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Treatment of sewage sludge contaminated with bacteria by using anolyte water

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ABSTRACT

The aim of this study is to evaluate the disinfection effect of anolyte water on some species of bacteria detected in sewage sludge, by using different concentrations of anolyte water and different incubation periods. The results showed that tested bacterial species showed different reduction in their numbers by increasing incubation periods. Also, the obtained results showed that anolyte water in concentration of 1 ml produced 100% inactivation for *E. coli*, *Staphylococcus sp.*, *Bacillus sp.*, and *Enterococcus sp*. after 30min exposure time. While, 2ml anolyte water produced complete inactivation for *E. coli*, *Staphylococcus sp.*, *Bacillus sp.* and *Enterococcus sp*. after 20 min exposure time. In conclusion anolyte water can be used safely for inactivation of some pathogens found in sewage sludge.

Key words*:* Sewage sludge, treatment, anolyte water, bacteria.

INTRODUCTION

Sludge is the solid material remaining after sewage treatment facilities purify wastewater produced from homes, business and industries. Obtaining cleaner water from treatment facilities means producing more sludge. The sludge produced from municipal wastewater treatment plants seems to be a problem in some countries, while in other countries is recognized as an environment friendly source of power rather than being a burden on the environment. Whether the sludge is used or disposed of, it is important to avoid creating additional environmental problems and to keep costs down. In the past the sewage sludge was disposal in the least troublesome, most affordable ways possible. It was dumped at sea, buried in landfills or burned in incinerator (Haller, 1999).

Sludge has a various contents such as inorganic substances and agriculture value components (organic substances, nitrogen, phosphorous and potassium) and lesser amount of calcium, sulphur and magnesium, and it may also contain pollutants such as heavy metals and pathogens (Rulkens, 2008).

Due to the increase in the cost of landfill and more strict environmental standards and increased public concern about air, land and water, new methods for sludge treatment was developed (Jun and Xuejun, 2005). The produced sludge from developing countries usually differs from which generated in developed ones due to divergent industrialization and public health levels. In developing countries, the metal and toxic content is usually much lower, while pathogens content is much higher (Rulkens, 2008 ; Zaini *et al.*, 2006). A lot of diseases spread due to the use of sludge from wastewater treatment plants without

further treatment, which cause harmful effects to human, animals and plants.

Sludge contains many different types of pathogens that are secreted by humans and animals through the feces. Feces originate from wastewater from households and from storm water, which can contain feces from birds, cats, dogs, etc. (Naturvårdsverket, 2002).

Sludge utilization in agriculture has been increased in recent years (Stone *et al.*, 1998). It is therefore, very important to control pathogens present in sewage sludge for protection of humans, animals and plants (Lepeuple *et al.*, 2004). Detection of pathogens in sludge is usually influenced by the level of treatment methods (Carrington, 2001).

The present disposal routes of sewage sludge represent a critical environmental issue in Egypt. Recently, there has been an increasing concern about sewage sludge management due to the environmental risks, which resulted from the fast expansion of wastewater treatment plants without equal attention in dealing with the produced sludge. Therefore, the pressing needs are to develop appropriate low cost methods to treat the sewage sludge to be safe and suitable for reuse in agriculture (Ghazy *et al.*, 2009).

Anolyte water was used as alternative of chlorine to avoid contamination of wastewater with pathogens (Perçin and Esen, 2009). It was produced from electrolyzing salt water through double chamber membrane electrolytic cell. It contains reactive ions (HOCl, ClO_2^-, ClO_3^- Cl^-) , dissolved oxygen, superoxide radicals which contribute to its powerful oxidizing properties. It is colorless liquid with pH value of 2-3. It is nontoxic and harmless to human (Leonov, 1999).

The current study aimed to evaluate the biocidal effect of anolyte water in treatment of sewage sludge from different forms of pathogens.

MATERIALS AND METHODS

1-**Anolyte water:**

Anolyte water was prepared according to **Wolf, (2012)** CEO W.P.C-Estonia and it was obtained from Egypt Envirolyte Ltd.

