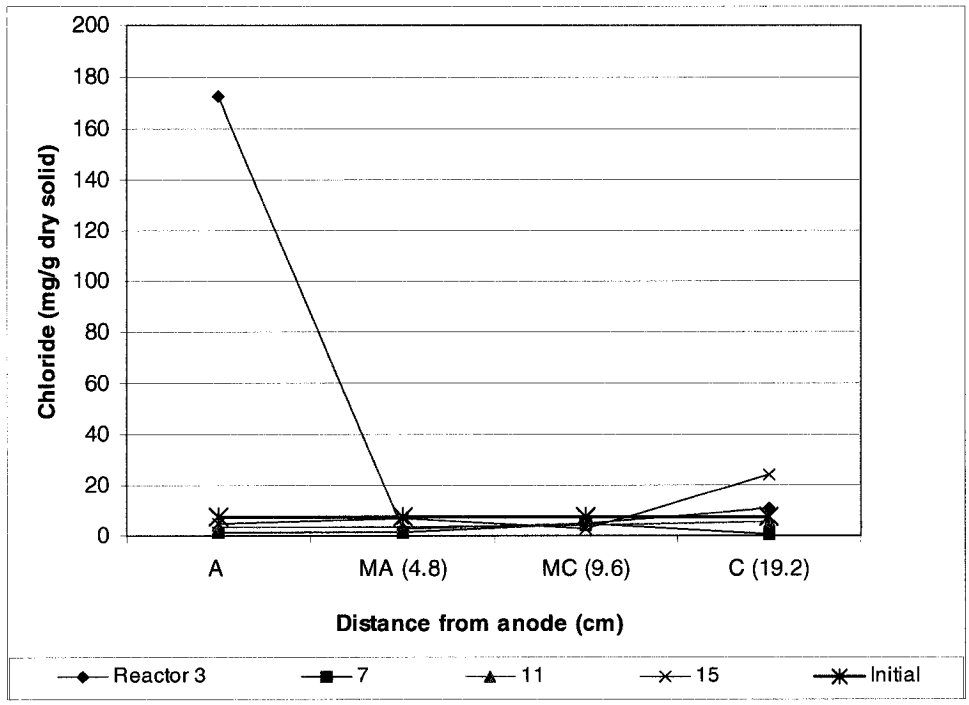


Figure 4-44: Chloride concentration in the reactors with the voltage gradient of 1.5 V/ cm

The general assessment of results shows that the content of chloride did not increase with addition of any enhancer. Electrical field influences on the chloride content in the reactors: the highest voltage gradient keeps chloride below the initial value. Also, chloride acts as an effective ionic element in continuation of the electrolysis reactions.

4-1-11- Effect on volatile fatty acids

Figure 4-45 illustrates the geometric mean of VFAs in EK treated biosolids. It can be concluded that:

- The highest reduction of these compounds was found in reactor 14 with value of 71.75 %.

- The results show the increase of VFAs in the most of reactors, namely 1, 2, 3, 5, 8, 10, 11, 12 and 16. Evaluation of experimental conditions (see Table 3-2) shows the existence of BioxyS/ TEAD in all named reactors except for the reactor 1 and 12. As mentioned in chapter 3, the reaction activity of Bioxy S/ TEAD is based on formation of pereactic acid. And based on given information on chapter 2, HACH method for measuring VFAs is expressing all VFAs as acetic acid. Therefore, it can be concluded that using the HACH method 8196 to measure VFAs in the EK reactors with concentrations of Bioxy S/ TAED is not a reliable method due to the interfering effects of Bioxy S/ TAED on the results.

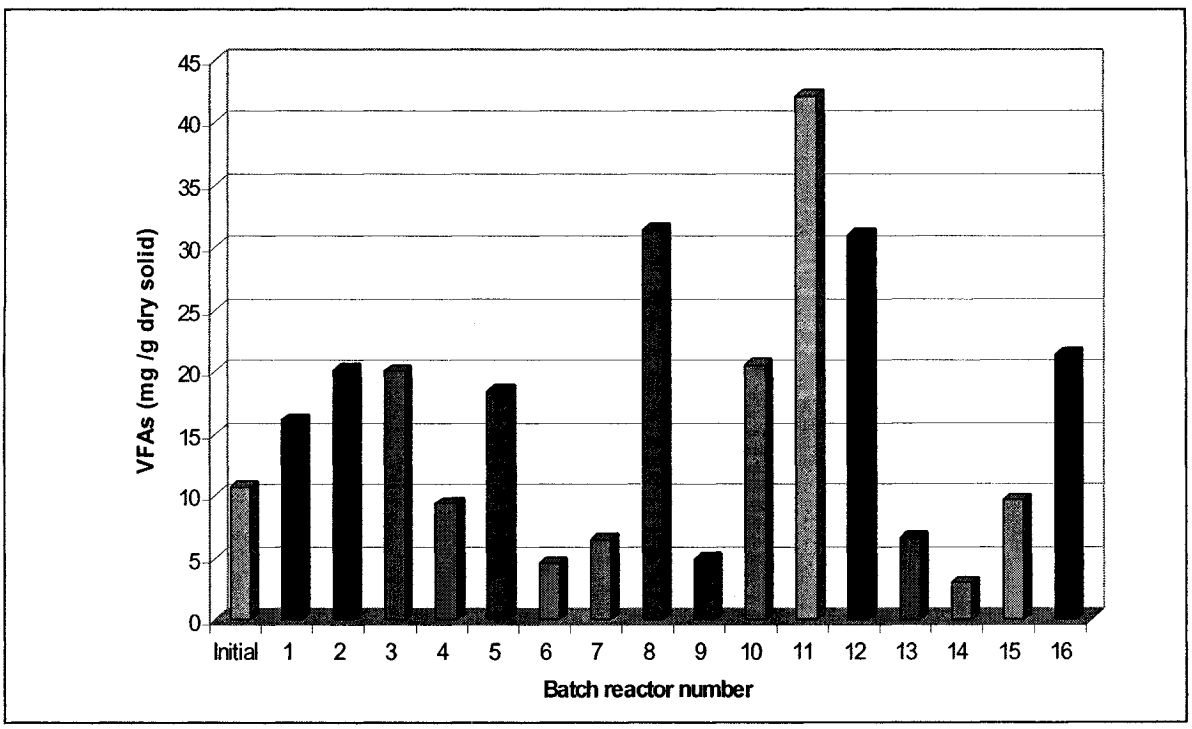


Figure 4-45: Comparison of VFAs content of each reactor with initial value

❖ Volatile fatty acids in the reactors with the voltage gradient of 0.5 V/cm

Table A-14 (appendix A) and Figure 4-46 presents data of volatile fatty acids in this group. The highest amount of VFAs in this group was related the reactors 13 (the anode area with amount of 251.23 mg/ g dry solid). The lowest value (1.26 mg/g dry solid) belonged to the reactor 9 in the anode area.

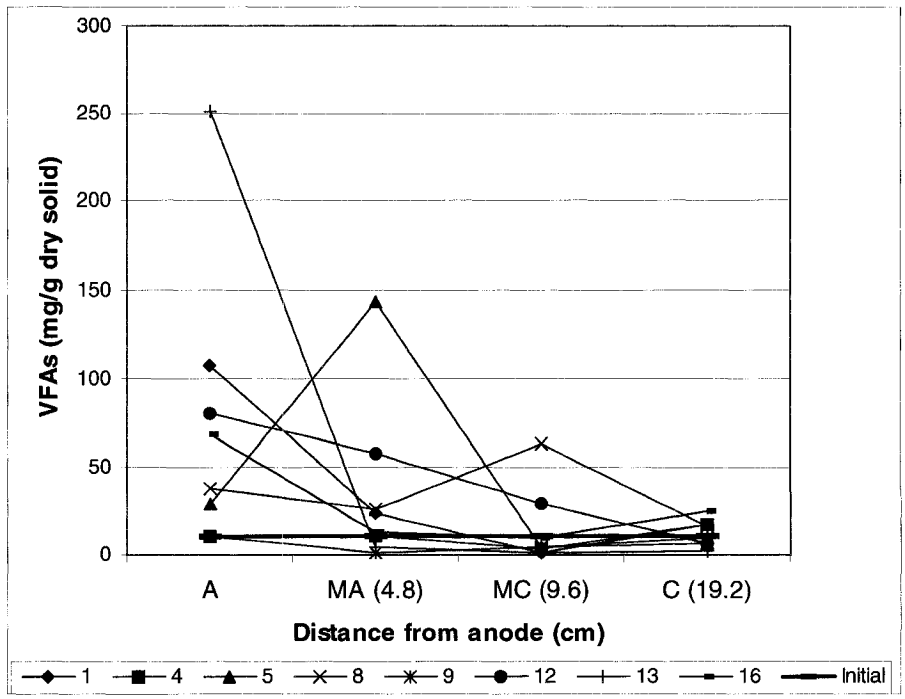


Figure 4-46: VFAs concentration in the reactors with voltage gradient of 0.5 V/cm

❖ Volatile fatty acids in the reactors with the voltage gradient of 0.7 V/cm

Table A-15 (Appendix A) and Figure 4-47 presents data of VFAs in the reactors with gradient voltage of 0.7 mV/ cm. The reactor 2 in the middle cathode area showed the highest value (106.2 mg/ g dry solid) of VFAs; however, the reactor 6 at the same area had the lowest amount (1.30 mg/ g dry solid).

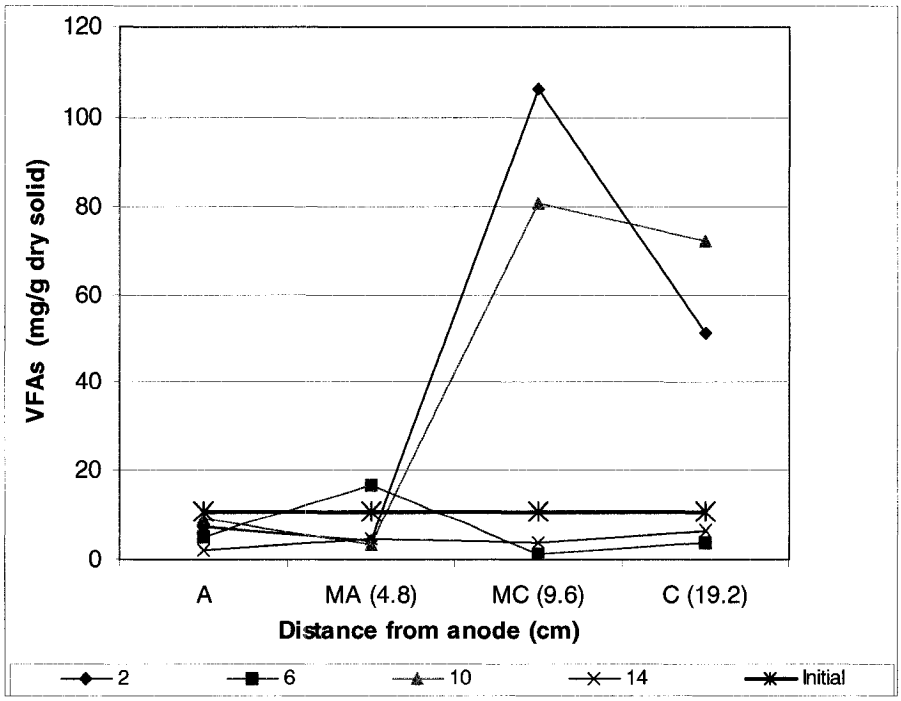


Figure 4-47: VFAs concentrations in the reactors with voltage gradient of 0.7 V/cm

❖ Volatile fatty acids in the reactors with the voltage gradient of 1.5 v/cm

The results of VFAs in the reactors has been shown in Table A-16 (Appendix A) and Figure 4-48. The highest amount of VFAs in this group which was found in anode area of the reactor 3 was 235.78, while the lowest amount (0.01>) belonged to cathode area of the reactor 15.

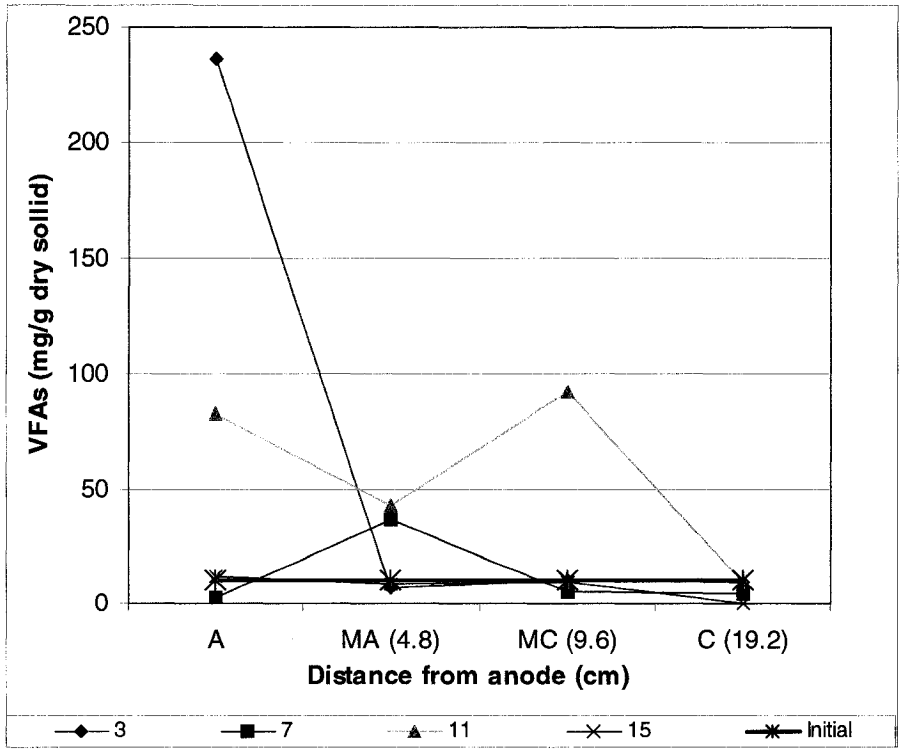


Figure 4-48: VFAs concentration in the reactors with voltage gradient of 1.5 V/cm

The general evaluation of the results implies that spite the analytical error (mentioned earlier), the decrease of VFAs was observed due to increase of voltage gradient. In the presence of the amphoteric enhancer, the highest amount of VFAs was found close to the anode area and in the absence of this agent the highest value of VFAs was found close to the cathode area showing the interference of the enhancers with VFAs and changing their properties.

4-1-12- Effects on electrical parameters

The voltage potential at each porbe-elecrode and the electrical current for each EK reactor were measured each 6 hours, and then according to the formula introduced in Eq.3.3 the resistance for each batch test was computed.

Table A-13 (Appendix A) demonstrates the resistance distribution of each batch trial. These results, which are average of resistance values for each probe-electrode during every day of running test, illustrate the biggest potential loss at the interface of biosolids and the anode, due to accumulation of solids particles in this area. This fact was observed for all the reactors.

The assessment of the results (Table A-13 and Figure 4-49, 4-50 and 4-51) of the resistance during three days running test is given below.

❖ Electrical changes at the first day of test

- The reactor 2 had the highest resistance gradient (44 Ω/cm) comprising to the other reactors (Figure 4-49).
- The resistance variation between cathode and anode for reactors 11, and 14 varied smoothly. In the other word, the reactor 11, and 14 had the lowest resistance gradient (13.90 and 9 Ω/cm, respectively)
- The highest resistance (110 Ω) was related to the reactor 15.
- The lowest resistance value (44 Ω) was observed in reactor 1.

