Increasing Sterilization Efficiency of Shredder Autoclave on Medical Waste Using Ultraviolet Light Device

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ABSTRACT

This work involved investigating bacterial pollution of medical wastes produced by shredder autoclave at some local hospitals in Iraq. A modulation has been introduced to ensure sterilization performance through the use of Ultraviolet light on the hazardous wastes after being processed by shredder autoclave with different retention times at optical density of 270 nm in order to control the full killing of all kind of microbial life according to the environmental limits. The bacteria recovered from the wastes were identified through routine microscopically and biochemical tests and the results revealed the following species: *Acinetobacter baumanii, Bacillus cereus, Clostridium difficilie, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results showed ability of these bacteria to re-generate in the produced solid medical wastes within the periods of 72 hours that reaches up to 20 cell / ml, while the use of UV light in a retention time of 15 min. was able to destroy all bacterial growth in the same period of 72 hours.

Introduction

Hospitals and health centers produce a diverse mix of medical waste during its daily activities. Some of these wastes are in the form of liquids thrown into the sewage and others are emitted in the form of gases from the laboratory nozzles. The major part of waste in the health sector contains chemical and biological residues. Each type of health waste is treated in a certain way, which varies according to the origion of waste being used. Many methods are used, such as incineration, landfill, heat and steam sterilization ….. Etc. to have a clean environment free of pollution and treat the environment from harmful effects and maintain the balance of ecosystem [1]. Overall, the term "medical waste" can be defined as a subset of wastes generated at health care facilities, such as hospitals, physicians' offices, dental practices, blood banks, vaccination procedures resulting from operating rooms and veterinary hospitals/clinics, as well as medical research facilities and laboratories. This health wastes can be classified into four types: infectious waste, hazardous waste, radioactive waste and other public wastes [2]. It's obvious that if these wastes were not properly treated, they might be considered a source of health risk [3].

Most of the medical waste is treated and destroyed using incineration method. This method has many risks and disadvantages, considering the large quantities of waste produced daily in hospitals. Burning is supposed to destroy the materials in where they are present and the infectious materials are onto it such as paper, cardboard, plastic, glass and metal. In this process acid gases are generated (by the existing chlorine) and toxic metals are released (from contaminants and additives found in paper, plastic and other materials). Dioxin and furan are formed from any chlorine found in waste [4].

This chemical problems are not the result of medical waste itself, but because of the unnecessary combustion of polyvinyl chloride (PVC), paper, tissue and other non-infectious substances leading to the generation of these toxic substances and the production of ashes that in turn require Special treatment is considered to be hazardous waste. A

group of scientists, after finding high levels of bacteria in gases from hospital waste incinerators, concluded that incinerators may not be the definite or optimal method for sterilizing medical waste [5].

In view of the reasons mentioned earlier, other ways to treat and destroy medical waste in a friendly safe manner to the environment were found, such as: Steam Sterilization which is environmentally safe, less expensive to operate and requires a few qualified technicians [1].

The infectious medical disposal devices is shredded into small parts enabling steam and heat to reach as many of these wastes as possible plus reducing their size but even this process is not enough and cannot eliminate the growth of microbes. New effective modifications were added during treatment processes such as ultraviolet sterilization and microwave. This radiation works against spore-forming bacteria and other microbes due to the lethal effects on cell walls and DNA hence preventing their replication and multiplication to further generations so there will be no growth and spores will be definitely killed [6].

The aim of this research was to find a suitable and environmentally safe way to treat and destroy medical waste by adding ultra violet technique to the shredder autoclave and measuring bacterial growth of the collected microbes from medical wastes with and with UV to evaluate its efficiency.

Materials and Methods
Sample Collection and processing

Medical wastes were collected from some local hospitals in Iraq including City of Medicine and Ibn Al Haytham Hospitals in Baghdad. Those samples were obtained in sterile plastic containers prepared for this purpose. They were transported with cool boxes to the laboratory to be further processed and investigate for bacterial contaminant. Processing were carried out within a maximum of two hours in order to preserve the actual number of microorganisms in the sample and to avoid damage to the samples. Modeling of medical waste samples was performed using Shredder Autoclave (CISA, Italy) before and after treatment with UV. light under pressure of approximately 2.5 bar and 135 ° C.