2-Microbial Examination:

Different concentrations of anolyte water (1 and 2 ml) were added to microbial species (*E. coli*, *Enterococcus sp.*, *Baccillus sp.* and *staphylococcus sp*.) to evaluate the biocidal effect of anolyte water at different incubation periods (zero time, 1min, 5min, 10min, 15min and 20min). These microbial species were prepared as follow, under sterilization condition one disk was taken from each microorganism and inoculated in sterile 5 ml tryptic soy broth and incubated at 37°C for 24 hours. After incubation, the inoculated broth was centrifuged at 5000 rpm for 15 minutes, and then the pellets were transferred to 5 ml sterile saline water. The centrifugation step was repeated three times. The obtained counts from saline water were adjusted at 95, 102, 110 and 150 cfu/ml for *E. coli.*, *Staphylococcus sp*, *Bacillus sp*. and *Enterococcus sp*., respectively by using poured plate count agar and dilution test tubes. One ml of saline water microbes was separately inoculated in an autoclaved water (1000 ml) to evaluate the biocidal effect of anolyte water. Anolyte water was applied to bacterial strains in concentrations 1 to 1 and 1 to 2 with exposure time from 1, 5, 10, 20 and 30 minutes. After the exposure to the anolyte water the viability of microorganisms was estimated according to APHA (2017).

The second part of the experiment was to evaluate the effectiveness of anolyte on untreated sewage sludge which collected from Abo Rawash wastewater treatment

plant. The samples were collected and transferred to the lab in ice box within 4 hours (APHA, 2017).

4 gm of solid sewage sludge was added to 100ml of dist. water (HBRC, 2015). Different volumes of anolyte water (100, 50, 25,10,5 ml) were added to the sewage sludge at different incubation periods (zero time, 5, 10, 15 and 20 min).

The effect of anolyte water was evaluated by application on some bacterial strains. The effect of 1ml of anolyte water to 1ml of the different stains (*E. coli*, *Staphylococcus sp.*, *Bacillus sp.*, *and Enterococcus sp.*) is represent in Table (1) and Figure (1). While Table (2) and Figure (2) show the effect of adding 2ml of anolyte water to 1ml of the different strains of bacteria.

RESULTS

Table (1): The effect of adding 1ml of anolyte water to 1ml of different strains of bacteria at different incubation periods.

Count(CFU/ml)						
Time / **Bacteria *sp.***	**control**	**1 min.**	**5 min.**	**10 min.**	**20 min.**	**30 min.**
E. Coli	95	65	51	33	12	0
Staphylococcus sp.	102	91	65	41	15	0
Bacillus sp	110	90	50	0	0	0
Enterococcus sp.	150	79	67	20	0	0

Table (2) the effect of adding 2ml of anolyte water to 1ml of different strains of bacteria at different incubation periods.

Count(CFU/ml)						
Time / **Bacteria *sp.***	**control**	**1 min.**	**5 min.**	**10 min**	**20 min.**	**30 min.**
E.Coli	95	43	18	0	0	0
Staphylococcus sp.	102	80	39	10	0	0
Bacillus sp.	110	40	20	0	0	0
Enterococcus sp.	150	64	31	11	0	0

The results indicated that using 1ml of anolyte water reduced 100% of *Bacillus sp.* after 10 min, *Enterococcus sp.* after 20 min and *E.coli* and *Staphylococcu sp.* after 30 min (Table 1 and Fig. 1).

On the other hand, adding 2ml of anolyte water reduced 100% of *E. coli* and *Bacillus sp.* after 10 min, *Staphylococcu sp.*and *Enterococcus sp.* after 20min exposure time (Table 2 and Fig. 2).