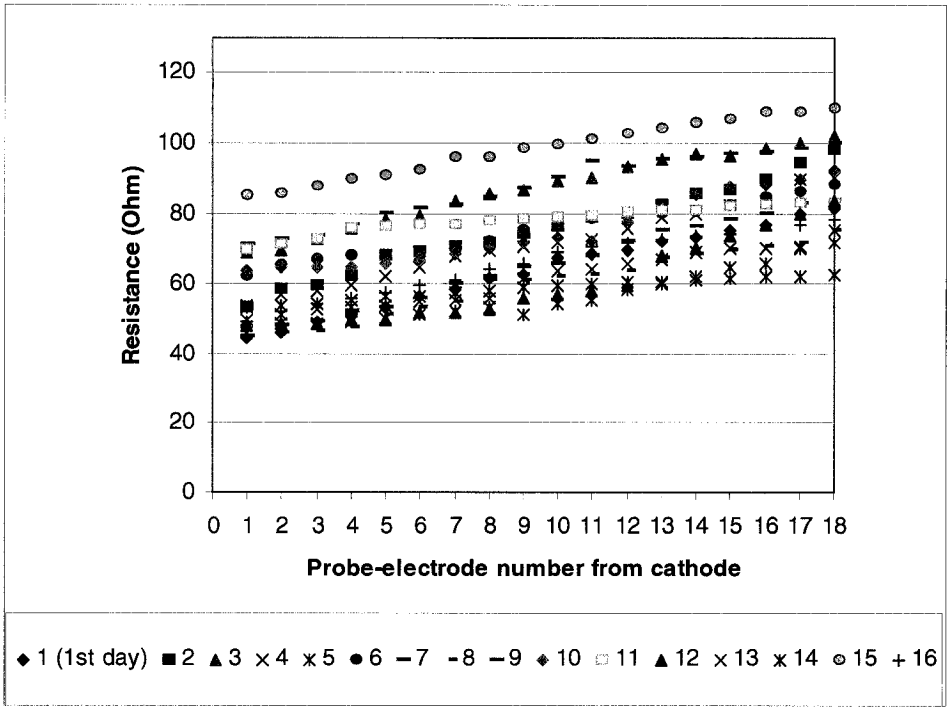


Figure 4-49: Resistance distribution in the batch reactors at the first day test

❖ Electrical changes at the second day of test

- The highest resistance gradient (75 Ω/cm) at the second day was related to the reactor 9 (Figure 4-50).
- Same as first day the reactor 14 showed a smooth resistance changes.
- The highest value of resistance (132.5 Ω) for the second day belonged to reactor 3.

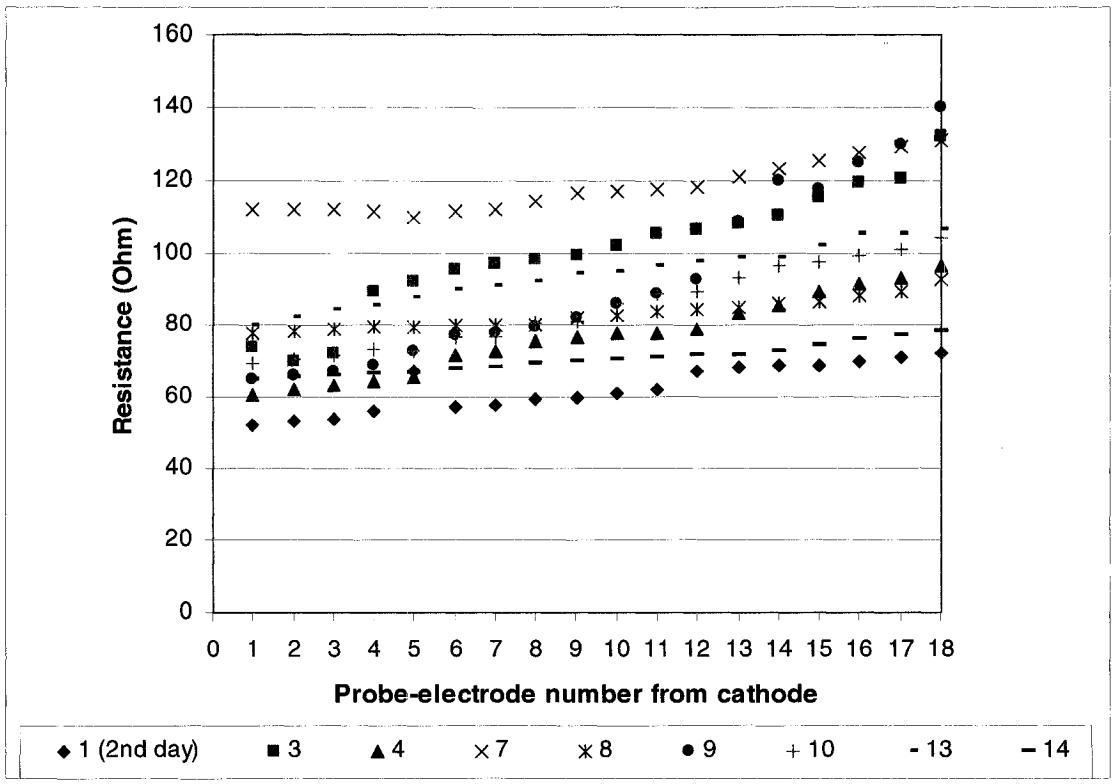


Figure 4-50: Resistance distribution in the batch reactors at the second day test

❖ Electrical changes in the third day of test

- The highest resistance (236 Ω) of third day belonged to the reactor 3. This reactor had the highest voltage gradient, the highest exposure time, the highest amount of the amphoteric agent with the highest percent of glutaraldehyde, and considerable percent of Bioxy S/ TAED (Figure 5-51).
- The highest resistance gradient (63 Ω/cm) was related to the reactor 9.
- All the reactors had a smooth resistance gradient except the reactor 9.

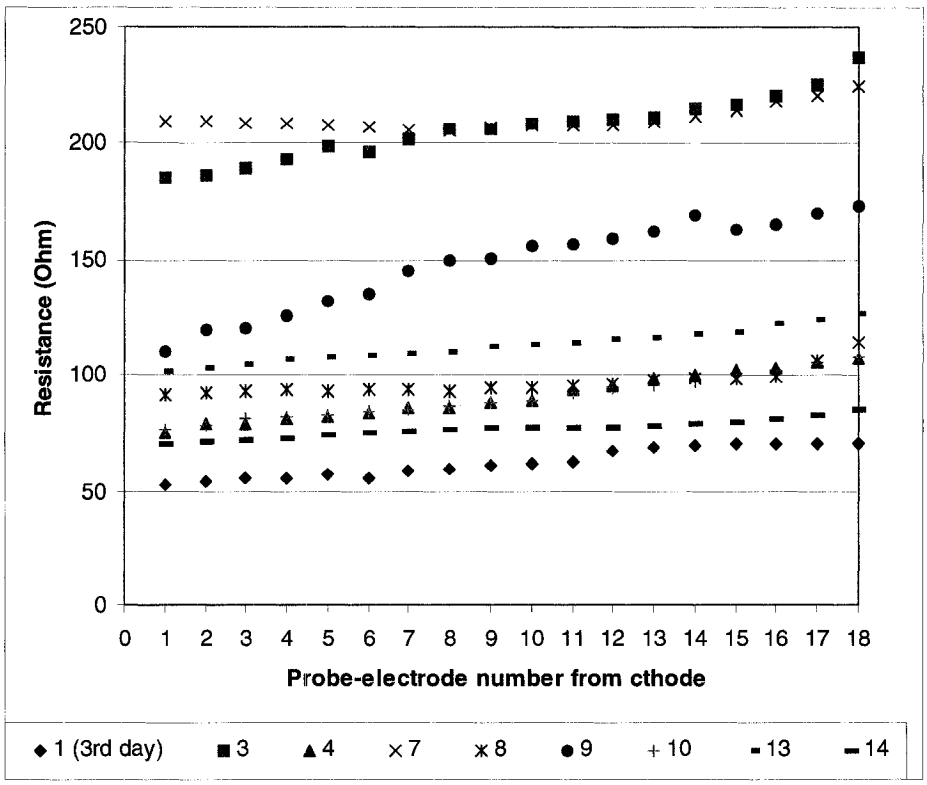


Figure 4- 51: Resistance distribution in the batch reactors at the third day test

- The resistance changes in the reactor 9 were much fluctuated. This obviously shows a flocculation process and transport of solids within the rectors.

Comparison of the resistance result of reactors within three days running experiments showed that:

- A combination of high voltage gradient, high exposure time, and high amount of glutaraldehyde, high percent of amphoteric agent, and Bioxy S / TAED caused high resistance values.
- The effect of different enhancer caused various resistance outlines distribution in the entire EK experiments. The resistance also changes due to dissociation and creating different conductivity conditions in each particular reactor. The

resistance is also affected by initiating electroosmotic and electrophoteric movement subsequently the differences between reactors increase with time.

4-2- Inactivation of C. perfringens spores and Reovirus

As mentioned earlier (Chapter 3), the experiments associated with this section were consisted of two phases. In the first phase, a set of designed experiments (see Table 3-2) was accomplished in order to determine the best inactivation condition. Based on the obtained results of this phase and to evaluate the inactivation effect of each factor separately a set of second experimental condition was designed (Table 3-3 and Table 3-4).

4-2-1- Phase I

The results of "phase I" is presented based on applied voltage gradient on each batch reactor.

❖ Pathogen inactivation in the reactors with voltage gradient of 0.5 V/cm

Table 4- 6, Figure 4-52 and Figure 4-53 present data obtained from the enumeration of *C.perfringens* spores and Reovirus in this group of the reactors. The reactor 5 with 2.37- log-unit reduction of *C. perfringens* spores had the highest value and the reactor 8 with 0.10-log-unit reduction had the lowest value. In this group all the reactors indicated a high value of Reovirus reduction. The lowest Reovirus $\log_{10}$ reduction was 7.63. As mentioned in literature review (2-3-1-1) according to USEPA, for biosolids to reach Class A, a three to five $\log_{10}$ unit reduction is acceptable.

Table 4-6: Counted amount of *C.perfringens* spores and Reovirus in the EK reactors with voltage gradient of 0.5 V/cm

EK reactor trail number		*C. perfringens* spores CFU /g dry solid	Reovirus PFU/ g dry solid	Total log_{10} reduction of*	
				C. perfringens spores	Reovirus
1	C	1.2×10^{5}	NA**	1.37	NA
	MC	4.8×10^{5}	NA		
	MA	9.3×10^{4}	NA		
	A	2.3×10^{5}	NA		
4	C	1.4×10^{4}	ND***	1.43	11
	MC	7.46×10^{2}	ND		
	MA	4.6×10^{4}	ND		
	A	1.1×10^{2}	ND		
5	C	ND	ND	2.37	7.63
	MC	ND	ND		
	MA	ND	6.2×10^{4}		
	A	7.1×10^{1}	ND		
8	C	2.3×10^{5}	ND	0.10	11
	MC	3.2×10^{5}	ND		
	MA	6.2×10^{4}	ND		
	A	7.1×10^{5}	ND		
9	C	ND	ND	1.71	11
	MC	3.7×10^{3}	ND		
	MA	3.9×10^{4}	ND		
	A	2.5×10^{4}	6.55×10^{2}		
12	C	ND	ND	0.19	11
	MC	1.03×10^{6}	ND		
	MA	1.4×10^{4}	ND		
	A	4.1×10^{4}	ND		
13	C	1.1×10^{4}	ND	1.06	11
	MC	ND	ND		
	MA	1.3×10^{5}	ND		
	A	1.9×10^{3}	ND		
16	C	7.1×10^{5}	ND	0.32	11
	MC	ND	ND		
	MA	ND	ND		
	A	9.7×10^{4}	ND		

*Geometric mean of three replicate experiments, **Non applicable. The reactor was not designed for Reovirus experiments, ***Non Detected

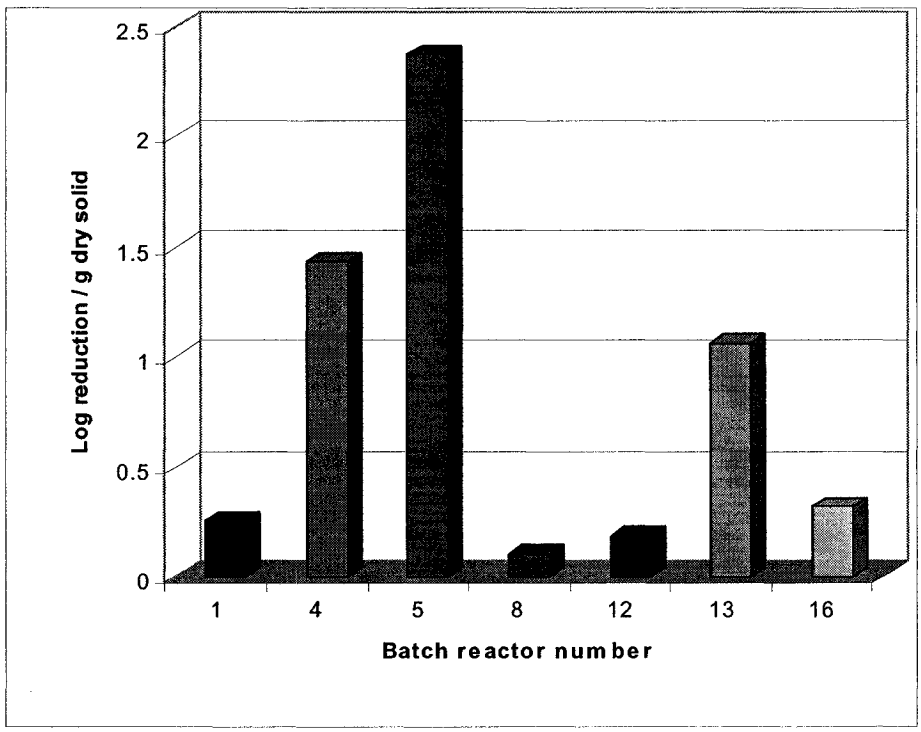


Figure 4-52: Log reduction of *C. perfringens* spores in the batch reactors with voltage gradient of 0.5 V/ cm

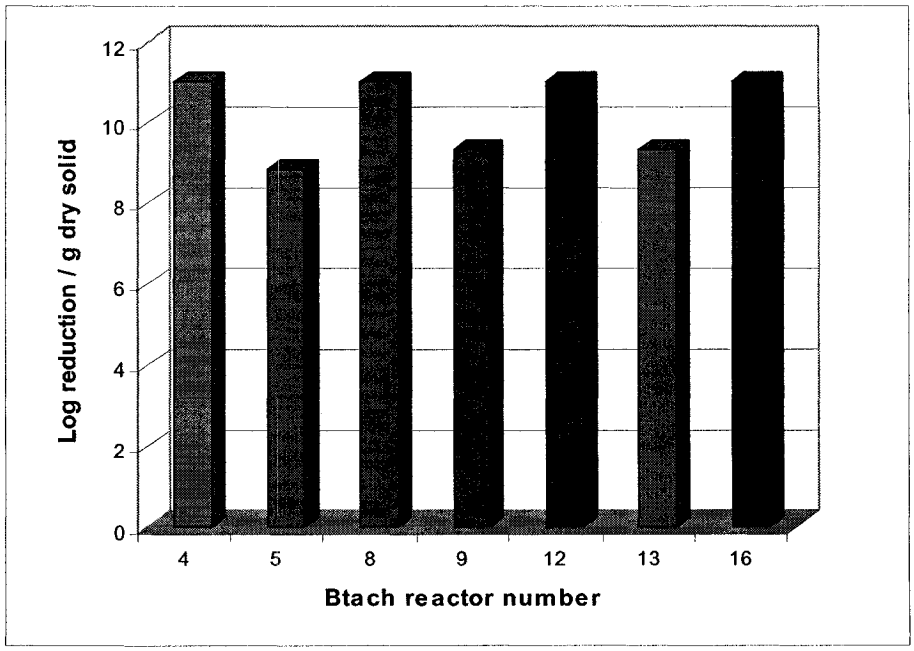


Figure 4-53: Log reduction of Reovirus in the batch reactors with voltage gradient of 0.5 V/ cm

❖ Pathogen inactivation in the reactors with voltage gradient of 0.7 v/cm

The result of this group (Table 4-7) show that the highest log_{10} reduction of *C. perfringens* spores belonged to the reactor 10 with 4.53-log_{10}-unit inactivation and the reactor 14 has the lowest value with 0.01-log_{10}-uint (Figure 4-54). In the case of Reovirus the reactor 10 (9.91-log_{10}-unit reduction) had the highest value, and the reactor 6 with log reduction of 5.81 showed the lowest value (Figure 4-55).

