applied sciences

MDPI

Article

Application of Sterilization Process for Inactivation of *Bacillus Stearothermophilus* in Biomedical Waste and Associated Greenhouse Gas Emissions

Cevat Yaman

Environmental Engineering, College of Engineering, Imam Abdulrahman Bin Faisal University, Dammam 34212, Saudi Arabia; cyaman@iau.edu.sa

Received: 26 June 2020; Accepted: 21 July 2020; Published: 23 July 2020

Abstract: This study investigated the biomedical waste collection, transportation, and treatment activities in the city of Kocaeli, Turkey. As an alternative to incineration technology, a steam autoclave was used to sterilize the biomedical waste. Information regarding the collection, transportation, treatment and associated greenhouse gas emissions (GHG) were also investigated. Prior to sterilization, biological indicator vials containing *Bacillus stearothermophilus* were placed in the center of the load to ensure that the pathogens were destroyed. GHG emissions were calculated based on the fuel consumed by the biomedical waste collection vehicles and the electricity/natural gas used at the sterilization plant. Results of this work revealed that the total biomedical waste generated per year increased from 1362 tons in 2009 to 2375 tons in 2019. The amount of biomedical waste generated per hospital bed was determined as 1.19 kg.bed^{-1}.day^{-1}. Results show that for efficient sterilization of biomedical wastes, the steam treatment system process should be operated at a contact time of 45 min, a temperature of 150 °C, and at a steam pressure of 5 bar. Biological indicator tests showed that the number of living *Bacillus stearothermophilus* decreased significantly, with removal rates greater than 6log$_{10}$. Finally, it was determined that the biomedical waste management activities generated a total of GHG emissions of 5573 ton CO_2 equivalency (tCO_2-e) from 2009 to 2019. Furthermore, the average global warming factor (GWF) was calculated to be 0.269 tCO_2-e per ton of biomedical waste generated. This study showed that the sterilization process is very effective in destroying the pathogens and the management of biomedical waste generates considerable amounts of GHG emissions.

Keywords: infectious waste; sterilization; biomedical waste; greenhouse gas; *Bacillus stearothermophilus*

---

1. Introduction

The appropriate management of biomedical waste is extremely important due to its significant environmental and health hazards. Recently, many attempts have been made to better manage the biomedical waste problem. Authorities define biomedical waste as the waste generated during the diagnosis and treatment of people and animals. If not properly handled, biomedical waste poses a great risk of infection through the spread of pathogens from health institutions into the environment [1]. Medical devices are now being manufactured for single use only, thus further increasing the amount of biomedical waste especially in developing countries. This will result in a rapid increase in biomedical waste amounts that should be disposed in a safe manner [2]. In the literature, there are different names for biomedical wastes such as hospital waste, regulated biomedical waste and infectious waste [3,4]. The terms infectious waste and biomedical waste are usually used for wastes that cannot be disposed of in a municipal solid waste landfill due to their pathogenic content. The safe disposal of biomedical wastes is of a great concern for the generators and the public. Different treatment methods can be applied in the treatment of biomedical waste. The main purpose in the treatment of biomedical wastes is to make it safe for human and environmental health. The methods used to make biomedical waste harmless can be grouped as incineration, sterilization, plasma pyrolysis, and microwaving. In the US, about 60% of biomedical waste is incinerated, 37% is sterilized, and the rest is treated by different methods [4]. Within the scope of medical waste statistics, it was reported that 89,545 tons of medical waste was collected from 1550 health institutions operating as of the end of 2018 in Turkey. The amount of medical waste collected in 2018 increased by 4% compared to the previous year. Of this amount, 92.3% of the medical waste collected was sterilized and 7.7% was sent to incineration facilities [5]. Alternative treatment methods to incineration have always been the focus of biomedical waste generators. For example, sterilization or autoclave methods use shredders to reduce the waste volume. Sterilization inactivates microorganisms by using the saturated steam and is commonly used to treat infectious biomedical waste [6,7]. Thermal processes are applied as low, medium and high temperature depending on the process temperature applied. As a method, the thermal process is applied as the wet (steam) and dry heat treatment. In dry heat treatment, heat is applied to biomedical waste without adding water or steam. Heat is delivered to the waste by conduction, convection or thermal radiation. The processing time and the temperature to be applied depend on the characterization and quantity of the biomedical waste treated. The process temperature to be applied should not be too high to prevent the volatile organic compounds that can be released from the plastic wastes but should be sufficient for the sterilization of waste [6]. The process of sterilization is the treatment of biomedical wastes with steam at high temperature and pressure. If the temperature and contact time are sufficient, this process inactivates many types of microorganisms. Biomedical waste containers are placed in a closed chamber and sterilized with steam for a certain time at the required pressure and temperature. As a general practice, biomedical waste is steamed at 121 °C for 30 min at 2 bar and approximately 99.99 percent of microorganisms are inactivated by this process [8–10]. Biomedical wastes can be landfilled together with municipal solid wastes after steam treatment and size reduction.