Bacterial Identification and Total Counting

Traditional bacterial identification were performed including microscopic, cultural characterization and biochemical tests under both aerobic and anaerobic incubation conditions [7].Total bacterial number of the studied samples being destructed was measured using the total plate count (T.P.C) method before and after the modified treatment following procedure described in Abbawi & Hassan [8] which involves adding 1ml of the bacterial isolate suspension to the nutrient agar petri dishes. Nutrient agar medium was prepared according to the manufacturer's instructions and pouring the suspension onto the dishes. The dishes were stirred long enough to mix the sample with the medium and incubated at 37 ° C for 24 hours and then counting the bacterial colonies developing

on the dish using Colony Meter and applying the formula: Total number of bacteria (cfu / ml) = number of colonies × inverted dilution factor.

Decimal serial dilution of waste solid and liquid materials were conducted pre and post- treatment with UV.

Knowing that dilution 10^{-3} was used for pre-treatment and 10^{-1} dilution was used for post-treatment for counting subsequent laboratory tests.

A bacterial suspension of medical waste was prepared prior to treatment by comparing turbidity to McFarland tube that represents 1.5×10^8 cells / ml measured at 600nm spectrophotometry with optical density OD = 0.5) and exposing this suspension to different radioactive intensities of 240,250,260,270,280 and 300 nm by changing the radiation intensity of a specific regulator for 15 minutes to test the appropriate radiation intensity and extracting the growth percentage by comparing it with the control tube.

Regarding the solid waste samples, various samples of different thicknesses included plastic, cloth and cotton residues, in which they were introduced into the sterilizer through a conveyor belt and passes through a spiral installation to stir these residues before entering the heat and pressure chamber. However, the solid wastes were continuously stirred during the experiment conditions to ensure full exposure of ultraviolet radiation for all visible parts of such wastes.

Samples of the current study were divided into four groups: the first group was cultured immediately after the sterilization process, and the second group, was cultured after 24 hours of sterilization and the third group, which was cultivated after 48 hours of sterilization and the fourth group was cultivated after 72 hours of the sterilization process for both the liquid and solid samples, to determine the viable numbers of existing bacteria at different intervals and give sufficient time and conditions for the growth of Sporulation. A special UVC UV-based UVC lamp device has been used for 270 nm for this range of lethal effect on microorganisms.

All four sample groups were subjected to different retention times, which were: (1 min, 5 min, 10 min and 15 min) after autoclave shredder sterilization and then measured the bacterial number produced after each time Retention to check the efficiency of the processing process.
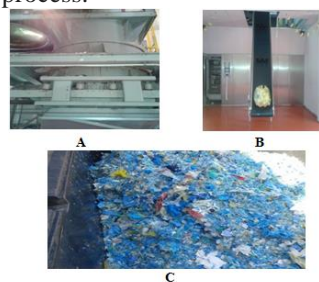

Figure (1): A: Shredder autoclave B: Transferring belt C: Medical wasted after being shredded and sterilized

Results and Discussion

The current work involved collecting and diagnosing of bacterial contaminant isolates from medical wastes of a couple of hospitals in Baghdad prior to treatment.

Those isolates were identified based on the results of the biochemical tests approved in [9 and 10] as shown in the following table

Table (1) bacterial species isolated from medical waste prior to treatment by autoclave shredder device

Characteristic Properties	Bacteria							
	Acinetobacter baumanii	*Bacillus cereus*	*Clostridium difficile*	*E. coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Catalase	+	+	-	+	+	+	+	+
Citrate	+	+	+	-	+	+	+	+
Coagulase	-	-	-	-	-	-	-	+
Gas Forming	-	-	-	+	+	+	+	-
Gelatin	-	+	+	-	-	+	+	+
Gram Stain	-	+	+	-	-	-	-	+
H_2S	-	-	+	-	-	+	-	-
Hemolysis	-	+	-	-	+	+	-	+
Indole	-	-	-	+	-	-	-	-
Motility	-	+	+	+	-	+	+	-
Methyl red	-	-	-	+	-	+	-	+
Oxidase	-	-	-	-	-	-	+	-
Urease	-	+	-	-	+	+	-	+
Glucose	+	+	+	+	+	+	-	+
DNase	-	+	-	-	-	+	-	+
Lactose	-	-	-	+	+	-	-	+
Lipase	+	-	-	-	-	+	+	+

The diversity of bacteria recovered indicates a high health risk of these pathogens in case they are present in the samples after treatment.