Table 4-7: Counted amount of *C.perfringens* spores and Reovirus in the EK reactors with voltage gradient of 0.7 V/cm

EK reactor trail number		*C. perfringens* spores CFU /g dry solid	Reovirus PFU/ g dry solid	Total log_{10} reduction of	
				C. perfringens spores	Reovirus
2	C	ND	ND*	1.34	9.41
	MC	1.9×10^{4}	ND		
	MA	ND	ND		
	A	5.8×10^{4}	3.36×10^{2}		
6	C	2.4×10^{3}	1.69×10^{2}	1.63	5.81
	MC	2.7×10^{4}	ND		
	MA	1×10^{5}	3.77×10^{2}		
	A	9.3×10^{2}	1.40×10^{2}		
10	C	ND	ND	4.53	9.93
	MC	1.6×10^{1}	1.3×10		
	MA	2.1×10^{1}	ND		
	A	1.4×10^{1}	ND		
14	C	1.6×10^{5}	ND	0.01	9.31
	MC	1.5×10^{5}	8.47×10^{2}		
	MA	1.5×10^{6}	ND		
	A	3.8×10^{5}	ND		
*Non Detected					

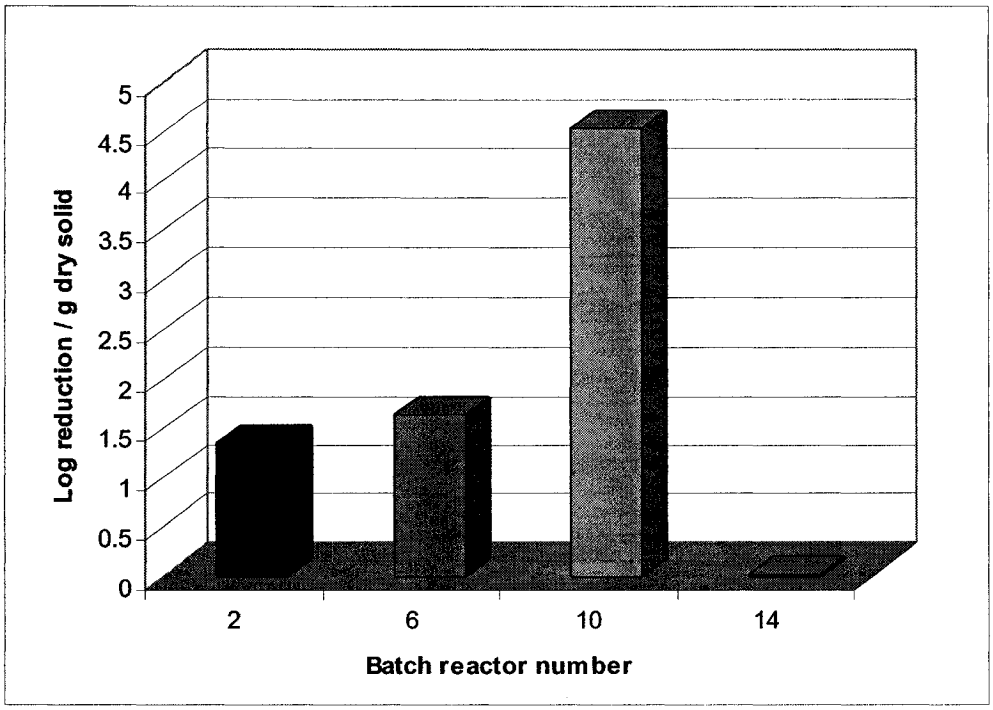


Figure 4-54: Log reduction of *C. perfringens* spores in the batch reactors with voltage gradient of 0.7 V/ cm

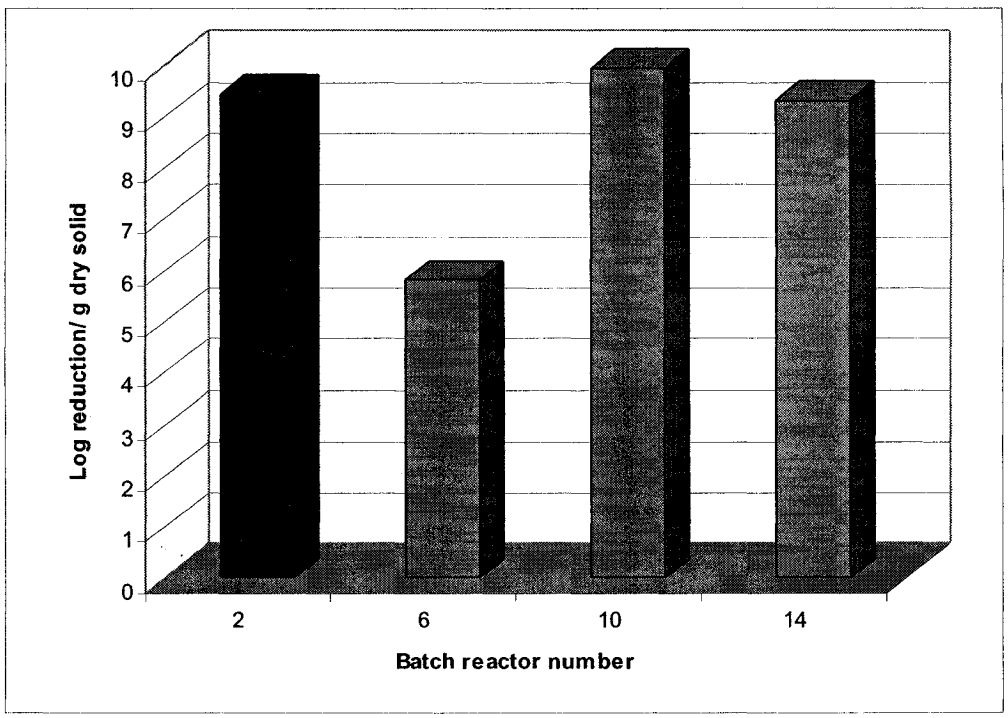


Figure 4-55: Log reduction of Reovirus in the batch reactors with voltage gradient of 0.7 V/ cm

❖ Pathogen inactivation in the reactors with voltage gradient of 1.5 v/cm

Results of this group indicted that the highest $\log_{10}$ reduction of *C. perfringens* spores belonged to the reactor 11 with 2.00 unit inactivation and the lowest value belonged to the reactor 7 with 0.62 unit reduction. For the Reovirus all the reactors showed a $\log_{10}$ reduction of 11 (Table 4-8, Figure 4-56 and Figure 4-57).

Table 4-8: Counted amount of *C.perfringens* spores and Reovirus in the EK reactors with voltage gradient of 1.5 V/cm

EK reactor trail number		*C. perfringens* spores CFU /g dry solid	Reovirus PFU/ g dry solid	Total $\log_{10}$ reduction of	
				C. perfringens spores	Reovirus
3	C	ND	ND*	1.15	11
	MC	ND	ND		
	MA	1.2×10^{5}	ND		
	A	2.7×10^{4}	ND		
7	C	5.2×10^{3}	ND	0.62	11
	MC	2.1×10^{5}	ND		
	MA	1.1×10^{4}	ND		
	A	1.8×10^{5}	ND		
11	C	ND	ND	2.00	11
	MC	2.8×10^{3}	ND		
	MA	ND	ND		
	A	1.4×10^{4}	ND		
15	C	ND	ND	1.58	11
	MC	ND	ND		
	MA	4.4×10^{4}	ND		
	A	ND	ND		
*Non Detected					

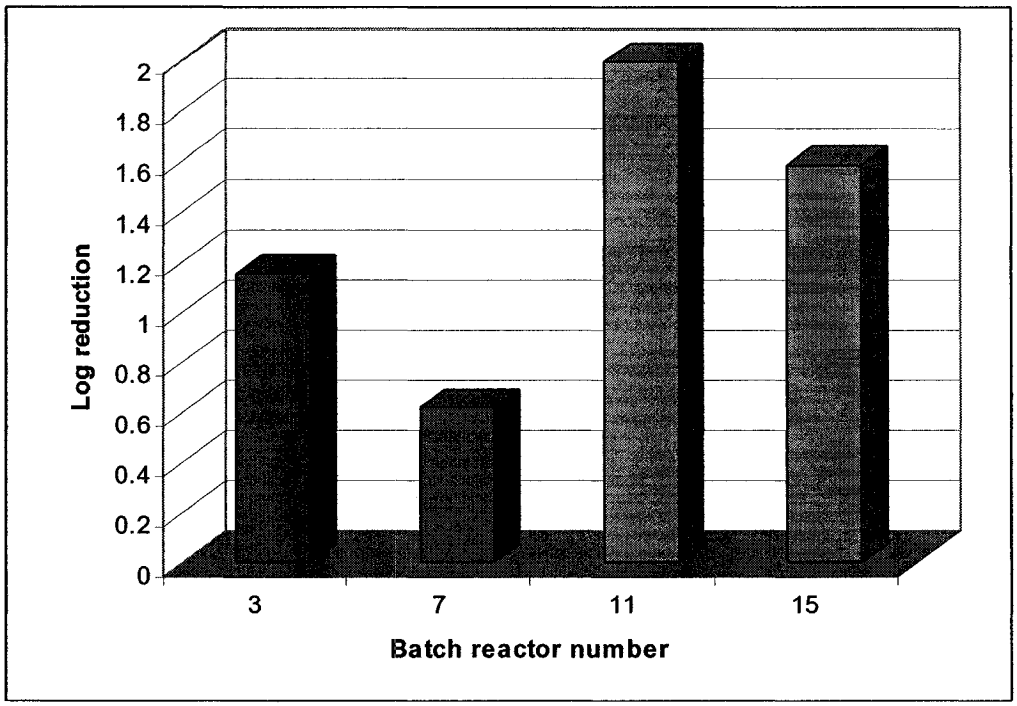


Figure 4-56: Log reduction of *C. perfringens* in the batch reactors with voltage gradient of 1.5 V/ cm

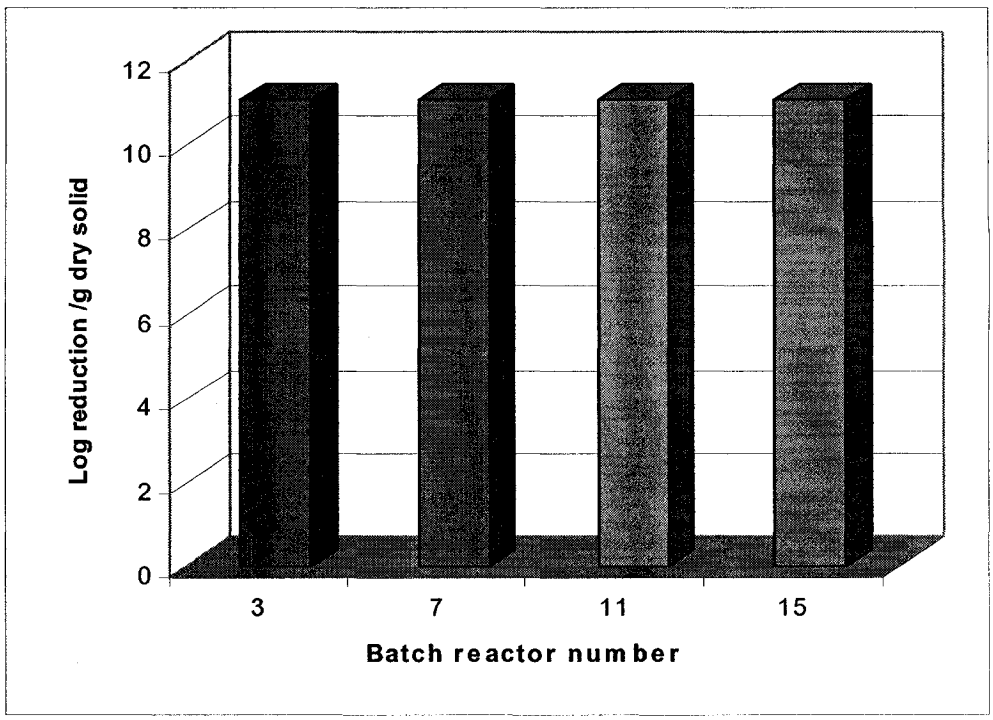


Figure 4-57: Log reduction of Reovirus in the batch reactor with voltage gradient of 1.5 V/ cm

❖ Running control test for *C.perfringens* spores and Reovirus

Table 4-9 shows the result of running control test in two different duration time of 1 day and 3 days.

Table 4-9: Counted amount of *C.perfringens* spores and Reovirus for control tests

	C. perfringens CFU /g dry solid	Reovirus PFU/ g dry solid	Total log_{10} reduction of	
			C. perfringens CFU /g dry solid	Reovirus
1 day	1.4×10^{5}	1.1×10^{7}	0.07	0.006
3 days	1.5×10^{5}	9×10^{6}	0.04	0.09

4-2-2- Phase II

❖ Running test S2a, S2b, S2c and S2d for *C.perfringens* spores

Refereeing to chapter 3, a set of experiments designed to understand the effect of each factors in EK complex system. These experiments were assigned with S2(n) symbol where (n) designed as a test code (see Table 3-3). Test S2a and S2c had similar condition as the reactors 14 and 10, therefore S2b and S2d were the tests to be run.

The results of running these tests presented 1.98 and 2.54 -log_{10}–units inactivation for S2d and S2d, respectively.

❖ Running part II of Phase II for *C.perfringens* spores

Results of running test according to Table 3-4 shows a highest log_{10} reduction of 2.98 for S10 (Table 4-10 and Figure 4-58).

Table 4-10: Log_{10} reduction of *C.perfringens* in phase II

Trial	Log_{10} reduction
S4	0.7
S5	1.68
S6	1.20
S9	0.6
S10	2.98
S11	1.05
S15	1.23

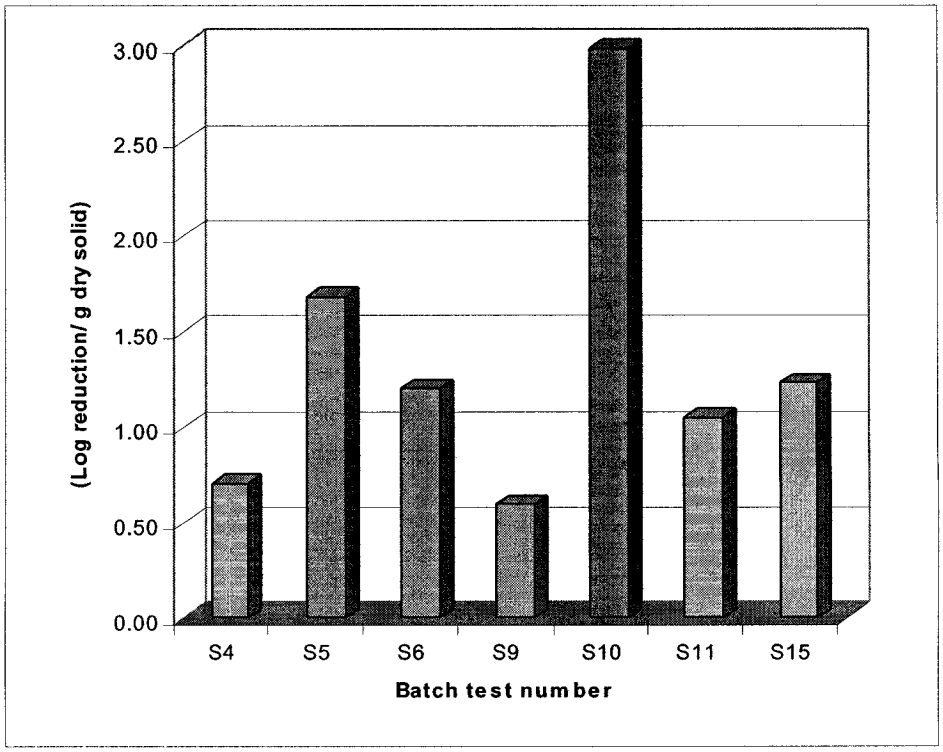


Figure 4-58: Log reduction of *C. perfringens* spores in the absence of EK

The results in phase II of experiments confirmed the synergistic effect of EK system with oxidant agents on inactivation of *C.perfringens* spores. As observed in the first part of phase II, the EK system without enhancement agent was not able to meet the

regulatory level of Class A biosolids. Also, application of oxidant agents in the absence of EK system show the low log unit reduction of *C.perfringens* spores.