Sterilization is the process of completely destroying all kinds of microbial life, including bacterial spores in biomedical wastes, by physical, chemical, and mechanical methods, or reducing the level of these microorganisms by 99.9999% (6 $\log_{10}$ reduction). Whether the biomedical wastes treated by sterilization are rendered harmless is tested using chemical and biological indicators. Chemical indicators are used in the autoclave sterilization of biomedical waste. When the sterilization is completed, color change must be detected in the chemical indicator carrier that has been autoclaved together with the waste. In the biological indicator test, the viability of the biological indicator is used to detect whether all potential infectious microorganisms have been destroyed in the sterilized waste. It is a tubular test indicator with *Bacillus stearothermophilus*, which is known to be the most resistant microorganism to heat. If the test result is negative, the sterilized biomedical waste is sent to the landfill, but if the test result is positive, the sterilization process should be repeated. Ananta, Heinz [11] reported that *Bacillus stearothermophilus* spores can be inactivated by high-pressure treatment, but only if it is applied at an elevated temperatures. Rajan, Pandrangi [12] also reported that, while the thermal inactivation of spores followed first-order kinetics, the Weibull model best described the inactivation of *Bacillus stearothermophilus* spores. Iciek, Papiewska [13] conducted a study to investigate the combined effect of temperature, pH and NaCl concentration on the thermal inactivation of *Bacillus stearothermophilus* and observed that the sterilization temperature and pH of the sterilized medium as well as the concentration of NaCl, had a significant effect on spore activation and destruction.

Greenhouse Gas Emissions

Global greenhouse gas (GHG) emissions have grown since pre-industrial periods, with a 70% increase between 1970 and 2004 [14]. Since pre-industrial times, increased greenhouse gas emissions from human activities have caused a significant increase in the atmospheric greenhouse gas concentrations. Between 1970 and 2004, global emissions of CO_2, CH_4, N_2O, hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulphur hexafluoride (SF6), described by their global warming potential (GWP), have increased by 70% from 28.7 to 49 Giga ton (Gt) CO_2-eq. CO_2 emissions grew by approximately 80% between 1970 and 2004, representing 77% of the total greenhouse gas emissions in 2004. Between 1970 and 2004, the greatest growth in global greenhouse gas emissions came from the energy supply sector, with an increase of 145%. The Intergovernmental Panel on Climate Change (IPCC) predicts that global greenhouse gas emissions will continue to increase over the next few decades [14]. However, IPCC also estimates that studies have demonstrated a significant economic potential for reducing global greenhouse gas emissions over the next decades that could balance the projected growth of global emissions or reduce emissions below current levels. GHG emissions will then need to peak and decline to stabilize greenhouse gas concentrations in the atmosphere. The lower the level of stabilization, the faster this peak and drop will have to occur. Reduction efforts over the next two to thirty years will have a major impact on opportunities to reach lower levels of stability.