Bacterial count examination showed heavy growth when culturing from diluent sample (10^{-3}) on Nutrient agar, blood agar, and brain heart infusion agar. Number of bacteria in the liquid sample was 217,000 colony forming units per ml (cfu/ml) (Fig. 2) while number of bacteria in the solid waste was 124,000 cfu/ ml (Fig. 3) this gives a clear indication of the extent of the bacterial contamination in the samples prior to treatment with the autoclave shredder.

Concerning with liquid waste samples after sterilization in the device without UV treatment, they were divided into four groups at different intervals and under sterile conditions. None of the studied groups showed any significant growth on cultured dishes while solid wastes after 24, 48 and 72 hours scored number counts of bacteria: 10, 20 and 20 cells / ml, respectively (Table 2).

Table (2) Viable Bacterial Count of Liquid and Solid Wastes Samples pre UV. Processing

Liquid medical waste samples		Solid medical waste samples (bacterial suspension)	
Culturing time intervals	Bacterial counts	Culturing time intervals	Culturing time intervals
Directly after sterilization	Zero (cfu/ml)	Directly after sterilization	Zero (cfu/ml)
After 24 hours	Zero (cfu/ml)	After 24 hours	10 (cfu/ml)
After 48 hours	Zero (cfu/ml)	After 48 hours	20 (cfu/ml)
After 72 hours	Zero (cfu/ml)	After 72 hours	20 (cfu/ml)

According to the bacterial suspension of medical waste which was prepared prior to treatment and exposing this suspension to different radioactive intensities as it was referred in the aforementioned chapter of material and method, by changing the radiation intensity of a specific regulator for 15 minutes to test the appropriate radiation intensity and extracting the growth percentage by comparing it with the control tube. The results revealed that the best bacterial killing rate of radiation intensity was at 270 nm that was adopted in subsequent experiments with different retention times. The percentage of bacterial growth was only 6% at 270 nm and 15 minutes of retention time (Figure 2).
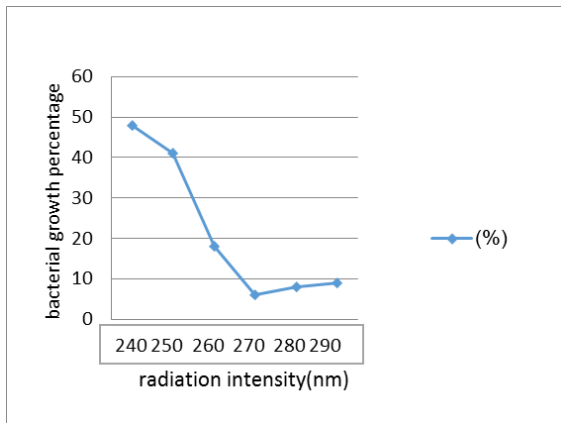
Fig. 2: Percentage of bacterial growth with different radiation intensities

Corresponding with UV treatment, liquid medical waste samples were excluded because it did not contain any bacterial cell. This was demonstrated by the results of the examination referred to in Table 1. The samples of the solid medical residues produced after treatment with the ultraviolet growth after divergent periods. Solid waste was treated with ultraviolet (UV) spectrum of 270 nm (with the highest inhibitory rate of bacterial growth) with a 1 minute retention time, bringing the number of bacteria to 20 cells / ml at planting time 48 and 72 hours respectively (Table 3).

Table (3) bacterial counts for solid samples after exposure to radiation at 1 minute retention time

Culturing time	Bacterial counts
Directly after sterilization	Zero (cfu/ml)
After 24 hours	10 (cfu/ml)
After 48 hours	20 (cfu/ml)
After 72 hours	20 (cfu/ml)

The treated medical waste retention time of 5 minutes again gave results similar to one-minute retention time as in Table (3). But in the case of solid medical waste been treated with ultraviolet light for a 10 minute retention period, the number of bacteria decreased to 10 cells / mL after 72 hours with no growth in the remaining periods as shown in Table 4.