4-2-3- Analysis of variance of the pathogen inactivation results

Table 4-11 shows the ANOVA of results from the first phase of experiments by using Qualitek-4 and with quality control type of "Bigger is Better". According to this data the contribution of the Bioxy S/ TAED on pathogen inactivation results of phase I is 62.16%. In the other word, the Bioxy S had the highest contribution on the inactivation of *C. perfringens* spore in EK system. The second effect was related to voltage gradient with 21% effectiveness.

Table 4-11: ANOVA of the results for inactivation of *C. perfringens* spores in enhanced EK system (Phase I)

	DOF (f)	Sum squrs. (S)	Variance	F. Ratio	Pure Sume	Percent P(%)
Voltage gradient	2	6.570	1.984	2.164	2.135	21.013
Glutaraldehyde	2	4.10	2.413	1.308	0.565	13.159
Exposure time	1	0.053	0.053	0.053	0	0
Amphoteric agent	2	1.138	0.569	0.62	0	0
Bioxy S/ TAED	3	19.434	0.916	2.632	4.49	62.159
Other/ Error	5	2.399	1.199			2.918
Total	15	33.694				100

Evaluation of result shows that inactivation of *C.perfringes* spores and Reovirus in the EK reactor is a complex process since there are different factors involved in the process, which attack numerous sporal and viral constituents, including spore coats, proteins, unsaturated lipids, respiratory enzymes, peptidoglycans and virus capsids.

All this evidence implies that EK system in the presence of a mixture of oxidant agents has a great impact on the inactivation process. These effects can be explained as follows:

Reactions created due to the EK phenomena help to produce effective oxidative zones, which neutralise protective systems inside and outside of *C. perfringens* and Reovirus. For *C. perfringens,* the spore coat is a primary protective barrier against the oxidant agent (Paustian, 2001); the capsid of viruses plays the same role for Reovirus (Olland, 2001). It seems that the condition in reactor 10 which showed the better result for in activation *C.perfringens* spores directs the oxidative stress to modify primary and secondary structures of spores and capsid's proteins; however further research in required to develop a full understanding of the effect of this applied condition on disinfection process in enhanced EK system. .

The chemical structure of glutaraldehyde has an effect on the dialdehyde on the surface of bacterial cells. Glutaraldehyde also interacts at the spore's surface and remains there; however, in an alkaline condition (the cathode zone), it could also penetrate the spore. The role of the alkalinity condition as an activator agent is in facilitating penetration and interaction of glutaraldehyde with components of the spore cortex. Under this condition, glutaraldehyde helps spore swelling and prepares the condition for act of oxidative zones.

In the case of Reoviruse, the main targets for glutaraldehyde agent are nucleic acid, proteins, and envelope constituents. The established reactivity of glutaraldehyde with proteins suggests that the viral capsid or viral-specific enzymes are vulnerable to glutaraldehyde treatment.

After the activity of glutaraldehyde it seems that Bioxy S/ TAED starts to act. This oxidant agent reacts with SASPs to leave the bacterial DNA unprotected and susceptible to other disinfectants. An oxidizing chemical pulls electrons away from the cell membrane, causing it to become destabilized and leaky. This results in the destruction of the integrity of the cell membrane, which leads to rapid death.

Furthermore, it has been observed that transition metals catalyze the formation of the hydroxyl radical: therefore, the addition of iron, copper, cobalt, chromium, or manganese increases the efficacy of oxidant agents and formation of hydrogen peroxide. However, because of the increased activity, metal contamination of biosolids will cause degradation and instability of the chemical structure of oxidant agents. In the EK system metals are attracted to the electrodes. This distribution of metals increases the effectiveness of oxidant agents and simultaneously prevents degradation due to the impact of metal concentration on the high activity of oxidant agents.

V- CONCLUSION

This research permitted to develop an enhanced electrokinetic (EK) technology for effective inactivation of pathogen bacteria and viruses within biosolids. This development can be recognized as a new method for the sustainable management of sewage sludge.

The results from this study demonstrated a successful, fast and easy process for inactivating *Clostridium perfringens* spores and Reovirus in order to upgrade biosolids to acceptable standard levels while electrokinetic dewatering. This new process offers several benefits over conventional methods. First, it is able to eliminate pathogens, such as *E. coli*, *Salmonella*, enteroviruses, parasites and, most importantly, the most resistance form of bacteria: spores. EK treated biosolids will usually reach a P2 rating in Quebec and Class A in the USA. Second, it facilitates both dewatering and volume reduction of biosolids. Third, it reduces the overall cost and time for treatment of biosolids. Fourth, it demonstrates a process capable of reducing the capital cost of WWTP by combining several different operational units, which are involved in dewatering and disinfections as well as metal and nutrient removal.

In this research, the application of different enhancing agents to the efficiency of EK in order to eliminate pathogens below threshold levels was examined. It is concluded that a combination of a higher concentration of glutaraldehyde (0.5% v/v) and a medium concentration (0.2% w/v) of Bioxy S/ TAED had the highest effects on the inactivation of *C. perfringens* spores using EK process. The result also showed that Reovirus was inactivated below threshold levels under all applied conditions.

The role of different enhancement agents in disinfecting biosolids and in changing the physicochemical properties of treated biosolids was observed. Analyzing the pH and ORP variations has led to a better understanding of the effect of two applied oxidant agents on inactivation of pathogens in the EK system. To introduce the optimum conditions, various issue were considered, such as quality of treated biosolids, the length of treatment and energy consumption. It can be concluded that the impact of enhanced EK technology on pathogen inactivation is due to the synergistic effect of Bioxy S/ TAED, glutaraldehyde and voltage gradient, while the application of the amphoteric agent does not improve this process. However, the amphoteric enhancer has important impact on pH gradient, TS%, ORP and electrical resistance. Electrical gradient has effect on nitrogen-ammonia concentration, sulfate and electrical resistance.

The treated biosolids revealed low pathogen content and an acceptable amount of macro- and micronutrients and can, therefore, be recycled for land application and distributed for public use. The leakage collected from the EK system can be returned to the WWT system, where it undergoes physical treatment to be reused in agricultural irrigation.

The results of this research open to a new technology, one that can recycle sewage sludge safely and efficiently and thus rank Canada at the avant-garde of countries currently practicing sustainable development principles. This technology has the potential to alter the management of biosolids and solve the waste disposal problem by facilitating effective disinfecting, fast dewatering, recycling of treated biosolids. The EK treatment reduces the amount of nutrients such as nitrogen, sulfate and phosphorous in biosolids, and consequently decreases the release of these elements to surface water, thereby

eliminating eutrophication of water bodies. By demonstrating that EK technology can successfully eliminate pathogens, this research provides a cost effective and efficient way to produce safe biosolids.

5-1- Contribution to Knowledge

- This research is the first study to assess the functionality of the electrokientic process on the inactivation of *C. perfringens* and Reovirus.
- This research assessed the optimal condition for the inactivation of *C. perfringens* in anaerobic biosolids
- This research is the first study to collect extensive results on the synergetic effects of different enhancement agents with EK technology.
- By showing the subsequent effects on *C. perfringens*, this research helps to explain the EK inactivation process.
- By showing the subsequent effects on Reovirus, this research helps to explain the EK inactivation process.

5-2- Suggestions for future research

- To investigate the effect of the same experimental conditions on a pilot scale.
- To evaluate the effect of different types of electrode material on the inactivation efficiency of the EK reactor.
- To investigate the biochemical reaction involved in the inactivation process on a molecular scale.
- To evaluate the impacts of different shapes of the EK cell on the inactivation results.
- To develop a mathematical model for inactivation microorganisms.
- To investigate the effect of reducer agents, such as sulfor dioxide, and/or a mixture of reducer and oxidizing agents in the EK technology for the inactivation of pathogens.

VI-REFERENCES

Acar, Y. B. and N. A. Alshawabkeh. (1993). "Principles of Electrikinetics remediation." *Environ. Sci. Technol,* 27(13), 2638-2647.

Acar, Y. B. and A. N. Alshawabkeh. (1994). "Modeling conduction phenomena in soils under an electric current."*Proc. of the 15th Int. Conf. on Soil Mechanics and Foundation Engineering, New Delhi, India* 2, Oxford and IBH Publishing Co., New Delhi, India, 669–672.

Acar, Y.B., R. J. Gale and A.N. Alshawabkeh. (1996). "Electrokinetic remediation. I: pilot-scale tests with lead-spiked kaolinite". *Journal of Geotechnical Engineering,* 122(3), 173-185.

Acar, Y.B., R. J. Gale, G. Putnam and I. Hamed. (1989). "Electrochemical processing of soils: its potential use in environmental geotechnology and significance of pH gradients". Envo Publishing, Bethlehem, PA, Shanghai, China, 25-38.

Adams, A. (2006). "Transport of nanoscale zero valent iron using Electrokinetic phenomena". Ph.D. thesis, University of Western Australia.

Adams, D. A. (1973). "Sensitization by ethylenediamineteretraacetate of Clostridium perfringens type A spores to germination by lysozyme". *Journal of Bacteriology,* 116(1), 500-502.

Adams, M.R. and M.O. Moss. (2000). *Food Microbiology.* Royal Society of Chemistry, Cambridge, UK.

Adriano, D.C. (1986). *Trace elements in the terrestrial environment.* Spring, Berlin.

Afolabi, P., K. G. Davies and P.S. O'Shea. (1995). "The electrostatic nature of the spore of Pasteuria penetrans, the bacterial parasite of root-knot nematodes". *J. Appl. Bacteriol.,* 79(3), 224-249.

Alshawabkeh, A. N., Y. Shen, K. Y. Maillacheruvu. (May 2004). "Effect of DC electric fields on COD in aerobic mixed sludge processes". *Environmental Engineering Science,* 21(No. 3), 321-329.

Alshawabkeh A. N. (2001). "Basic and application of electrokinetic remediation". Course material, universidade Federal do Rio de Janiro (COPPE-UFRJ).

Alshawabkeh, A. N., R.J Gale E. Ozsu-Acar and R. M. Bricka. (1999). "Optimization of 2-D electrode configuration for electrokinetic remediation." *Journal of Soil Contamination,* 8(6), 617-635.

American Type Culture Collection (ATCC). (2006). "Clostridium perfringens". http://www.atcc.org/common/catalog/wordSearch/results.cfm (January, 20, 2006).

ANSI/AAMI. (2005). "Chemical sterilization and high-level disinfection in health care facilities". American National standard. ANSI/AAMI ST58:2005.

Apedaile, E. and D. Cole. (2002). "Health aspects of biosolids land application". ROPEC, city of Ottawa.

Apedaile, E. (2001). "A perspective on biosolids management". *The Canadian Journal of Infectious Disease and Medical Microbiology,* 12(4).

Arkhippov, A, P. L. Freddolino and K. Schulten. (2006). " Stability and dynamic of virus capsid described by coarse-grained modeling". *Structure*, 14(12), 1767-1777.

Barker, T. B. (2005). *Quality by experimental design.* Chapman & Hall/CRC, New York.

Barich, D. (2005). "*Clostridium*". http://biology.kenyon.edu/Microbial_Biorealm/bacteria/gram-positive/clostridium/clostridium.htm. May 26, 2006).

Bates, J.R. (1997). *Clostridium perfringens. In: Food-borne microorganisms of public health importance.* AIFST (NSW branch), Sydney, Australia.

BCMAFF (B. C. Ministry of Environment, Fishers and Food). (1996). "Composting factsheet". Order No. 382.500-5. Agdx 537/727.

Bean, C. L. and K. Belanger. "Pathogens in biosolids: Evaluation of Clostridium perfringens as an indicator organisms to assess the efficiency of biosolids disinfection processes." *Rep. No. 2003NH205,* University of Hampshire.

Bendell, T., G. Wilson and R. M. G. Millar. (1991). *Taguchi methodology within total quality.*Bedford. IFS.

Berggren, I., M. Johansson and A. Albihn. "Survival of pathogenic bacteria and parasites in stored sewage sludge." http://www.ramiran.net/doc04/SESSION%203.pdf (May 2006, 2006).

BEST (Board on Environmental Studies and Toxicology). (2002). *Biosolids Applied to Land Advancing Standards and Practices.* THE NATIONAL ACADEMIES PRESS, Washington, D.C.

Blais, J.F., R.D. Tyagi, C.J. Auclair and M.C. Lavoie. (1992). "Indicator bacteria reduction in sewage sludge by a metal bioleaching process." *Wat. Res.,* 26 487-495.

Black, M.I. , P. V. Scarpino, C. J. O'Donnell, K. B. Meyer, J. V. Jones and E. S. Kaneshiro. (1982). "Survival rates of parasite eggs in sludge during aerobic and anaerobic digestion". *Appl Environ Microbiol,* 44(5), 1138-1143.

Brewster J., J. A. Oleszkiewicz, K.M. Coombs and A. Nartey (2005). "Enteric virus indicators: Reovirus versus polivirus". *Journal of Environmental Engineering, 131(7), 1010-1013.*

Buck, K. M. (2001). "The Effects of Germicides on Microorganisms". http://www.infectioncontroltoday.com/articles/191clean.html (July 07, 2007).

Buehler, M. F. J. E. Surma and J. W. Virden. (1994). " Transport of radioactive ions in soil by electrokinetics". PNL-SA-24683. U. S. Department of Energy. Illinois.

Burke, D.A. (2000). "Class A Biosolids with the AGF Pasteurization Process". *WEFTEC 2000, Water Environment Federation 73rd Annual Conference and Exposition* to be presented at Oct 14-18, 2000, Anaheim, CA.

Buyske, S. and R. Trout. (2006). "Robust design and Taguchi methods". http://www.stat.rutgers.edu/~buyske/591/lect10.pdf (16 May, 2006).

Byamukama, D., R. L. Mach, F. Kansiime, M. Manafi and A. H. Farnleitner. (2005). "Discrimination efficacy of fecal pollution detection in different aquatic habitats of a high-altitude tropical country, using presumptive Coliforms, *Escherichia coli*, and *Clostridium perfringens* spores". *Applied and Enviromental Microbiology*, 71(1), 65-71.

Cauwenberghe, L. V. (1997). "Electrokinetic". *Rep. No. TO-97-03,* GWRTAC, Pittsburg.

CGER (Commission on geosciences, environment and resources). (1997). *Use of reclaimed water and sludge in food crop production,* NATIONAL ACADEMY PRESS, Washington, D.C.

Chappell, J. D., A. E. Prota. (2002). "Crystal structure of reovirus attachment protein σ1 reveals evolutionary relationship to adenovirus fiber". *The EMBO Journal*, 21, 1-11.

Charbonneau, H., M. Hébert and A. Jaouich. (2001). "Portrait de la valorisation agricole des MRF au Québec. Partie 2: Contenu en éléments fertilisants et qualité environnementale". *Vecteur,* 34(1), 56-60.

Chen, Y., S. Miyata, S. Makino and R. Moriyama. (1992). "Molecular characterization of a germination-specific muramidase from Clostridium perfringens S40 spores and nucleotide sequence of the corresponding gene." *Journal of Bacteriology,* 179(10), 3181-3187.

Chen, P. and Y. P. Ting. (1995). "Effects of heavy metal uptake on the electrokinetic properties of Saccharomyces cerevisae". *Biotechnology Letters,* 17(1), 107-112.