The generation, transportation and disposal practices of wastes potentially generate greenhouse gas emissions [15]. Total GHG emissions resulting from waste management activities in the world are about 1.3 $GtCO_2$-e, corresponding to about 2.8 percent of total GHG emissions [14]. Approximately, 3.3% of total greenhouse gas emissions originate from waste management activities in Turkey [16]. The total greenhouse gas emissions of Kocaeli city for 2016 were calculated as 25.1 million tons of CO_2-e. Of the total greenhouse gas emission, 65.3% of total emissions were from fixed sources, 17.4% from industrial processes, 15.0% from transportation, 1.4% from land use and 0.9% from waste management [17]. The collection, transportation and transfer of waste is not included in waste management activities, but in the estimation of mobile greenhouse gas resources (cars, trucks) [17]. The units of GHG emissions are converted into CO_2 equivalency (CO_2-e) in order to better identify and evaluate GHG emissions. Another term commonly used to describe GHG emissions is called global warming factor (GWF). The GWF identifies the amount of GHG emissions generated per ton of biomedical waste collected, transported and sterilized. GWF used in this study is based on a 100-year time period as reported in the recent IPCC assessment report [18]. In the literature, there is no study that investigated the GHG emissions from biomedical waste collection and treatment systems in the city of Kocaeli, Turkey. This study had two main objectives. The first objective was to verify if efficient biomedical waste treatment can occur under standard operating parameters in steam treatment systems in the city of Kocaeli, Turkey. The second objective was to investigate the greenhouse gas (GHG) emissions generated during the transportation and treatment of biomedical waste.

2. Materials and Methods

2.1. Description of the Study Area

This study was conducted in the city of Kocaeli, which has a population of 1,875,493 and is located in the northwest of Turkey (Figure 1). According to the Turkish Statistical Institute, 81,024 tons of biomedical waste were collected and treated by sterilization, incineration and other methods in 2016 in Turkey [5]. Based on this biomedical waste amount, about 450 million healthcare facility visits were recorded in 2016. Generally, biomedical wastes are segregated and placed in 10-L durable red plastic bags in the study area. Sharps and needles are first collected in yellow rigid plastic boxes and then placed in red plastic bags. As a safety rule, 1/3 of the capacity of the bags is always left empty. After tying the bags securely, they are temporarily stored in designated rooms and collected daily by licensed collection vehicles. Kocaeli Metropolitan Municipality has 9 biomedical waste collection vehicles operating for the 27 healthcare institutions and other small clinics. The collected biomedical waste is transported to an 8 $ton.day^{-1}$ capacity sterilization plant located at the Kocaeli landfill site.

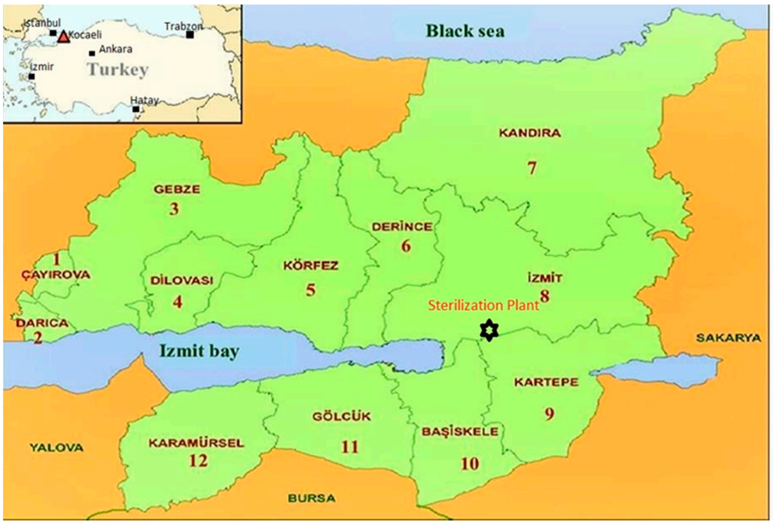

Figure 1. Location map of the study region [19].

2.2. Collection and Transportation of Biomedical Waste

The biomedical waste management system implemented in Kocaeli city includes the disposal of all biomedical wastes originating from all public hospitals, private hospitals, dialysis centers, family health centers, laboratories and district municipalities within the borders of the municipality. Biomedical wastes, excluding pathological and hazardous wastes, generated in the boundaries of Kocaeli city and district municipalities are collected and sterilized at the biomedical waste sterilization facility within the scope of national biomedical waste regulation and then disposed in the solid waste landfill. Biomedical waste amounts from the public hospitals, private hospitals, dialysis centers, family health centers and laboratories in the study area are given in Table 1.

Table 1. Amounts of biomedical waste generated in Kocaeli city in 2019.