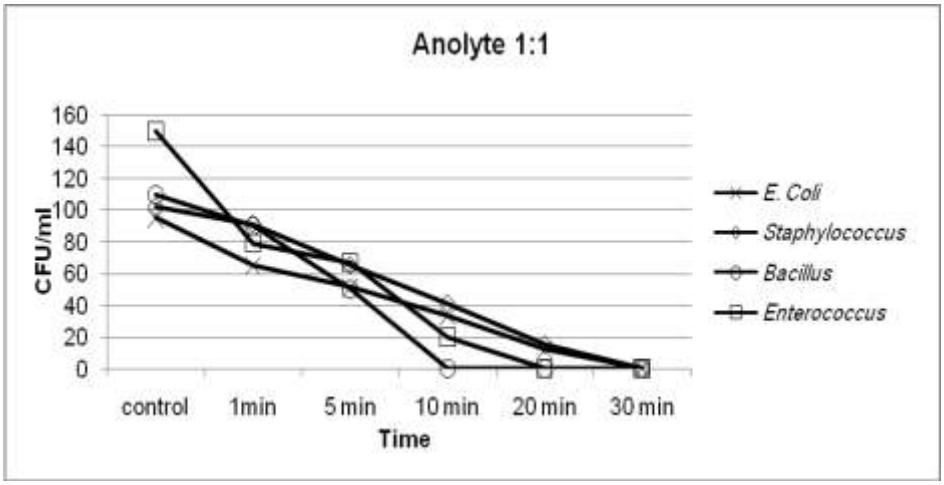

Fig. 1. The effect of anolyte water with concentration (1/1) on some strains of bacteria at different incubation periods.

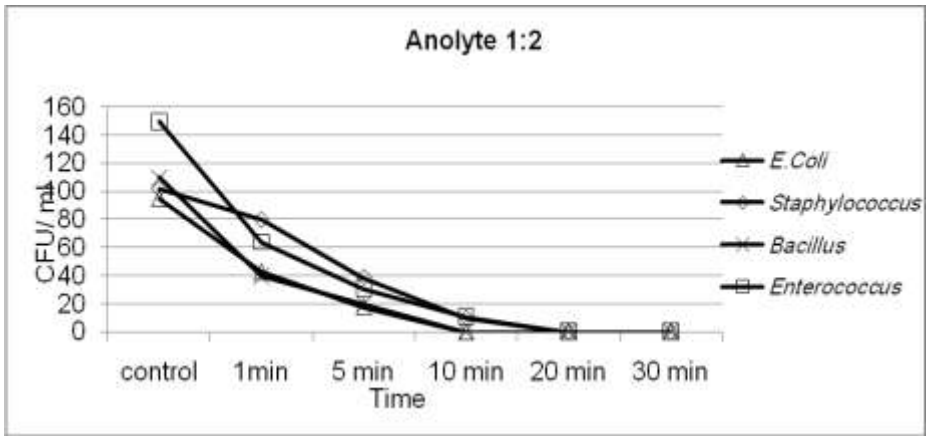

Fig. 2. The effect of anolyte water with concentration (1/2) on some strains of bacteria at different time incubation periods

Anolyte water was applied to sewage sludge from Abo Rawash treatment plant, results of total colifrom and fecal coliform are represented in Tables (3 & 4).

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Table 3: The effect of different concentrations of anolyte water on Total Coliform at different incubation periods on sewage sludge from Abo Rawash treatment plant

Total coliform Count (MPN/100ml)					
Time / Conc.	**Zero time**	**5 min**	**10 min**	**15 min**	**20 min**
100/100	160000	8000	0	0	0
50/100	160000	33000	14000	0	0
25/100	160000	32000	22000	8000	0
10/100	160000	23000	0	0	0
5/100	160000	16000	16000	11000	0

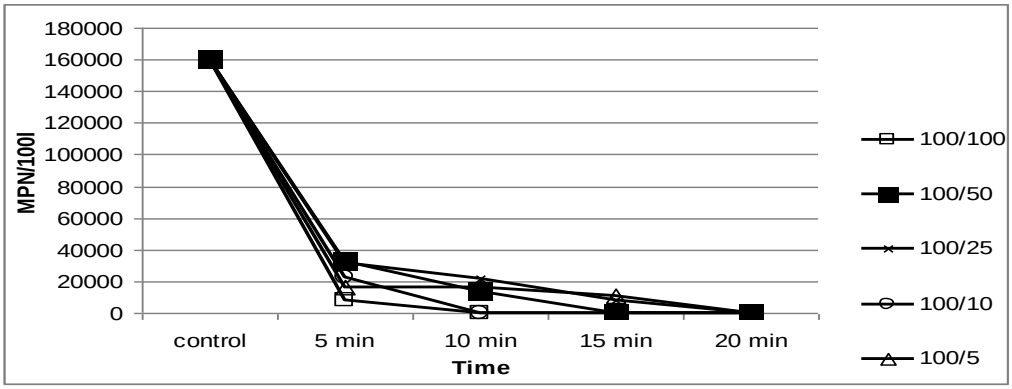

Fig. 3: Effect of different concentrations of anolyte water with different incubation periods on sewage sludge from Abo Rawash treatment plant on Total Coliform