Chun, M.-S., M. S. Shim and N. W. Choi. (2006). "Fabrication and validation of a multi-channel type microfluidic chip for electrokinetic streaming potential devices". *Lab Chip,* 6, 302-309.

Choudhury A. and M. Elektorowicz. (1997). "Enhanced electrokinetic methods for lead and nickel removal from contaminated soils". 32nd Central Symposium on Water Pollution Research, Burlington, ON.

Collins, Y. E. and Stotzky G. (1992). "Heavy metals alter the electrokinetic properties of bacteria, yeast and clay minerals". *Applied and Environmental Microbiology,* 58(5), 1592-1600.

Committee on Toxicants and Pathogens in Biosolids Applied to Land, National Research Council. (2002). *Biosolids Applied to Land: Advancing Standards and Practices.* National Academy Press. Washington DC.

Coombs, K. M., S.-C. Mak and L. D. Petrycky-Cox. (1994). "Studies of the major Reovirus core protein o'2: reversion of the assembly-defective mutant tsC447 is an intragenic process and involves back mutation of Asp-383 to Asn." *Journal of Virology,* 68(1), 177-186.

CPEO. (2002). "Electrokinetics." http://www.cpeo.org/techtree/ttdescript/elctro.htm (May 9, 2006).

Craven, S. E. (1988). "Activation of Clostridium perfringens spores under conditions that disrupt hydrophobic interactions of hydrophobic interaction of biological macromolecules". *Applied and Environmental Microbiology,* 54(8), 2042-2048.

Craven, S. E. and L.C. Blankensip. (1985). "Activation and injury of Clostridium perfringens spores by alcohols". *Applied and Environmental Microbiology,* 50(2), 249-256.

Dalton, C., A.D. Goater, J.P.H. Burt and H.V. Smith. (2004). "Analysis of parasites by electrorotation". *Journal of Applied Microbiology,* 96(1), 24.

Dagher, F. (2006). "BIOXY S, solid preacetic acid- sanitizer". Atomes Company, Unpublished Report.

DeFlaun, M. F. and C. W. Condee. (1997). Electrokinetic transport of bacteria". *Journal of Hazardous Materials,* 263-278.

De'portes, I., J.-L. Benoit-Guyod, D. Zmirou and M.-C. Bouvier. (1998). "Microbial disinfection capacity of municipal solid waste (MSW) composting". *Journal of Applied Microbiology,* 85(2), 238-246.

Dixon, J.B. and S.B. Weed. (1989). *Minerals in soil environment.* Soil Science Society of America, Madison. WI.

Doyle, E. (2002). "Survival and growth of Closrtidium perfringens during the cooling step of thermal processing of meat products". http://www.meatami.com/Template.cfm?Section=OtherPathogens&CONTENTID=2290&TEMPLATE=/ContentManagement/ContentDisplay.cfm (May 26, 2006).

Drees, K. P., M. Abbaszadegan and R. M. Maier. (2003). "Comparative electrochemical inactivation of bacteria and bactriophage". *Water Research,* 37, 2291-2300.

Duncan, C. L. and D. H. Strong (1968). "Improved medium for sporulation of Clostridium perfringens". *Applied Microbiology,* 16(1), 82-89.

Elektorowicz, M. (1995). "Technical requirement related to the electorokinetic removal of contaminants from soil." ASCE/CSE Joint Conference on Enviromental Engineering, Pittsburg, USA.

Elektorowicz, M., E. Safaei, J. Oleszkiewicz and R. Reimers. (2007). "Electrokinetic remediation of biosolids through inactivation of Clostridium perfringens spores". *proceedings of the Sixth Symposium and Status Report on Electrokinetic Remediation* EREM.Virgo, Spain.

Elektorowicz, M., J. Huang and J. Oleszkiwicz. (2007a). "Study of aerobic and anaerobic sludge disinfection using the Electrokinetic method". 42nd CENTRAL Canadian Symposium on Water Quality Research Canada.

Environment Canada. (2000). "CH2MHill Canada. Biosolids Generation, Treatment, Use and Disposal in Canada. Opportunities for Energy Recovery and Greenhouse Gas Reduction Report".

(EDQ) Environnement de Quebec- Direction du milieu rural. (2004). "Guidelines for the beneficial use of fertilizing residuals". Envirodoq ENV/2004/0020.

EnviroTools. (2004). "Electrokinetic Remediation of Metals". (11/10, 2006) http://www.envirotools.org/factsheets/Remediation/Electrokinetics_Remediation.doc.

Epstein, E. (2003). *Land application of sewage sludge and biosolids.* Lewis, Boca Raton.

Eriksen, L., P. Andreasen and B. Ilsoe (1996). "Inactivation of Ascaris suum eggs during storage in lime treated sewage sludge". *Water Research,* 30(4), 1026-1027-1029.

Esmaeily, A. (2002). "Dewatering, metal removal, pathogen elimination and organic matter reduction in biosolids using Electrokinetic phenomena". MASc thesis, Concordia University, Montreal, Canada. 1-103.

Esmaeily A., M. Elektorowicz, S. Habibi, and J. Oleszkiewicz. (2006). "Dewatering and coliform inactivation in biosolids using electrokinetic phenomena". *Journal of Environmental Engineering and Science,* 5(3), 197-202.

FDA/Center for Food Safety & Applied Nutrition. (2005)".Foodborne Pathogenic Microorganisms and Natural Toxins Handbook". http://vm.cfsan.fda.gov/%7Emow/chap11.html (May 26, 2006).

Flores-Rodriguez, N. and G. H. Markx. (2006). "Flow-through devices for the ac electrokinetic construction of microstrued materials". *J. Micromech. Microeng,* 16, 349-355.

Fujioka, R. S. and L. K. Shizumura. (1985). "Clostridium perfringens, a reliable indicator of stream water quality". *J. Water Pollut. Control Fed.,* 57 986-992.

Gerba C.P., L. Pepper and L.F. Whitehead. (2002). "A risk assessment of emerging pathogens of concern in the land application of biosolids". *Water Science and Technology,* 46(10), 225-230.

GIUES. (2007)Clostridium perfringens. www.giuseppesansone.it/global/disinfez.htm.

Guzev V.S. and D.G. Zviagintsev.(1973). "Electrokinetic properties of the cells of microorganisms and their taxonomy". *Mikrobiologia,* 42(4), 708-712.

HACH.(2007). *Water Analysis Handbook Procedures.* 5th Edition. HACH Company. Colorado. USA

Hakimpour, M. (2001). "Development of a hybride electrokinetic system for the simultaneous removal of heavy metals and PAHs from clayey soil". M.A.Sc. thesis, Concordia University, Montréal, Canada.

Hall, J.E. and A. Zmyslowska. (1997). "Development of sludge treatment and disposal strategies for large conurbations: case study -Warsaw". *Water Science and Technology,* 36(11), 291-298.

Harman, E. (2003). "The thesis and the book- A guide for first-time academic authors." 104.

Harpold, D. J., B. L. Wasilauskas and M. L. O'Conner. (1985). "Rapid identification of Clostridium species by high-pressure liquid chromatography". *American Society for Microbiology,* 22(6), 962-967.

Hayes, C. S., B. Illades-Aguiar, L. Casillas-Martinez and P. Setlow. (1998). "In vitro and in vivo oxidation of methionine residues in small, acid-soluble spore protein from Bacillus species. *J Bacteriol*, 180, 2694-2700.

Heritage, J. (2006)."Medical Microbiology - A brief introduction". http://www.bmb.leeds.ac.uk/mbiology/ug/ugteach/dental/tutorials/introduction/bacteria.html.

Hester, R. E. and R. M. Harrison. (2001). *Food safety and food quality, Issues in environmental science and technology,* (15). RS.C Publishing. UK. 166 pp.

Hill, R. T., I. T. Knight, M. S. Anikis and R. R. Colwell. (1993). "Benthic distribution of sewage sludge indicated by Clostridium perfringens at a deep-ocean site". *Applied and Environmental Microbiology,* 59(1), 47-51.

Hiemenz, P. and C.-R. Raj. (1997). *Principles of Colloid and Surface Chemistry (Principles of Colloid and Surface Chemistry).* 3rd edition: Marcel Dekker, 650 pp.

Hodko D. , G. D. Hitchens, T. D. Rogers, J. W. Magnuson, and J. K. Dillon. "Engineered bioremediation of contaminated Soil". http://www.nasatech.com/Briefs/July01/KSC12045.html (May, 16, 2006).

Hook, D., B Jalaludin and G. Fitzsimmons. (1996). "*Clostridium perfringens* food-borne outbreak: an epidemiological investigation". *Australian and New Zealand Journal of Public Health,* 20(2), 119-122.

HPA (Health Protection Agency). (2005a). "Enumeration of Clostridium perfringens by membrane filtration". *Rep. No. W5i3.1,* Standards Unit, Evaluations and Standards Laboratory on behalf of the Regional Food, Water and Environmental Microbiologists Forum, UK.

HPA (Health Protection Agency). (2005). "Lactose gelatin medium". *Rep. No. MSOP 36i3.1,* Standards Unit, Evaluations and Standards Laboratory, UK.

Huang J., M. Elektorowicz, and J. Oleszkiwicz. (2007). "Dewatering and disinfection aerobic and anaerobic sludge using an electrokinetic (EK) system". IWA SPECIALIST CONFRENCE. Moncton, Canada.

Hurwitz, A. (2006). "What are Taguchi designs?" *Engineering statistics handbook,* Paul Tobias and Ledi Trutna, ed., NIST/SEMATECH.

Ibanez, J. G. (2004). "Electrochemistry Encyclopedia". http://electrochem.cwru.edu/ed/encycl/art-e02-environm.htm (May/9th, 2006).

ICR Microbial Laboratory Manual. (1996). EPA/600/R-95/178. Office of Research and Development. Washington DC.

Kalinowski, D.P., L. E. Edsberg, R. A. Hewson, R. H. Johnson and M. S. Brogan. (2004). "Low-Voltage direct current as a fungicidal agent for treating Onychomycosis". *Journal of the American Podiatric Medical Association,* 94(6), 555-572.

Karathanasis, A. D. (2006). "Comments on:Transport of biosolids in waste-amended soils". *Journal of Environmental Quality,* 35, 1-2.

Keh, H. J., W. C. Chen. (2006)."Sedimentation velocity and potential in concentrated suspensions of charged porous spheres". *Journal of Colloid and Interface Science*, 296(2), 710-720.

Kim, J.-O. , J.-T. Junga, I.-T. Yeomb, and G.- H. Aohc. (2007). "Electric fields treatment for the reduction of membrane fouling, the inactivation of bacteria and the enhancement of particle coagulation". *Desalination,* 202(1-3), 31-37.

Kim S.O., S.H. Moon, K.W. Kim and S.T. Yun. (2002). "Pilot scale on the ex-situ electrokinetic removal of heavy metals from municipal wastewater sludges". *Wat. Res,* 36, 4765-4774.

Kiremidjian, L. and G. Stotzky (1973). "Effects of natural microbial preparations on the electrokinetic potential of bacterial cells and clay minerals". *Applied Microbiology,* 25(6), 964-971.

Klobutcher, L. A., K. Ragkousi and P. Setlow. (2006). "The Bacillus subtilis spore coat provides "eat resistance" during phagocytic predation by the protozoan Tetrahymena thermophila". *Proc Nati Acad Sci U S A*, 3(103), 165-170.

Kunkle, A. C. (1974). "Electrokinetic separation of solid particles from aqueous suspension thereof". (USpatent 4,003,811).

Lageman, R. and W. Pool (2001). "Thirteen years eletro-reclamation in The Netherlands". *proceedings of the Third Symposium and Status Report on Electrokinetic Remediation—EREM 2001* Karlsruhe. 1, 1–17, April 18–20 (2001).

Labbe, R. G. (1990). "Isolation and characterization of Clostridium perfringens spores-lytic enzymes". DTIC. http://stinet.dtic.mil/.

Lear, G., M. J. Harbottle, C. J. van der Gast, S. A. Jackman, C. J. Knowles, G. Sills and I. P. Thompson. (2004). "The effect of electrokinetics on soil microbial communities". *Soil Biology and Biochemistry,* 36, 1751-1760.

LeBalnc, T. R. (2007). "Regulation biosolids and biosolids management in Canada". IWA SPECIALIST CONFRENCE. Moncton, Canada.

Leland, M. V. and G. M. Zang. (1997). "Electrokinetic soil remediation: impact of aqueous phase properties on soil surface charge and electroosmosis efficincy." US Environmental Protection Agency Risk Reduction Engineering Laboratory. REP. 36895. 202-206.

Levinson, H.S. and F.E. Feeherry. (1978). "Kinetics of heat activation of spores of Clostridium perfringens." *Spores VII,* G. Chambliss and J.C Vary, ed., American Society for Microbiology, Washington, 34-40 pp.

Lewis, A. (2006). "Regulatory framework for biosolids management in Canada". Greater Vancouver Regional District. Burnaby, BC, Canada.

Luo, Q., H. Wang, X. Zhang and Y. Qian. (2005a). "Effect of direct electrical current on the cell surface properties of phenol-degrading bacteria". *Applied and Environmental Microbiology,* 71(1), 423-427.

Luo, Q., H. Wang, X. Zhang and Y. Qian. (2005). "The use of non-uniform electrokinetics to enhance in situ bioremediation of phenol-contaminated soil". *Journal of Hazardous Materials B121,* 187-194.

Lytle, D.A., Johnson C.H., Rice E.W. (2002). "A systematic comparison of the electrokinetic properties of environmentally important microorganisms in water". *Colloids and Surfaces B: Biointerfaces,* 24(2), 91-101.

Mackes, M. C. and E.R. Rhodes. (2004). "Evaluation of bacteriological indicators of disinfection for alkaline treated biosolids". *J. Environ. Eng. Sci.,* 3, 231-236.

Manalang, P. (1927). "Significant drop in vitality after 5 minutes at 55 C." *Philip. J. Sci,* 33 249-257.

Manokararajah, K. (2003). "Electrokinetic remediation of a nitrate-contaminated soil". PhD thesis, University of Manitoba, Winnipeg.

Mansouri, A., S. Bhattacharjee, D.Y. Kwok and L.W. Kostiuk. (2005). "Evolution of streaming potential in a finite length microchannel". *Nanotech,* 1, 744-747.

McClane, B.A. (1997). *Clostridium perfringens. In Food Microbiology: fundamentals and frontiers.* ASM Press, Washington, D.C., USA.

McLeod, B. R., A. R. Liboff and S. D. Smith. (1992). "Biological systems in transition: Sensitivity to extremely low frequency field". *Electro Magnetbiology*, 11, 29-42.

McNamara, A. M. and C. P. Lattuada. (1998). Examination of meat and poultry products for Clostridium prefringens. *USDA/FSIS Microbiology Laboratory Guidebook*. 3rd Edition.

McGinley, S. (1998). "Clostridium perfringens *"New ways to type strains of a deadly bacteria"*. http://ag.arizona.edu/pubs/general/resrpt1998/clostridium.html (May 20, 2006).

Merlo, R.P., R. S. Trussell, W. S. Hermanowicz and D. Jenkins. (2007) "Sludge properties of the submerged membrane bioreactor and the activated sludge process". http://www.trusselltech.com/media/11.pdf.

Meyer, M. and J. L. Tholozan. (1999). "A new growth and in vitro sporulation medium for Clostridium perfrigens". *Letters in Applied Microbiology,* 28, 98-102.

Mitchell, J.K. (1993). *Fundamentals of soil behaviour.2.* John Wiley & Sons Inc., New York.

Mitchell, J.K. (1991). "Conducted phenomena: from theory to geotechnical practice". *Geotechnique,* 41(3), 299-340.

(MICB) Microbiologybytes. (2007). "Reoviruses". http://www.microbiologybytes.com/virology/Reoviruses.html (08/17, 2007).