Health Centers	Number of Beds	Biomedical Waste Amount (kg)	Biomedical Waste Per Bed (kg/bed.year)
Gebze Fatih	326	112,777	1.033
Danca Farabi	350	130,433	1.039
Kocaeli Public	335	137,765	1.119
Izmit Seka	305	99,950	1.235
Golcuk Necati Celik	175	60,835	0.984
Kandira M. Kazim Dinc	52	19,131	1.044
Korfez	52	17,973	1.105
Karamursel	45	20,942	1.038
Dilovasi	25	12,586	1.398
Anadolu Sağlık Merkezi	201	134,297	1.994
VM Biomedicalpark	121	75,681	2.006
Cihan	120	41,534	1.878
Gebze Biomedicalpark	118	69,980	1.039
Yuzyıl	112	57,551	1.781
Akademi	110	34,321	1.543
Konak	107	30,329	0.937
Medar Golcuk	101	15,870	0.851
Korfez Marmara	79	30,264	0.472
Kocaeli Private	75	27,906	1.150
Acibadem	61	32,700	1.117
Gebze Merkez	56	34,487	1.610
Gebze Medar	75	39,507	1.849
Romatem Physical Therapy and Rehabilitation (FTR) Hospital	27	1937	1.582
Hospital Park Darica	20	8898	0.215
Cagin Goz	25	1972	1.336
Dunya Goz	11	2062	0.237
Kocaeli University	727	358,451	1.481
Total	**3811**	**1,610,139**	**Average = 1.19**

In the study region, biomedical wastes are accumulated separately, where they occur, without being mixed with other wastes. Sharps, needles, infectious wastes, hazardous wastes and pathological wastes are collected in appropriate containers, which are compatible with the waste. The collected biomedical waste is first taken to the temporary biomedical waste storage of the health institution and then delivered to the biomedical waste collection vehicle. Once the biomedical waste is brought to the disposal site, the sharps, needles and infectious wastes are subjected to sterilization, while the pathological wastes and hazardous wastes are incinerated at the hazardous waste incineration plant located near the sterilization plant. The biomedical waste management system used in this study is given in Figure 2.

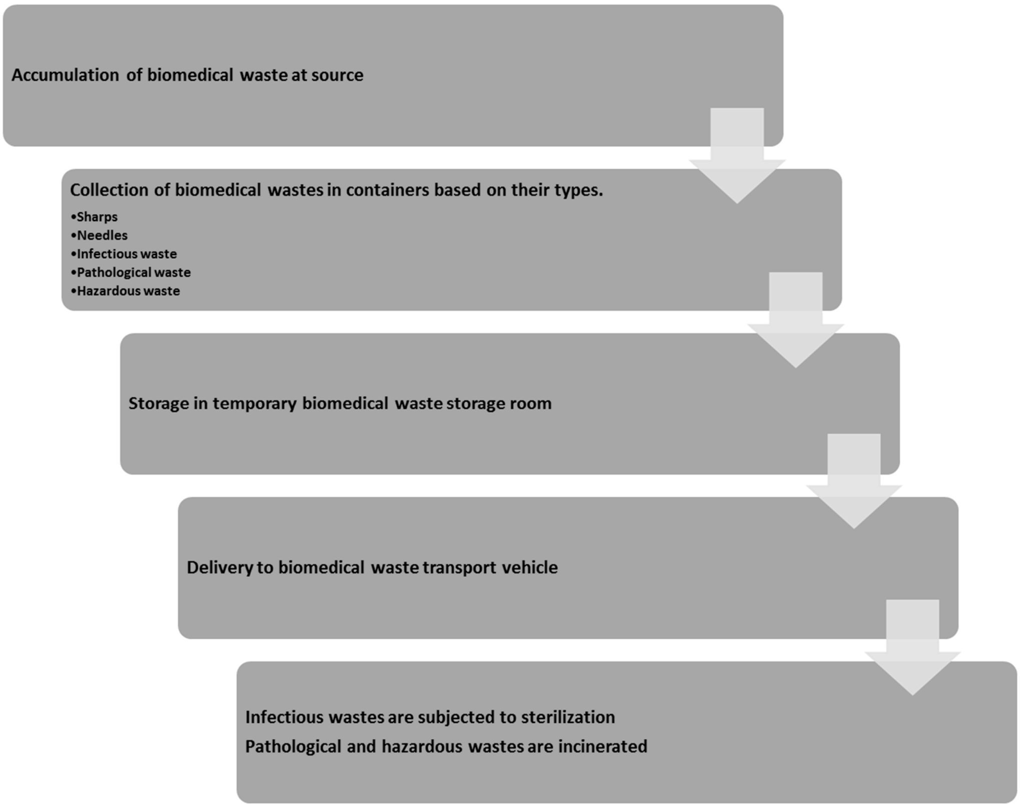

Figure 2. The biomedical waste management system, displaying the relevant steps.