Table 4: The effect of different concentrations of anolyte water on Fecal Coliform at different incubation periods on sewage sludge from Abo Rawash treatment plant

Fecal coliform Count (MPN/100ml)					
Time / Conc.	**Zero time**	**5 min**	**10 min**	**15 min**	**20 min**
100/100	160000	8000	0	0	0
50/100	160000	9000	7000	0	0
25/100	160000	13000	12000	8000	0
10/100	160000	14000	0	0	0
5/100	160000	23000	16000	1000	0

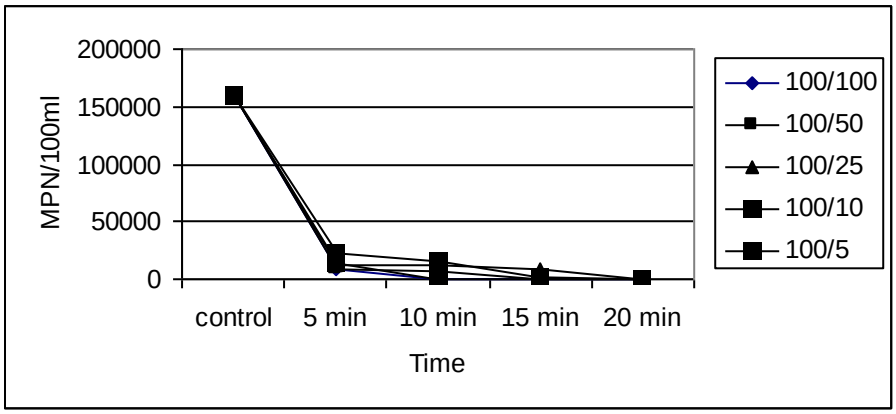

Fig. 4: Effect of different concentrations of anolyte water with different incubation periods on sewage sludge from Abo Rawash treatment plant on fecal Coliform

It was obvious from Table (3) that total coliform count before treatment 160000 MPN/100ml reduced to 8000, 33000, 32000, 23000, 16000 MPN/100ml with concentrations 100, 50, 25, 10 and 5ml of anolyte water, respectively after 5min. On the other hand, the total coliform at concentrations 100/100 and 100/10 was zero after 10min. After 20 min the total coliform was zero at all concentrations. Table (4) and Figure (4) illustrate that fecal coliform count before treatment 160000 MPN/100ml reduced to 8000, 9000, 13000, 14000, 23000 MPN/100ml with concentrations 100, 50, 25, 10 and 5ml of anolyte water, respectively after 5min.The count of fecal coliform become zero at concentrations 100/100 and 100/10 after 10min. After 20 min the total coliform was zero at all concentrations.

DISCUSSION

The present results indicated that using 1ml of anolyte water reduced 100% of *E.coli* and *Staphylococcu sp.* after 30 min, *Enterococcus sp.* after 20min and *Bacillus sp.* after 10 min and adding 2ml of anolyte water reduced 100% of *E. coli* and *Bacillus sp.* after 10 min, *Staphylococcu sp.*and *Enterococcus sp.* after 20 min exposure time.

Also, the anolyte water at concentration (1/1) after 30 min killed the investigated bacteria; while anolyte water at concentration (1/2) get rid of the bacteria after 20 min.Some studied reported that, the efficacy of disinfectants depended on the concentration of biocide, organic matters, pH and counts of microorganisms as well as types which they play a role in efficacy of anolyte water (EPA, 2001; WHO, 2001; 2008).

Samast *et al.* (2008) reported that anolyte water is more efficient, low cost, and nontoxic and wide spectrum of usage. In addition it kills bacteria, viruses, fungi and parasites quickly and can be used for disinfection of hard surfaces and water systems. Moreover, Perçin and Esen (2009) demonstrated that how anolyte water is obtained and discussed its efficiency.