Moore, R.S., H. D. Taylor, M. M. Reddy and L. S. Sturman. (1982). "Adsorption of reovirus by minerals and soils". *Applied and Environmental Engineering,* 44(4), 852-859.

Murrel, W.G. (1989). *Clostridium perfringens in food-borne micro-organisms of public health significance.* J.M. Executive Printing Service Pty Ltd. http://www.nzfsa.govt.nz/science/data-sheets/clostridium-perfringens.pdf

Murtsovkin, V. A. (1995). "Role of electrokinetic phenomena in bacterial motion." *Colloid Journal of the Russian Academy of Sciences,* 57(3), 346-353.

Nason, E. L., J. D. Wetzel, S. K. Mukherjee, E. S. Barton, B. V. V. Prasad and T. Dermody. (2001). "A monoclonal antibody specific for reovirus outer-capsid protein σ3 inhibits σ1-mediated hemagglutination by steric hindrance". Journal of Virology, 75(14), 6625-6634.

(NGSMI) National guide to sustainable municipal infrastructure. (2003). "Biosolids Management Programs". *Rep. No. 1.0,* NRC-CNRC, Canada.

NSEL (Nova Scotia Environment and Labor (. (2004). Guidelines for land application and storage of biosolids in Nova Scotia.

NMSU, New Mexico State University Chem 435. (2007). "Electric Double Layer". http://www.chemistry.nmsu.edu/studntres/chem435/Lab14/double_layer.html (17/08, 2007).

Oleszkiewicz, J.A. and D.S. Mavinic. (2002). "Wastewater biosolids: an overview of processing, treatment, and management." *J. Environ. Eng. Sci.,* 1 75-88.

Olland, A. M., J. J. Jané-Valbuena, L. A. Schiff, M. L. Nibert and S. C. Harrison. (2001). "Structure of the reovirus outer capsid and dsRNA-binding protein σ3 at 1.8 Å resolution". *European Molecular Biology Organization,* 20(5), 979-989.

Paustian, T. (2007). "Inclusions and other internal structures". http://lecturer.ukdw.ac.id/dhira/BacterialStructure/Inclusions.html 2007).

Payment, P., M. Trudel and R. Plante. (1985). "Elimination of viruses and indicator bacteria at each steps of treatment during preparation of drinking water at seven water treatment plants". *Appl Environ Microbiol,* 49, 1418-1428.

Payment, P. and E. Franco. (1993). "Clostridium perfringens and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts". *Applied and Environmental Microbiology,* 59(8), 2418-2424.

PDEP. (2006). Pennsylvania Department of Environmental Protection. http://www.dep.state.pa.us/dep/biosolids/training/sewagesludge/menu.htm (08/17, 2007).

Pennathur, S. and J.G. Santiago (2006). "Electrokinetic transport in nanochannels. 2. Experiments". *Anal. Chem.,* 1, 78(3), 972.

Plym-Forshell, L. (1995). "Survival of salmonellas and Ascaris suum eggs in a thermophilic biogas plant". *Acta Vet Scand.,* 36(1), 79-85.

Podol'skaya, V. I., L. N. Yakubenko and Z. R. Ul'berg. (2001). "Electrokinetic and transmembrane potentials as factors of cell adaptation to cyanide compounds". *Colloid Journal,* 63(4), 498-504.

Ponugoti, P. R., M. F. Dahab, and R. Surampalli. (1997). "Effects of different biosolids treatment systems on pathogen and pathogen indicator reduction". *Water Environment Research,* 69(7), 1195-1206.

Qin, B., Q. Zhang, Swanson B. G. and Pedrow P.D. (1994). "Inactivation of microorganisms by pulsed electric fields of different voltage waveforms". *Transaction in Dielectric and Electrical Indurstries,* 1(6), 1047-1057.

Raihan, H. M. (2006). "Application of electrokinetics to control metal transports in biosolids in a cold climate". MASc thesis, Concordia University, Montreal, Canada. 1-156.

Raju, D., P. Setlow and M.R. Sarker. (2007). "Antisense RNA-Mediated decreased synthesis of small, acid-soluble spore proteins leads to decreased resistance of *Clostridium perfringens* spores to moist heat and UV radiation". *Appl. Environ. Microbiol, Doi 10.1128/AEM.02500-06.*

Raju, D., M. Waters, P. Setlow and M. R. Sarker. (2006). "Investigating the role of small, acid-soluble spore proteins (SASPs) in the resistance of *Clostridium perfringens* spores to heat". BMC Microbiol, 6:50.

Reimers, R.S., J. A. Oleszkiewicz, D. B. Dwight and E. L. Faulmann. (2002). "PEC application for the national classification of the J-VAp process to the category of PFRP". Unpublished report.

Reimers, R. S., M.D.Little, and A.Lopez and K.K.Mbela. (1991). "Final testing of the synox municipal sludge treatment for PFRP approval—Phase II. Report to Synox Corporation". Bethesda, MD, by School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA. Unpublished report.

Reimers, R. S., F.Mussari, W. Schmitz. (2005) "Sludge treatment process." (USPatent 20050279706).

Rittirong, A., J. Q. Shang, M. A. Ismail and M. F. Randolph. "Effect of electric field intensity on electro-cementation of caissons in calcareous sand". *2007,* 17(1), 74-79.

Rhodehamel, J. E. and H. M. Stanley. (2001). "Bacteriological Analytical Manual Online". http://www.cfsan.fda.gov/~ebam/bam-16.html (May 11, 2006).

Roberts, T.A., A.C. Baird-Parker and R.B. Tompkin. (1996). *Micro-organisms in Food 5.* Blackie Academic & Professional, London.

Roy, R. K. (2001). *Design of experiments using the Taguchi approach: 16 steps to product and process improvement.* Wiley, New York.

Saveyn, H. (2006). "In situ determination of solidosity profiles during activated sludge electrodewatering". *Water Res.,* 40, 2135-2142.

Schmidt, C.A., M. C. Barbosab and M. S. de Almeida. (2007). "A laboratory feasibility study on electrokinetic injection of nutrients on an organic, tropical, clayey soil". *Journal of Hazardous Materials,* 143(3), 655-661.

Schepart, B. S. (1995). *Bioremediation of pollutants in soil and water.* ASTM. Philadelphia. USA.

Schramm, L. (2007). "Colloids."*Kirk-Othmer Encyclopedia of Chemical Technology".* John Wiley & Sons.

Schut, L. (2005). "Sewage Biosolids - managing urban nutrients responsibly for crop production". http://www.omafra.gov.on.ca/english/nm/nasm/info/brochure.htm (July 23, 2006).

Schultz, D. S. (1997). "Electroosmosis technology for soil remediation: laboratory results, field trial and economic modeling, Electrochemical Decontamination of Soil and Water". Special Issue of *Journal of Hazardous Material*, 55(1–3), 81–92.

Setlow, B., D. Sun and P. Setlow. (1992). "Interaction between DNA and alpha/beta-type small, acid-soluble spore proteins: a new class of DNA-binding protein". *J Bacteriol.,* 174(7), 2312-2322.

Sidhu, J., R.A. Gibbs, G. E. Ho, and I. Unkovich. (2001). "The role of indigenous microorganisms in suppression of salmonella regrowth in composted biosolids". *Water Research,* 35(4), 913-920.

Skanavis, C. and W.A. Yanko. (2001). "Clostridium perfringes as a potential indicator for the presence of sewage solids in marine sediments". *Marin Pollut. Bull.,* 42(1), 31-35.

Smith, S., V. Juneja and D. W. Schaffner. (2004). "Influence of survival methodological factors on the growth of Clostridium perfrigens in cooling rate challenge studies". *Journal of Food Protection,* 67(6), 1128-1132.

Smith, G. L., B. J. Murphy and M. Law. (2003). "Vaccinia virus motility". *Annu Rev Microbiol,* 57, 323-342.

Shing, M. and K. M. Coombs. (1996). "Assembly of the reovirus outer capsid requires μ1/σ3 interactions which are prevented by misfolded σ3 proteins in reovirus temperature-sensitive mutant tsG453". *Virus Res.*, 46, 19–29.

Shugar, G. J. and J. T. Ballinger. (1981). "Chemical Technicians' Ready Reference Handbook". McGrow-Hill. 1500.

Strauch, D. (1991). "Survival of pathogenic micro-organisms and parasites in excreta, manure and sewage sludge". *Rev. Sci. Tech.,* 10(3), 813-846.

Summer, M. E. and W.P. Miller. (1996). "Cation exchange capacity and exchange coefficients". *Methods of soil analysis Part 3. Chemical methods,* Sparks D.L., ed., Soil Science Society of America, Madison WI, 1201-1229.

Tenenbaum, D. (1997). "The beauty of biosolids". *Environmental Health,* 105(1).

Toze, S. (2004). "Reuse of effluent water- benefits and risks". Proceeding of 4th international Corp Science Congress. http://www.cropscience.org.au/icsc2004/pdf/2086_toze.pdf (May, 6, 2006).

Theil, K. W. and C. M. McCloskey. (1995). "Rotavirus shedding in feces of gnotobiotic calves orally inoculated with a commercial rotavirus-coronavirus vaccine". *J Vet Diagn Invest*, 7, 427-432.

Tsvid, E.E., I. I. Shkop, I. N. Pozmogova and E.M. Shul'govskaia. (1981). "Electrokinetic properties of microorganisms as dependent on culture conditions". *Mikrobiologiia,* 50(4), 659-664.

(UIU) University of Illinois at urban-Champaign. "Theoretical and computational biophysics group". http://www.ks.uiuc.edu/Highlights/index.cgi?section=2007.

United States Environmental Protection Agency (Office of Research and Development). (1996). "ICR Microbial Laboratory Manual". *Rep. No. EPA/600/R-95/178.*

US EPA. (1999). "Biosolids Generation, Use, and Disposal in the United States." *Rep. No. EPA530-R-99-009,* U.S. Environmental Protection Agency.

US EPA. (1995). "A guide to the biosolids risk assessments for the EPA Part 503 Rule". EPA832-B-93-005 50.

Van Ham, M. D., M. A. Teshima and P. R. Plouffe. (2007). "Source management initiatives and factors influencing biosolids quality". IWA SPECIALIST CONFRENCE. Moncton, Canada.

Venczel, L. V., M. Arrowood, M. Hurd and M. D. Sobsey. (1997). "Inactivation of Cryptosporidium pavum Oocysts and Clostridium perfringens spores by a mixed-oxidant disinfectant and by free chlorine". *Applied and Environmental Microbiology,* 63(4), 1598-1601.

Veitch, M., C. Ferreira, K. Densten, S. Heaton and J. Griffith. (1996). "Catering on the move-investigating a typical outbreak of gastroenteritis". *Communicable Diseases Intelligence,* 20(12), 279-282.

Virkutyte, J., M. Sillanpaa and P. Latostenmaa. (2002). "Electrokinetic soil remediation–critical overview". *Science of the Total Environment,* 289(1-3), 97-171.

Virkutyte, J., M. Sillanpaa, P. Latostenmaa and J. Martissius. (2004). "Electrode layout and process kinetics of electroremoval of copper from sand". *International Journal of Surface Mining, Reclamation and Environment,* 18(3), 220-231.

Wang, J. and J. Wang. (2007). "Application of radiation technology to sewage sludge processing: A review". *Journal of Hazardous Materials,* 143(1-2), 2-7.

Wang, J.Y. (2004). "Processing dewatered sewage sludge using electrokinetic technology". *Water Sci. Technol.,* 50(9), 205-211.

Warnes, S. I. and C. W. Keevil. (2004). "Desk studies on feasibility of horizontal standard rapid methods for detection of Clostridium perfrigens and entrococci in Sludge, Soil improvers, Growing Media and Biowastes". *Rep. No. Horizontal-4.* *http://www.ecn.nl/docs/society/horizontal/hor4_enter_clos.pdf*

Wise, D. L., D. J. Trantolo, E. J. Cichon. H. I. Inyang and U. Stottmeister. (2000). *Remediation Engineering of Contaminated Soils*. CRC Press. New York. 1008.

Wright, J. (2006) "Biosolid Recycling and Food Safety Issues". http://www.rsc.org/images/Is015003_tcm18-27605.pdf.

Yang, L. G. Nakhla and A. Bassi. (2005). "Electro-kinetic dewatering of oily sludges". *Journal of Hazardous Materials*, 125(1-3), 130-140.

Yeung, A.T. (1994). "Electrokinetic flow processes in porous media and their applications Advances in porous media". *Advances in porous media,* Elsevier, The Netherlands, 309-395.

Yuana, C. and C.H. Weng. (2006). "Electrokinetic enhancement removal of heavy metals from industrial wastewater sludge". *Chemosphere,* 65(1), 88-96.

Zhou, D.-M., C.-F. Deng. L. Cang and A. N. Alshawabkeh. (2005). "Electrokinetic remediation of a Cu–Zn contaminated red soil by controlling the voltage and conditioning catholyte pH". *Chemosphere*, 61(4), 519-527.

VII-APPENDIXES

Appendix A

Table A-1: The pH and ORP values for the reactors with voltage gradient of 0.5 V/cm

EK reactor trail number	Code	pH	ORP (mV)
1	Catholyte	10.67	-220.80
	C	7.22	-35.60
	MC	7.07	-27.30
	MA	6.72	-7.70
	A	6.41	8.80
	Anolyte	4.58	108.90
4	Catholyte	9.79	-186.50
	C	9.21	-153.90
	MC	8.63	-120.60
	MA	8.40	-107.50
	A	6.08	27.50
	Anolyte	6.07	27.70
5	Catholyte	10.48	-216.30
	C	9.03	-140.00
	MC	8.99	-136.00
	MA	8.86	-123.80
	A	8.64	-120.50
	Anolyte	5.94	33.70
8	Catholyte	10.43	-222.90
	C	9.34	-160.50
	MC	8.97	-140.00
	MA	7.16	-37.30
	A	5.29	74.20
	Anolyte	7.71	7.71
9	Catholyte	10.80	-243.00
	C	9.44	-166.70
	MC	8.54	-115.00
	MA	8.49	-112.30
	A	6.77	-14.00
	Anolyte	6.47	3.20
12	Catholyte	10.38	-219.40
	C	9.38	-163.20
	MC	8.86	-133.00
	MA	8.69	-123.80
	A	3.92	155.80
	Anolyte	2.46	242.50

Continue .....