2.3. Sterilization of Biomedical Waste

In this study, a steam autoclave was used to sterilize the pathogens. Autoclaving is an efficient wet heat treatment and disinfection process. The steam autoclave was operated during this study at a contact time of 45 min, a temperature of 150 °C, and at a steam pressure of 5 bar. The minimum time required for contact depends on factors such as the temperature applied, the moisture content of the waste and the penetration of steam into the waste [9]. The following steps were applied during the biomedical waste treatment in this study. 1—Pre-heating: hot steam was injected into the jacket of the autoclave to pre-heat the autoclave; 2—Waste loading: waste containers were loaded into the autoclave. During the loading, the chemical and biological indicators were placed in the middle of the waste load to monitor the sterilization effectiveness. The autoclave door was closed and sealed; 3—Air discharge: air was discharged through pre-vacuuming; 4—Steam treatment: steam was injected into the autoclave chamber until reaching the required temperature; 5—Steam discharge: steam was discharged from the autoclave, by using a condenser; 6—Waste unloading: the treated waste was removed together with the chemical and biological indicator strips; and 7—Mechanical treatment: the treated waste was introduced into a shredder before the final disposal in the Kocaeli landfill.

2.4. Biological and Chemical Testing

The chemical indicator used at every charge was in the form of a strip and was removed from the autoclave tank together with the biomedical waste at the end of each treatment period. The chemical indicator was used if the tank had reached a sufficient temperature by changing the color on the strip. The biological indicator used in the control of the sterilization process was applied for one charge a day as stated in the Turkish biomedical waste regulation. According to the Turkish biomedical waste regulation, sportive bacteria *Bacillus stearothermophilus* or *Bacillus subtilis* standard origins should be used as biological indicators, because these microorganisms are more resistant to high humidity and high temperatures than other disease-causing microorganisms. A minimum reduction of 4 $\log_{10}$–6 $\log_{10}$ is required in *Bacillus stearothermophilus* or *Bacillus subtilis* bacteria spores for the sterilization process to be considered valid. To control this, a certain number of *Bacillus stearothermophilus* spore-inoculated test strips were placed in the middle of the waste in a heat-resistant and vapor permeable container and the system was operated under normal conditions. At the end of the process, the test strips were removed from the waste. At the same time, at least one untreated biological indicator strip was also cultured in parallel as the positive control and incubated for 48 h at 30 °C for *Bacillus stearothermophilus*.

Since it is difficult to determine whether all microbial activities were completely destroyed, a probability function was defined at the end of sterilization based on the number of microorganisms that have survived. This function is often referred to as the reduction of the microorganisms that are most resistant to the sterilization process. Inactivation used today is defined as $\log_{10}$ reduction. This is defined as the difference between the logarithmic numbers of test organisms that can survive before and after the sterilization process and can be expressed by the formula as follows:

$$\log_{10}\ (\mathrm{cfu/g})\ \mathrm{R} = \log_{10}\ (\mathrm{cfu/g})\ \mathrm{TO}\ -\ \log_{10}\ (\mathrm{cfu/g})\ \mathrm{OS} \tag{1}$$

where $\log_{10}$ (colony forming unit (cfu).g^{-1}) R is the logarithmic number of reduction (R) of test organisms, $\log_{10}$ (cfu.g^{-1}) TO is the number of test organisms (TO) tested in the sterilization unit, $\log_{10}$ (cfu.g^{-1}) OS is the number of test organisms that survived (OS) after sterilization, and cfu.g^{-1} is the microorganism colony formation in 1 g of waste. At the end of the sterilization period, the tank was discharged and the sterilized wastes were loaded into the shredder. Then, the shredded wastes were disposed of in a municipal soil waste landfill located near the sterilization plant.