Tallinn (2008) and Perçin and Esen (2009) reported that anolyte water is harmless on human tissue, does not form toxic product and it is also considered to be safe as well as it kills any microbes during seconds. In addition, Marais and Rawhani (2001) and Mikhailov *et al.* (2009) concluded that anolyte water is a fair and a universal germicidal agent alternative of disinfectant and can be applied as sanitation, pre-sterilizing in water treatment. Moreover, Miomir *et al.* (2014) showed that water-

disinfection appliance from electrolyzing salt can be used in water systems where it is successful as biocidal in natural water which contains some microbial (*Enterobacter*, *Citrobacter*, *Bacillus*, *Aeromonas*, *Streptococcus*, *E. coli*, *Aeromonas*, *Pseudomonas aeruginisa* and Sulfate-reducing *clostridium*. On the other hand, Sergi *et al.* (2015) observed that, oxidizing agent from active chlorine ions which are produced from electrolyzed water (Anolyte & Catholyte) play importance role in removal some organic contaminants from wastewater during treatment.

Among the steps of water treatment is using chlorine in many parts of the world, especially developing countries, to kill microbes in water moves through the ecosystems until reached downstream users (WHO, 1984). Lechevallier *et al.* (1988) found that in water *Klebsiella pneumonia* can be resistant to chlorine from 2 to 10 fold if chlorine is at 0.3 ppm in 30 min contact time. Reasoner *et al.* (1989) some types of bacteria (such as *Enterobacter and Citrobacter*) as well as potentially opportunistic pathogens (such as *Aeromonas and Pseudomonas)* in water which were treated with chlorine and collected from distributed systems.

Damian and Jeanne (2007) noticed that *Mycobacterium. aurum*, followed by *Staphylococcus. epidermidis* and *E. coli.* (Log_{10} 3 to 4 CFU/mL) were survived in water with initial free chlorine concentrations of 0.2, 0.4, and 0.8 ppm. In addition, USEPA, (2004) and WHO, (2008) reported that, chlorine is to be less effective on viruses and some microbial during the wastewater treatment, this due to survival microorganisms in effluent and affected on sources of drinking water. So they refer using ozone and ultraviolet light as disinfectant to killing microorganisms are recommended. Also, Majumder, (2004) recorded in samples collected from different of wastewater treatment Plants, the removal ratio of heterotrophic total bacterial count after treatment were ranged from 80 to 90% , while these were 98 % after treated with disinfecting material (chlorine or ozone). Park *et al.* (2008) concluded that present organic matter reduces the effectiveness of acidic electrolyzed water for reducing microorganisms on the surfaces of lettuce and spinach. Moreover, Bohra *et al.* (2012) reported high bacterial content in wastewater due to the organic matter which is produced from human activity and animal wastes.

Conclusion

The treatment of sewage sludge with 5ml of anolyte water can make disinfection to fecal and total coilform after 20min. Also, anolyte water can be used as a better biocide than other used disinfectants in sewage sludge treatment and reduce pathogens.

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معالجة مياه الصرف الصحي الملوثة بالبكتيريا باستخدام مياه الانوليت

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المستخلص

ا لهدف من هذه الدراسة هو تقييم تأثير التطهير بأستخدام مياه الانوليت على بعض أنواع البكتيريا الم تواجدة في حمأة مياه المجاري ، باستخدام تركيزات مختلفة من مياه الانوليت وأوقات حضانة مختلفة. أظهرت النتائج أن الأنواع البكتيرية المختبرة أظهرت انخفاضاً في أعدادها من خلال زيادة فترات الحضانة. أيضا ، أظهرت النتائج التي تم الحصول عليها أن مياه الانوليت في تركيز 1 مل قتلت 100 ٪ من *E. coli* ، *Staphylococcus sp*، *Bacillus sp* ، *Enterococcus sp* بعد وقت التعرض 30 دقيقة. في حين أن 2 مل من مياه الانوليت قتلت 100 ٪ من *E. coli* ، *Staphylococcus sp* ، *Bacillus sp* ، *Enterococcus sp* بعد 20 دقيقة من وقت التعرض.

وقد أمكن استنتاج انه يمكن استخدام مياه الانوليت بأمان لتثبيط بعض مسببات الأمراض الموجودة في حمأة الصرف الصحي.