Table (4) bacterial counts for solid samples after exposure to radiation at 10 minutes retention time

Culturing time	Bacterial counts
Directly after sterilization	Zero (cfu/ml)
After 24 hours	Zero (cfu/ml)
After 48 hours	Zero (cfu/ml)
After 72 hours	10 (cfu/ml)

After treatment with UV. for 15 min., bacterial growth has been completely eliminated from all cultures within the four groups intervals. All living organisms in the samples were eliminated by the combination of factors; heat, pressure of the shredder and ultraviolet spectrum after sterilization. (fig. 4) shows elimination of growth in the third petri dish with 15 min. UV. exposure. Also (Fig. 5) and (fig.6) shows elimination of growth on MacConkey and

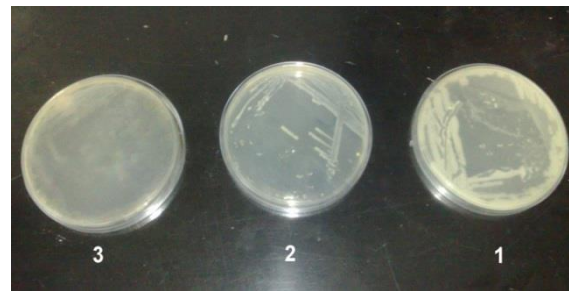brain heart infusion agar with the same retention time respectively.

Fig. 4: Bacterial growth elimination at the third dish on nutrient agar within 15 min. compared to pre-exposed dishes (1,2)

Fig. 5: Complete bacterial growth elimination within 15 min exposure on MacConkey agar

Fig. 6: complete Bacterial growth elimination within 15 min exposure on brain heart infusion agar

Ultra violet spectrum of type C, which has a wavelength of 100 to 280 nm is called UVC. This spectrum affects microorganisms such as bacteria, molds, yeast and primates by destroying and killing them, or by weakening the microorganism's ability to multiply and multiply. Radiation on DNA by causing chemical reactions to acid-forming thymine bases to prevent the division and multiplication of DNA or by separating and breaking cellular molecular bonds and free radicals, which have a significant impact on the cellular state of the microorganism through interaction with each other and Production of toxic and suspended compounds for cellular work and thus the death of the organism [11].

The highest killing rate was obtained at 270 nm due to the fact that the effect of ultraviolet light was not directly on the DNA of the bacterial cells, since the

highest kill rate was not obtained from the wavelength of 260 nm, since it is possible The effect of lethal radiation is directly on the di-picolinic acid (DPA), whose peak absorption is close or identical to the wavelength of 270 nm Especially if we know that this acid is about 5-15% of the dry weight of the spores of bacteria, which is responsible for the thermal resistance of some bacteria as it enters the structure of the cell wall of bacteria and combines with the ions of other chemical elements of the wall in the form of carboxylic groups, Free hydroxyl resulting from exposure to radiation, causing the disintegration of the biological wall and the death of the microorganism [12].

The destruction and killing of microorganisms depends mainly on the state of the medium in which these organisms exist, ie the host's state, whether liquid or solid. In the case of the medium liquid, the effect of heat and pressure reaches all living organisms. Suitable to protect the microorganism from inappropriate conditions of heat and pressure or protect its spheres when the growth conditions are inappropriate [13,14]. The role of ultraviolet radiation affects all that exists in the center of steel, where the impact of these rays to all parts of solid waste on the conveyor belt to the pressure tank and thus kill or reduce the reproductive capacity of the microorganism [15].