2.5. Greenhouse Gas Emissions

In order to determine the total diesel fuel used by the collection vehicles, an average fuel consumption of 0.5 L per km traveled was selected [20]. Fruergaard, Astrup [21] reported that for each 1 L of diesel fuel, 0.5 kg $\mathrm{CO_2}$-e was generated for provision and 2.7 kg $\mathrm{CO_2}$-e for combustion, which gives a total of 3.2 kg $\mathrm{CO_2}$-e.L^{-1}. These values were selected to calculate the total GHG emissions in this study. Upon determining the total amount of GHG emissions, a GWF for each year was estimated by dividing the total amount GHG emissions by the yearly total collected and treated biomedical waste. The amounts of GHG emissions resulting from electricity consumption at the sterilization plant was determined by using the emission factor of 0.480 kg $\mathrm{CO_2}$-e.kWh^{-1} provided for Turkey by the International Energy Agency (IEA) [22]. The amount of GHG emissions from natural gas consumption at the sterilization plant was determined by using the conversion factor of 10.34 kWh.m^{-3} natural gas (1000 Btu.ft^{-3}) for electricity/natural gas energy equivalence [23].

3. Results

3.1. Quantification of Biomedical Waste

Results of this study showed that the total biomedical waste generated per year increased from 1362 tons in 2009 to 2375 tons in 2019 (Table 2). The amount of biomedical waste generated per hospital bed varied between 0.21 and 2.00 kg.bed^{-1}.day^{-1} with an average value of 1.19 kg.bed^{-1}.day^{-1} as of

December 2019. This range seems to be similar compared to a study conducted in Istanbul, in which the daily averages of biomedical waste amount per hospital varied from 0.43 to 1.68 kg.bed^{-1}.day^{-1} [20]. The average diesel fuel consumed per kg of medical waste collected and transported was calculated as 0.041667 L kg^{-1}. In addition, the average electricity and natural gas consumed at the sterilization plant was calculated as 0.00944 kWh kg^{-1} and 0.02687 m^3 kg^{-1}, respectively.

Table 2. Amounts of biomedical waste collected and treated from 2009 to 2019 and the associated fuel usages.

Year	Biomedical Waste Generated (kg.year^{-1})	Trip Number to Sterilization Plant	Consumed Diesel (L.year^{-1})	Electric Consumed at the Sterilization Plant (kW.year^{-1})	Natural Gas Consumed at the Sterilization Plant (m^3.year^{-1})
2009	1,361,545	1362	56,731	12,855	37,205
2010	1,350,605	1351	56,275	12,751	36,904
2011	1,572,606	1573	65,525	14,846	42,968
2012	1,755,567	1756	73,149	16,572	44,789
2013	1,758,089	1758	73,254	16,596	47,670
2014	1,850,428	1850	77,101	17,467	50,552
2015	1,946,386	1946	81,099	18,373	53,433
2016	2,164,089	2164	90,170	20,427	56,315
2017	2,231,941	2232	92,998	21,067	59,196
2018	2,356,404	2356	98,184	22,241	62,078
2019	2,375,297	2375	98,971	22,420	64,959
Total	**20,722,957**	**20,723**	**863,457**	**195,616**	**556,068**

3.2. Inactivation of Bacillus Stearothermophilus

In order to determine whether all the potentially infectious microorganisms were destroyed in the waste from the sterilization process, it needed to be checked whether the biological indicator microorganisms treated with the waste remained alive or dead. According to the Turkish biomedical waste regulation, a minimum reduction of between 4 $\log_{10}$ and 6 $\log_{10}$ is required in *Bacillus stearothermophilus* bacteria spores for the sterilization process to be considered valid. This was done by placing a certain number of *Bacillus stearothermophilus* spores containing an inoculated test indicator in the middle of the waste in a heat-resistant and vapor-permeable tube. At the end of the charge, the tube containing *Bacillus stearothermophilus* was taken from the waste, and the appropriate medium described by the producer of the biological indicator was plated on an agar medium. Meanwhile, at least one biological indicator that had not been subjected to sterilization was cultured as a positive control and incubated for 48 h at 55 °C for *Bacillus stearothermophilus*. In the chemical indicator test, when the result of the examination was negative, these biomedical wastes were re-sterilized by adding a biological indicator. These wastes were kept in biomedical waste temporary storage area until the biological indicator tests were completed. Even if there was no microbial reproduction as a result of the biological indicator, these wastes were re-sterilized. In this study, biological indicator tests showed that, with a contact time of 45 min, a temperature of 150 °C, and at a steam pressure of 5 bar, the number of living *Bacillus stearothermophilus* decreased significantly. Daily bioindicator tests showed that the removal rates for *Bacillus stearothermophilus* were always greater than 6 $\log_{10}$.