.....Table A-1 Continued

EK reactor trail number	Code	pH	ORP (mV)
13	Catholyte	10.54	-223.90
	C	9.38	-158.20
	MC	8.80	-124.90
	MA	8.45	-104.80
	A	4.65	108.50
	Anolyte	2.44	230.50
16	Catholyte	9.30	-156.70
	C	9.24	-153.90
	MC	8.06	-86.30
	MA	8.11	89.30
	A	8.19	-79.40
	Anolyte	7.37	-40.00

Table A-2: The pH and ORP values for the reactors with voltage gradient of 0.7 V/cm

EK reactor trail number	Code	pH	ORP (mV)
2	Catholyte	10.40	-211.20
	C	9.56	-167.30
	MC	8.70	-118.30
	MA	8.51	-107.90
	A	6.59	-2.80
	Anolyte	6.00	28.70
6	Catholyte	10.44	-213.20
	C	9.54	-159.40
	MC	8.80	-123.70
	MA	8.67	-116.60
	A	2.17	239.40
	Anolyte	6.37	9.50
10	Catholyte	10.07	-195.70
	C	9.47	-162.20
	MC	8.50	-108.50
	MA	8.1	-86.60
	A	4.92	92.30
	Anolyte	4.16	133.40
14	Catholyte	10.28	-207.20
	C	9.56	-167.30
	MC	7.66	-61.30
	MA	6.53	1.80
	A	4.96	90.30
	Anolyte	3.89	149.70

Table A-3: The pH and ORP values for the reactors with voltage gradient of 1.5 V/cm

EK reactor trail number	Code	pH	ORP (mV)
3	Catholyte	10.97	-241.20
	C	9.40	-159.10
	MC	8.90	-129.60
	MA	8.75	-120.40
	A	3.10	197.80
	Anolyte	1.86	266.90
7	Catholyte	10.26	-209.40
	C	9.54	-166.80
	MC	9.00	-135.00
	MA	8.83	-124.80
	A	7.31	-35.00
	Anolyte	8.78	-121.50
11	Catholyte	9.80	-182.70
	C	9.23	-148.60
	MC	8.25	-91.10
	MA	7.91	-71.30
	A	5.67	56.60
	Anolyte	7.64	-54.80
15	Catholyte	10.35	-215.00
	C	9.04	-137.70
	MC	8.61	-112.20
	MA	8.07	-80.50
	A	7.71	-59.50
	Anolyte	5.88	44.70

Table A-4: Nitrogen-ammonium, nitrite and nitrate values for the reactors with voltage gradient of 0.5 V/cm

EK reactor trail number	Code	Unit	Nitrogen-Ammonia	Nitrite	Nitrate
1	C	mg/ g	7.37	0.06	0.02
	MC	mg/ g	0.07	0.13	<0.01
	MA	mg/ g	1.73	0.03	0.02
	A	mg/ g	4.69	0	0
	G	mg/ g	1.43	0.06	<0.01
	Catholyte	mg/ L	1190	0.1	0.01
	Anolyte	mg/ L	170	0.51	<0.01
4	C	mg/ g	2.68	0.03	6.50
	MC	mg/ g	7.46	0.03	5.00
	MA	mg/ g	18.19	0.01	2.62
	A	mg/ g	2.00	<0.01	<0.01
	G	mg/ g	5.19	0.01	0.53
	Catholyte	mg/ L	1090	1.70	0.1
	Anolyte	mg/ L	1430	0.44	0.1
5	C	mg/ g	21.06	<0.01	<0.01
	MC	mg/ g	210.25	<0.01	<0.01
	MA	mg/ g	46.77	0.20	436.5
	A	mg/ g	12.73	0.01	0.01
	G	mg/ g	40.30	0.02	0.06
	Catholyte	mg/ L	1300	0.11	0.1
	Anolyte	mg/ L	NL*	NL	NL
8	C	mg/ g	24.40	0.01	<0.01
	MC	mg/ g	26.66	<0.01	0.02
	MA	mg/ g	2.89	<0.01	0.1
	A	mg/ g	93.36	0.02	<0.01
	G	mg/ g	20.47	0.01	0.02
	Catholyte	mg/ L	1410	0.1	0.3
	Anolyte	mg/ L	NL	NL	NL
9	C	mg/ g	42.13	0.03	<0.01
	MC	mg/ g	21.86	0.01	<0.01
	MA	mg/ g	29.33	0.03	0.01
	A	mg/ g	5.36	0.01	0.01
	G	mg/ g	19.51	0.02	<0.01
	Catholyte	mg/ L	1540	0.23	0.8
	Anolyte	mg/ L	2100	1.00	0.2

Continue .....

.....Table A-4 Continued

EK reactor trail number	Code	Unit	Nitrogen-Ammonia	Nitrite	Nitrate
12	C	mg/ g	27.22	<0.01	<0.01
	MC	mg/ g	18.50	0.01	<0.01
	MA	mg/ g	80.84	<0.01	<0.01
	A	mg/ g	234.97	0.31	0.22
	G	mg/ g	55.62	0.02	0.01
	Catholyte	mg/ L	4900	<0.01	<0.01
	Anolyte	mg/ L	1400	<0.01	0.1
13	C	mg/ g	11.12	<0.01	<0.01
	MC	mg/ g	20.30	<0.01	0.63
	MA	mg/ g	15.57	<0.01	<0.01
	A	mg/ g	86.63	<0.01	<0.01
	G	mg/ g	23.50	<0.01	0.63
	Catholyte	mg/ L	3600	0.1	0.1
	Anolyte	mg/ L	1500	0.01	0.2
16	C	mg/ g	65.78	0.04	0.02
	MC	mg/ g	35.65	0.01	1.50
	MA	mg/ g	35.05	0.01	<0.01
	A	mg/ g	55.50	0.01	<0.01
	G	mg/ g	46.22	0.02	0.06
	Catholyte	mg/ L	1800	0.1	0.3
	Anolyte	mg/ L	1400	0.35	0.4

* NL = Not enough sample

Table A-5: Nitrogen-ammonium, nitrite and nitrate values for the reactors with voltage gradient of 0.7 V/cm

EK reactor trail number	Code	Unit	Nitrogen-Ammonia	Nitrite	Nitrate
2	C	mg/ g	0.58	<0.01	<0.01
	MC	mg/ g	3.82	<0.01	<0.01
	MA	mg/ g	13	<0.01	<0.01
	A	mg/ g	7.15	<0.01	<0.01
	G	mg/ g	3.80	<0.01	<0.01
	Catholyte	mg/ L	3100	<0.01	<0.01
	Anolyte	mg/ L	630	<0.01	<0.01
6	C	mg/ g	21.10	<0.01	<0.01
	MC	mg/ g	55.74	0.02	<0.01
	MA	mg/ g	55.60	0.02	<0.01
	A	mg/ g	2.51	0.01	<0.01
	G	mg/ g	20.12	0.01	<0.01
	Catholyte	mg/ L	12,000	0.22	<0.01
	Anolyte	mg/ L	1660	0.66	<0.01
10	C	mg/ g	22.22	0.04	0.01
	MC	mg/ g	15.56	<0.01	<0.01
	MA	mg/ g	15.40	0.44	0.02
	A	mg/ g	1.27	<0.01	0.01
	G	mg/ g	9.06	0.01	0.01
	Catholyte	mg/ L	510	0.06	0.30
	Anolyte	mg/ L	270	0.01	0.40
14	C	mg/ g	20.40	0.01	0.01
	MC	mg/ g	82.32	<0.01	<0.01
	MA	mg/ g	27.42	<0.01	<0.01
	A	mg/ g	2.18	<0.01	<0.01
	G	mg/ g	17.79	0.01	<0.01
	Catholyte	mg/ L	1970	0.2	30
	Anolyte	mg/ L	590	0.01	<0.01

Table A-6: Nitrogen-ammonium, nitrite and nitrate values for the reactors with voltage gradient of 1.5 V/cm

EK reactor trail number	Code	Unit	Nitrogen-Ammonia	Nitrite	Nitrate
3	C	mg/ g	32.80	0.01	<0.01
	MC	mg/ g	40.35	0.01	<0.01
	MA	mg/ g	33.80	0.01	<0.01
	A	mg/ g	99.03	0.3	<0.01
	G	mg/ g	45.82	0.03	<0.01
	Catholyte	mg/ L	6000	0.06	<0.01
	Anolyte	mg/ L	600	0.12	10.00
7	C	mg/ g	13.09	<0.01	<0.01
	MC	mg/ g	3.09	<0.01	<0.01
	MA	mg/ g	56.03	0.03	0.01
	A	mg/ g	2.18	0.01	<0.01
	G	mg/ g	8.89	0.01	<0.01
	Catholyte	mg/ L	4600	0.13	<0.01
	Anolyte	mg/ L	1500	0.19	0.1
11	C	mg/ g	20.11	0.01	0.01
	MC	mg/ g	4.06	<0.01	<0.01
	MA	mg/ g	16.86	0.01	0.01
	A	mg/ g	6.68	<0.01	0.01
	G	mg/ g	9.80	0.01	0.01
	Catholyte	mg/ L	980	0.08	0.2
	Anolyte	mg/ L	790	0.01	0.3
15	C	mg/ g	39.08	0.02	0.04
	MC	mg/ g	13.13	0.01	<0.01
	MA	mg/ g	22.59	1.29	<0.01
	A	mg/ g	20.34	0.13	0.02
	G	mg/ g	22.04	0.07	0.01
	Catholyte	mg/ L	950	0.65	0.4
	Anolyte	mg/ L	1320	0.8	0.4

Table A-7: Phosphate, sulfate and chloride values for the reactors with voltage gradient of 0.5 V/cm

EK reactor trail number	Code	Unit	Phosphate	Sulfate	Chloride
1	C	mg/ g	9.76	16.85	17.90
	MC	mg/ g	0.42	1.57	1.57
	MA	mg/ g	6.06	8.64	1.08
	A	mg/ g	2.95	23.90	2.81
	G	mg/ g	2.93	8.60	3.04
	Catholyte	mg/ L	27.70	140.00	70.00
	Anolyte	mg/ L	N	260	4300
4	C	mg/ g	6.49	1.14	2.68
	MC	mg/ g	4.99	3.17	7.45
	MA	mg/ g	2.62	7.08	4.59
	A	mg/ g	1.18	1.11	4.78
	G	mg/ g	3.16	2.31	4.58
	Catholyte	mg/ L	268	170	400
	Anolyte	mg/ L	110	40	350
5	C	mg/ g	2.95	6.27	9.90
	MC	mg/ g	3.93	0.04	0.62
	MA	mg/ g	50.2	15.59	74.83
	A	mg/ g	2.11	7.04	7.86
	G	mg/ g	5.93	2.31	7.76
	Catholyte	mg/ L	120	10	160
	Anolyte	mg/ L	NL	NL	NL
8	C	mg/ g	6.19	57.82	19.87
	MC	mg/ g	10.08	33.32	0.30
	MA	mg/ g	3.15	0.01>	2.46
	A	mg/ g	3.86	93.36	3.37
	G	mg/ g	5.26	56.45	2.66
	Catholyte	mg/ L	224	130	190
	Anolyte	mg/ L	NL	NL	NL
9	C	mg/ g	22.20	3.29	2.94
	MC	mg/ g	31.05	5.27	0.99
	MA	mg/ g	154.40	14.46	6.91
	A	mg/ g	6.60	3.93	1.19
	G	mg/ g	28.96	5.61	2.21
	Catholyte	mg/ L	1064	400	290
	Anolyte	mg/ L	5440	600	320

Continue .....

.....Table A-7 Continued

EK reactor trail number	Code	Unit	Nitrogen-Ammonia	Nitrite	Nitrate
12	C	mg/ g	10.81	22.07	1.03
	MC	mg/ g	163.71	84.12	1.85
	MA	mg/ g	189.97	440.93	8.45
	A	mg/ g	4272.57	4307.81	50.91
	G	mg/ g	194.72	243.72	5.35
	Catholyte	mg/ L	1670	18000	140
	Anolyte	mg/ L	9430	4000	220
13	C	mg/ g	8.28	107.39	0.92
	MC	mg/ g	44.64	13.52	1.14
	MA	mg/ g	119.35	233.65	1.94
	A	mg/ g	3733.86	1732.65	121.28
	G	mg/ g	113.30	155.73	3.97
	Catholyte	mg/ L	2580	19000	370
	Anolyte	mg/ L	10880	18000	320
16	C	mg/ g	166.17	400.40	7.15
	MC	mg/ g	75.80	26.26	3.37
	MA	mg/ g	75.46	18.45	4.06
	A	mg/ g	104.96	120.65	6.27
	G	mg/ g	99.94	69.65	4.98
	Catholyte	mg/ L	2864	390	243
	Anolyte	mg/ L	4830	22000	260

Table A-8: Phosphate, sulfate and chloride values for the reactors with voltage gradient of 0.7 V/cm

EK reactor trail number	Code	Unit	Phosphate	Sulfate	Chloride
2	C	mg/ g	15.69	0.26	0.58
	MC	mg/ g	91.15	6.66	3.82
	MA	mg/ g	270	3.88	16.19
	A	mg/ g	15.47	4.37	2.35
	G	mg/ g	49.45	2.33	3.04
	Catholyte	mg/ L	1128	30	130
	Anolyte	mg/ L	4270	640	159
6	C	mg/ g	20.02	2.02	2.29
	MC	mg/ g	130.48	91.34	2.42
	MA	mg/ g	147.59	35.81	2.4
	A	mg/ g	0.14	2.37	0.42
	G	mg/ g	15.32	11.19	1.54
	Catholyte	mg/ L	4710	1200	20
	Anolyte	mg/ L	9550	1200	20
10	C	mg/ g	4.26	7.11	27.20
	MC	mg/ g	1.49	8.16	4.34
	MA	mg/ g	0.76	96.10	4.69
	A	mg/ g	1.23	0.7	1.97
	G	mg/ g	1.57	7.92	5.75
	Catholyte	mg/ L	110.30	30	170
	Anolyte	mg/ L	21.50	40	4230
14	C	mg/ g	4.53	2.24	2.91
	MC	mg/ g	2.11	435.84	26.63
	MA	mg/ g	2.24	9.14	5.94
	A	mg/ g	1.98	45.70	9.63
	G	mg/ g	2.55	13.25	5.33
	Catholyte	mg/ L	324	200	120
	Anolyte	mg/ L	108	1100	6100

Table A-9: Phosphate, sulfate and chloride values for the reactors with voltage gradient of 1.5 V/cm

EK reactor trail number	Code	Unit	Phosphate	Sulfate	Chloride
3	C	mg/ g	42.22	63.42	11.32
	MC	mg/ g	87.43	47.08	4.7
	MA	mg/ g	57.19	54.08	2.70
	A	mg/ g	1511.8	2357.8	172
	G	mg/ g	133.67	139.69	12.55
	Catholyte	mg/ L	1830	120	120
	Anolyte	mg/ L	11140	10000	300
7	C	mg/ g	1.51	0.65	0.78
	MC	mg/ g	2.57	14.70	4.54
	MA	mg/ g	122.08	16.34	1.400
	A	mg/ g	0.03	5.45	1.64
	G	mg/ g	1.96	5.41	1.69
	Catholyte	mg/ L	3130	4300	140
	Anolyte	mg/ L	5050	400	21
11	C	mg/ g	3.23	4.12	5.57
	MC	mg/ g	0.65	0.54	4.06
	MA	mg/ g	1.79	2.21	3.59
	A	mg/ g	1.33	1.57	3.39
	G	mg/ g	1.50	1.67	4.08
	Catholyte	mg/ L	35	40	60
	Anolyte	mg/ L	42.60	60	1450
15	C	mg/ g	6.81	60.95	24.38
	MC	mg/ g	2.07	5.53	2.76
	MA	mg/ g	4.35	19.43	7.04
	A	mg/ g	4.94	23.76	4.49
	G	mg/ g	4.17	19.86	6.79
	Catholyte	mg/ L	150	400	200
	Anolyte	mg/ L	191	3000	130

Table A-10: Volatile fatty acids value for the reactors with voltage gradient of 0.5 V/cm

EK reactor trail number	Code	Unit	VFAs
1	C	mg/ g	16.85
	MC	mg/ g	1.57
	MA	mg/ g	23.76
	A	mg/ g	106
	G	mg/ g	16.10
	Catholyte	mg/ L	10
	Anolyte	mg/ L	3160
4	C	mg/ g	17.62
	MC	mg/ g	3.54
	MA	mg/ g	11.29
	A	mg/ g	10.67
	G	mg/ g	9.32
	Catholyte	mg/ L	380
	Anolyte	mg/ L	NL
5	C	mg/ g	6.27
	MC	mg/ g	4.37
	MA	mg/ g	143.43
	A	mg/ g	28.99
	G	mg/ g	18.38
	Catholyte	mg/ L	320
	Anolyte	mg/ L	NL
8	C	mg/ g	15.72
	MC	mg/ g	62.41
	MA	mg/ g	25.66
	A	mg/ g	38.38
	G	mg/ g	31.36
	Catholyte	mg/ L	930
	Anolyte	mg/ L	NL
9	C	mg/ g	10.05
	MC	mg/ g	4.17
	MA	mg/ g	1.26
	A	mg/ g	10.61
	G	mg/ g	4.86
	Catholyte	mg/ L	640
	Anolyte	mg/ L	970

Continue .....