Through the results obtained in this study is the extent of the significant contamination in pre-treatment samples and the effectiveness of wet heat sterilization in reducing the number of microorganisms but not their lack, which is required for safe and non-infectious waste [16] The bacterial pathogens isolated in Table 2 and the serious diseases they cause in the final results of the medical waste. In other words, any bacterial growth following the sterilization process may be colonized by one of these bacterial species and thus the process of sterilization is incorrect. The appearance of bacterial growth in the current study samples after different periods of time and its lack immediately after treatment is due to the fact that the microorganism may produce spores and enclosures surrounding itself when the living conditions are inadequate, and when conditions improve after an appropriate period of time, Covers [17], This has been demonstrated by the current study of the emergence of living organisms after 72 hours, either to be composed of spore formation organisms or have secured a suitable environment within the molecules of solid waste may be due to the interruption of power at times and delay the work of the treatment system, causing this imbalance in the system of sterilization, This should be noted, in particular, that the resulting waste may be treated as municipal waste and may still contain some of the hazardous bacterial species referred to in Table 2.

The reason for the disappearance of growth after the treatment of these wastes with the UV spectrum is that the lethal effect of these rays reaches the different parts of the microorganisms' habitat and at the appropriate time of retention to cause damage that prevents the reproduction of the organism and multiply its numbers through its effect on the DNA or through the cellular bonds that it breaks and the free radicals that it generates, which can replace the cell's cellular wall or produce toxic substances within the micro cell cytoplasm [18].

The results of the present study coincided with several studies, including the study of Garcia [3] during his study on the effect of ultraviolet light on the purification of medical waste, and the study of Munshi [19] in an applied study of the effects of ultraviolet radiation on bacterial growth in reverse osmosis systems of water filters. Walker [20] pointed in their study about disinfection of medical equipment used in surgeries that the use of ultraviolet irradiation was effective in eliminating microbial spores, as well as the study of Awodele [21] on the pathways of medical waste management in a number of Nigerian hospitals. Results obtained from this study, together with Liltved & Landfald, [22] examined their study of reducing the number of infectious pathogens in fish tissue using ultraviolet radiation.

Conclusions

Through the results of this study, the process of sterilization and treatment of medical waste reduces the number of pathogenic bacteria to a few limits but at the same time does not work to terminate the presence of bacterial pathogen permanently. Microorganisms have the potential to re-grow and proliferate after extended periods of time after wet-heat sterilization through blackboards or solid waste from a microbial environment. The introduction of transformative processes and development of the system, such as the use of ultraviolet light contributed to the termination of bacterial presence of all kinds even after later periods of time. The study recommends the use of other methods of sterilization such as microwave, ozone and hydrogen peroxide, and special bacterial tests to demonstrate the efficiency of the treatment of samples at intervals of time after sterilization to ensure the complete absence of residues from any living organisms and ensure that all agricultural communities have lost their ability to Development of microbiological populations.

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زيادة كفاءة التعقيم للنفايات الطبية الناجمة عن التعقيم بجهاز الموصدة المقّطعة باستخدام الأشعة فوق البنفسجية

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الملخص

تضمنت هذه الدراسة الكشف والتحري عن التلوث البكتيري للنفايات الطبية الناجم عن التعقيم بجهاز الموصدة القاطع لدى بعض المستشفيات المحلية في العراق. تم إدخال تعديل على عمل الجهاز لضمان أداء التعقيم من خلال استخدام الإشعاع فوق البنفسجي على النفايات الخطرة بعد معالجته بواسطة الموصدة القاطعة وذلك في أوقات احتجاز مختلفة بالكثافة البصرية من 270 نانومتر بغية السيطرة والقتل الكامل لجميع أنواع النمو الميكروبي التي قد تنتج في هذه النفايات الطبية وفقًا للمعايير البيئية. وقد بين الفحص المجهري واختبارت الخصائص الزرعية والكيموحيوية التقليدية المستخدمة في التشخيص ان الانواع البكترية المعزولة من النفايات الطبية تتضمن: *Acinetobacter baumanii, Bacillus cereus, Clostridium difficilie, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* و *Staphylococcus aureus*. أظهرت نتائج المقارنة قدرة البكتيريا المسببة للأمراض القابلية على إعادة نموها وتضاعفها في النفايات الطبية الصلبة المنتجة في غضون 72 ساعة لتصل إلى 20 خلية لكل مل ، في حين أن استخدام الأشعة فوق البنفسجية في زمن الاحتجاز 15 دقيقة قد تمكن من احداث تدمير كامل للنمو البكتيري خلال نفس الفترة (72 ساعة)