.....Table A-10 Continued

EK reactor trail number	Code	Unit	VFAs
12	C	mg/ g	6.99
	MC	mg/ g	28.90
	MA	mg/ g	56.95
	A	mg/ g	79.81
	G	mg/ g	30.97
	Catholyte	mg/ L	430
	Anolyte	mg/ L	1730
13	C	mg/ g	1.65
	MC	mg/ g	0.95
	MA	mg/ g	4.67
	A	mg/ g	251.23
	G	mg/ g	6.54
	Catholyte	mg/ L	230
	Anolyte	mg/ L	1120
16	C	mg/ g	25.17
	MC	mg/ g	9.75
	MA	mg/ g	12.55
	A	mg/ g	68.04
	G	mg/ g	21.40
	Catholyte	mg/ L	170
	Anolyte	mg/ L	600

Table A-11: Volatile fatty acids value for the reactors with voltage gradient of 0.7 V/cm

EK reactor trail number	Code	Unit	VFAs
2	C	mg/ g	51.05
	MC	mg/ g	106.2
	MA	mg/ g	4.08
	A	mg/ g	7.40
	G	mg/ g	20.12
	Catholyte	mg/ L	270
	Anolyte	mg/ L	2860
6	C	mg/ g	3.84
	MC	mg/ g	1.30
	MA	mg/ g	16.77
	A	mg/ g	5.05
	G	mg/ g	4.54
	Catholyte	mg/ L	970
	Anolyte	mg/ L	310
10	C	mg/ g	72.23
	MC	mg/ g	80.65
	MA	mg/ g	3.39
	A	mg/ g	8.92
	G	mg/ g	20.49
	Catholyte	mg/ L	40
	Anolyte	mg/ L	620
14	C	mg/ g	6.28
	MC	mg/ g	3.87
	MA	mg/ g	4.57
	A	mg/ g	2.18
	G	mg/ g	3.00
	Catholyte	mg/ L	390
	Anolyte	mg/ L	1100

Table A-12: Volatile fatty acids value for the reactors with voltage gradient of 1.5 V/cm

EK reactor trail number	Code	Unit	VFAs
3	C	mg/ g	9.15
	MC	mg/ g	10.22
	MA	mg/ g	7.30
	A	mg/ g	235.78
	G	mg/ g	20.03
	Catholyte	mg/ L	470
	Anolyte	mg/ L	130
7	C	mg/ g	4.35
	MC	mg/ g	4.79
	MA	mg/ g	36.64
	A	mg/ g	2.18
	G	mg/ g	6.39
	Catholyte	mg/ L	130
	Anolyte	mg/ L	100
11	C	mg/ g	9.45
	MC	mg/ g	92.42
	MA	mg/ g	43.38
	A	mg/ g	82.76
	G	mg/ g	42.08
	Catholyte	mg/ L	1770
	Anolyte	mg/ L	1830
15	C	mg/ g	0.01>
	MC	mg/ g	9.26
	MA	mg/ g	8.50
	A	mg/ g	12.14
	G	mg/ g	9.85
	Catholyte	mg/ L	250
	Anolyte	mg/ L	NL

Table A-13: Resistance distribution in different days of test for each batch reactor

Rea.	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1	44.3	46.3	49.10	51.3	53.10	56.4	58.7	61.8	62.8	67.3	68.3	69.2	72.1	73.1	75.1	76.5	79.6	81.3
	2	54	53.1	54	54	67	57.3	58	59.3	59.8	60.9	62.5	67.3	68.2	69	69	69.9	71	72
	3	53	54.3	55.6	55.6	57.1	56	59.3	59.9	61.2	61.9	62.7	67.9	68.9	70	70.3	70.8	70.9	71
	4	54	55	56	57	61	58.10	59.6	60	60.90	61	62.9	68	69	75	76	79	80	83
	5	63	68	69.6	73.2	78	76.1	76.9	77.4	78.1	79.3	83.2	85.3	86.4	89.4	91.2	96.3	97.5	99
	6	78	79.6	80.2	83.6	88.3	84	86.7	89.4	91.2	92.6	96.5	99.6	100.3	102.5	109.3	110.3	115.3	118.3
	7	83	78	79.9	82	89.3	86.7	89.7	96.2	96.8	98	99.6	103.6	106.5	108.3	109.3	112.5	114.5	115.9
	8	96	98.3	99.6	102.3	112.3	106.3	108.3	109.7	110.2	114.3	116.5	118.6	119.3	119.9	121.3	125.3	126.9	130
	9	112.3	119.5	125.3	126.3	132.6	134	136.3	139.7	143.6	148.9	149.1	150.2	156.3	157.3	163.5	165	169.9	173
2	1	53.6	58.6	59.4	62.3	68	68.9	70.3	71.5	74.3	75.9	78.6	79.6	82.3	85.3	86.3	89.6	94.2	98
3	1	69	69.3	72.3	75.6	78.4	79.5	83.2	85.4	86.4	89	89.9	93.4	95.4	96.7	96.3	98.6	99.9	102
	2	74	79.9	81.3	89.3	92.4	95.6	97.5	98.6	99.6	102.3	105.3	106.8	108.6	110.3	115.3	119.3	120.8	132.5
	3	110	111.5	112.5	114.5	116.8	117.8	118.9	119.7	120.6	125.8	129.9	131.5	135.6	136.9	137.8	138.9	140.2	142
4	1	53.9	56.9	58.1	59.6	62.3	64.5	67.8	69.5	70.6	71.5	72.3	75.6	78.9	79.6	82.3	83.5	89.6	89.9
	2	60.3	62.3	63.5	64.5	65.3	71.5	72.6	75.6	76.9	77.6	77.9	78.9	83.6	85.6	89.3	91.5	93.6	96.8
	3	75.6	78.9	79.3	81.2	82.6	83.6	85.9	86.3	88.6	89.6	94.3	95.6	98.6	100.2	102.3	103.6	105.6	106.9
5	1	46	48.6	48.9	49.3	50.2	51.3	52.3	53.6	51.5	54.6	55.6	58.6	59.9	62.2	64.5	65.9	69.8	71.5
6	1	62.3	65.4	66.8	67.8	68	67.9	70.3	72	75	76	78	79.2	79.8	80.5	82.2	84.3	86.2	87.9
7	1	71	723	73.5	76.8	79.8	81.2	82.3	84.5	86.9	89.9	94.6	93.4	95.4	95.8	96.7	97.5	98.6	99.9
	2	112.3	112.2	112.1	11.5	110	11.7	112	114.5	116.5	117.2	117.9	118.6	121	123.6	125.6	127.9	129.6	131
	3	210	209.8	209.2	208.5	208.1	207.5	205.6	206	207	207.9	208	208.4	210	212.2	214.6	218.5	220.3	224.5

Continue .....

.....Table A-13 Continued

Rea.	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
8	1	45.3	45.9	46.7	47.9	51.2	53.6	54.8	61.2	61	62.4	62.8	63.5	67.2	68.5	69.5	70.3	71.5	75
	2	78	78.5	78.9	79.2	79.6	79.9	80.1	80	82.1	82.9	83.9	84.5	84.8	85.9	86.7	88.3	89.5	93
	3	92	92.6	93	93.6	93.2	93.6	94	93.5	94.9	95	95.8	96.4	98	98.6	98.90	99.6	106	114
9	1	45.3	48.3	49.1	52.1	53.2	56	59.9	62	65	66	68	72	75	76	78	79.9	83	84
	2	65	66	67	69	73	77	77.8	79.5	82	85.89	88.88	93	109	120	118	125	130	140
	3	110	119.5	120.3	125.6	132	135	145	150	151	156	157	159	162	169	163	165	169.9	173
10	1	63.75	64.68	64.94	65	65.88	66.87	68.5	69.7	71.33	73	72.1	77.8	81.3	85.6	87.3	88.3	89.7	92.3
	2	69.44	70.6	71.56	73.2	72.93	76.69	76.67	80.82	81.3	85.9	89	89.5	93.5	96.5	97.9	99.6	101.2	104.3
	3	76.8	78.6	81.3	82.5	83.2	84.5	85.2	86.8	88.5	89	92.3	94.5	95.6	96.9	99.8	10.3	105.6	108
11	1	69.2	71.2	72.6	75.6	75.9	76.5	76.9	77.5	78.2	78.9	79.3	80.5	80.67	80.9	81.6	82.1	82.9	83.1
12	1	48	48.5	48.9	49.6	49.9	51.6	51.9	52.9	55.9	56.8	57.8	59.6	67.8	69.9	73.6	76.8	79.8	83.6
13	1	49.5	51.2	52.6	53.4	53.9	54.8	55.3	55.9	61.5	63.5	64.2	65.8	66.7	68.7	69.7	70.14	70.5	75.3
	2	80	82.5	84.6	85.7	87.8	89.9	91.2	92.5	94.5	95.2	96.5	97.6	98.8	98.9	102.3	105.6	105.7	106.8
	3	102	103.5	104.5	106.8	107.9	108.9	109.4	110.2	112.3	113.6	114.5	115.6	116.5	117.9	118.9	122.3	124.5	126.9
14	1	53.6	53.9	54.6	55.2	56.3	56.6	57.4	58.2	58.9	59.4	60.3	60.5	60.8	61.2	61.5	61.9	62.3	62.6
	2	65	65.5	66	66.5	66.9	67.9	68.5	69.4	70.2	70.5	70.9	71.5	71.4	72.9	74.5	75.9	77.5	78.2
	3	71	71.8	72.5	73.2	74.5	75.2	76.1	76.9	77.5	77.8	78	77.5	78.6	78.9	79.6	81.5	82.9	85.6
15	1	85	85.5	87.5	89.4	90.4	92.4	95	96	98.5	99.5	101	102.3	104.3	105.6	106.8	108.8	108.9	110
16	1	49	52.3	54.6	55.8	57.6	59.8	61.2	64.3	65.8	68.9	70.60	71.5	72.5	73.5	73.9	76.8	76.9	78

Appendix B

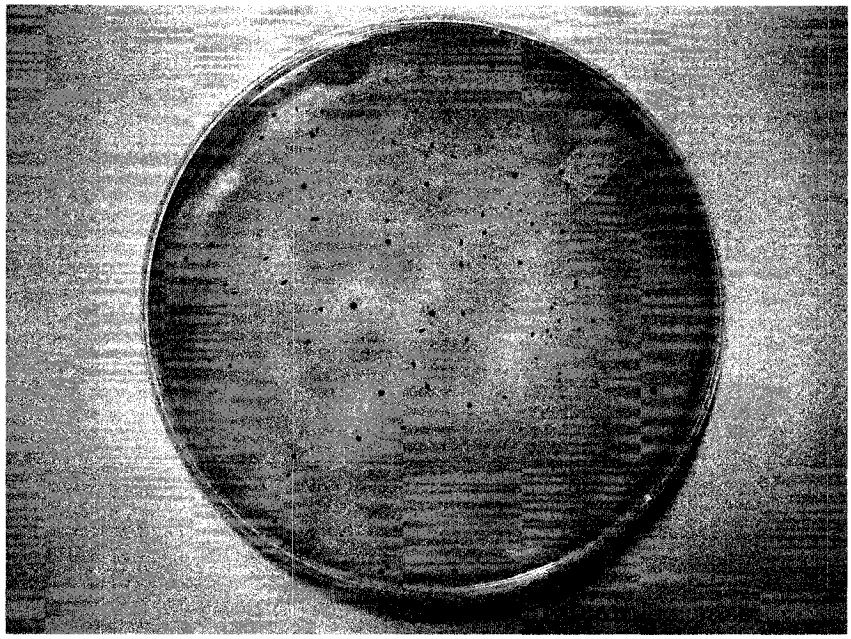


Figure B-1: *C. perfringens* colonies on TSC agar

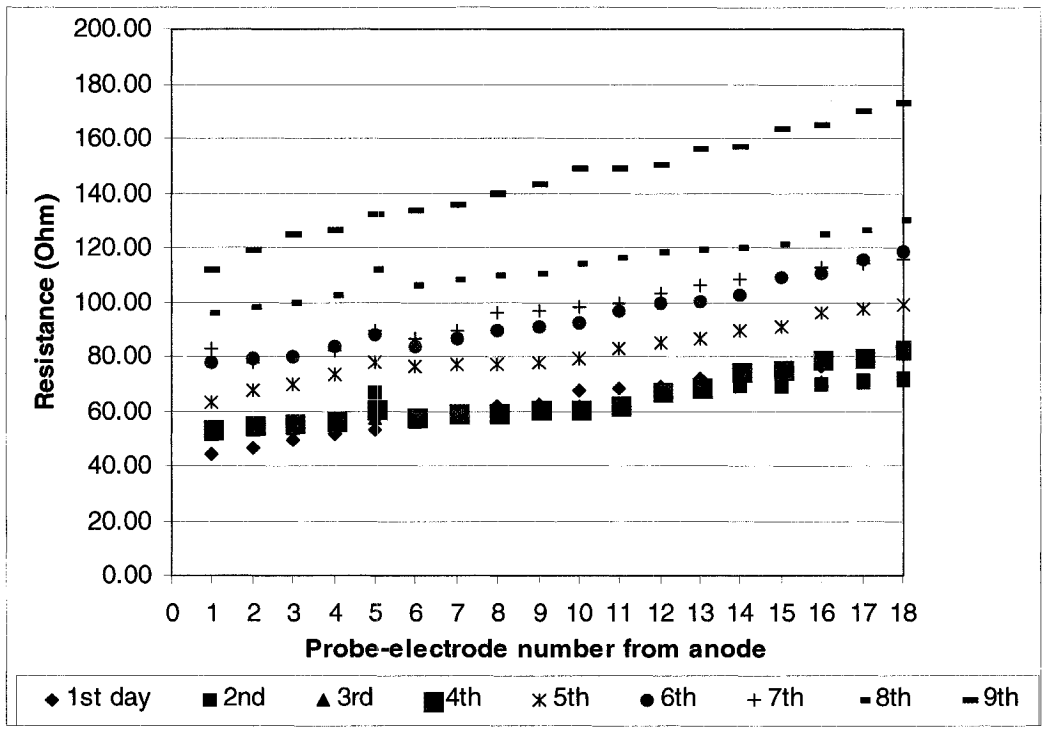


Figure B-2: Resistance distribution for the reactor 1 during 9 days of running test

VIII-GLOSSARY

Bacteriophage: Viruses that have a specific affinity for and infect bacteria

Bioremediation: Any process that uses microorganisms, fungi, green plants or their enzymes to return the environment altered by contaminates to its original condition

Cryomicroscopy: A form of electron microscopy (EM) where the sample is studied at cryogenic temperatures

Decimal reduction time: the time required at a specific temperature and under specified conditions to reduce a microbial population by one decimal time required at a certain temperature to kill 90% of the organisms

Endospores: A dormant, tough, and non-reproductive structure produced by a small number of bacteria

Electric double layer: The variation of electric potential near a surface

Fungicide: An agent that destroys fungi

Factorial designs: An experiment whose design consists of two or more factors, each with discrete possible values or "levels", and whose experimental units take on all possible combinations of these levels across all such factors.

In vitro: Observable in an artificial environment

Geometric mean: a collection of positive data is defined as the nth root of the product of all the members of the data set, where n is the number of members

Gram-positive: Those bacteria that retain a crystal violet dye during the Gram stain process

kGy: 1000 grays, an SI unit used to measure the absorbed dose of radiation

Log_{10} unit reduction: A10-fold or one decimal or 90% reduction in numbers of recoverable bacteria

Microflora: The bacteria and fungi that inhabit an area

Norwalk agent: A strain of epidemic gastroenteritis that appears to be related to the calciviruses

Onychomycosis: The invasion of nail plate by fungus

Porous media:	A porous material is a solid (often called frame or matrix) permeated by an interconnected network of pores (voids) filled with a fluid (liquid or gas
pI	The isoelectric point (pI) is the pH at which a molecule or surface carries no net electrical charge
Refractile	Granules within cells that scatter (refract) light
Tortuosity:	the single most important characteristic of flow through porous media
T number:	Numbering of the icosahedral asymmetric units
Vector:	An insect or animal which carries a disease from one animal or plant to another
Water activity:	The energy state of water in a substance. It is defined as the vapor pressure of water divided by that of pure water at the same temperature