3.3. Greenhouse Gas Emissions

A total of 20,722,957 kg (≈20,723 tons) biomedical waste was generated in the study area between 2009 and 2019. It was confirmed by the authorities that each biomedical waste collection and transport vehicle carried approximately 1 ton of waste in each trip to the sterilization plant. Thus, the total trip numbers between 2009 and 2019 were 20,723, which included a round-trip drive from the first collection point to the sterilization plant and back to the same collection point. It was calculated, based on the information provided by the authorities, that approximately 863,457 L of gasoline was consumed by the biomedical waste collection vehicles between 2009 and 2019. For the calculation of fuel usage, an average diesel consumption of 0.5 L per 1 km traveled was selected, which was also recommended by Korkut [20]. Yearly average electricity and natural gas consumptions at the sterilization plant were provided by the plant operator as shown in Table 2. The total amounts of electricity and natural gas consumed between 2009 and 2019 were 195,616 kW and 556,068 m^3, respectively. Table 3 summarizes the basic data and parameters used in the GHG calculation [21–23].

Table 3. The basic data and parameters used in the global greenhouse gas (GHG) calculation.

Fuel	Unit GHG Equivalency	Consumed between 2009 and 2019	Total GHG Generated in the Study
Diesel	3.2 kg CO_2-e L^{-1}	863,457 L	2763 tCO_2-e
Electricity	0.480 kg CO_2-e kWh^{-1}	195,616 kWh	93.9 tCO_2-e
Natural gas	4.96 kg CO_2-e m^{-3}	556,068 m^3	2758 tCO_2-e

Figure 3 shows the amount of GHG emissions and global warming factors (GWFs) generated during the transportation and treatment of biomedical waste from 2009 to 2019. The amounts of yearly GHG emissions from the consumption of diesel fuel were calculated based on the emission factor of 3.2 kg CO_2-e.L^{-1} [21]. The total amount of GHG emissions generated from the biomedical waste collection and transportation vehicles between 2009 and 2019 was calculated as 2763 tCO_2-e.$year^{-1}$. The International Energy Agency (IEA) reported that 1 kWh of electricity consumption in Turkey can generate 0.480 kg CO_2-e [22]. Thus, by using the emission factor of 0.480 kg CO_2-e.kWh^{-1}, yearly GHG emissions from electricity consumption varied between 6.12 and 10.76 tCO_2-e.$year^{-1}$. The total amount of GHG emissions generated from the electricity usage at the sterilization plant between 2009 and 2019 was calculated as 93.9 tCO_2-e.$year^{-1}$. The conversion factor for the electricity/natural gas energy equivalence was taken as 10.34 kWh.m^{-3} natural gas (1000 Btu.ft^{-3}) [23]. Thus, by using the emission factor of 0.480 kg CO_2-e.kWh^{-1} in this study, yearly GHG emissions from natural gas usage varied between 183.16 and 322.41 tCO_2-e.$year^{-1}$. The total amount of GHG emissions generated from the natural gas usage at the sterilization plant between 2009 and 2019 was 2758 tCO_2-e.$year^{-1}$. Finally, the total amount of GHG emissions resulting from the use of diesel fuel for waste collection and transportation vehicles, and electricity and natural gas uses at the sterilization plant, was calculated as 5573 tCO_2-e.$year^{-1}$ from 2009 to 2019 (Figure 3). The GWF values varied between 0.265 and 0.272 tCO_2-e.ton^{-1}, with an average value of 0.269 tCO_2-e.ton^{-1} of biomedical waste collected, transported and sterilized. It can be concluded from Figure 3 that the greater the amount of biomedical waste collected and sterilized, the more GHG emissions generated. As the amount of biomedical waste has increased over time, the amounts of GWF decreased from 0.272 to 0.265 tCO_2-e.ton^{-1}.

Figure 4 shows the amount of GHG emissions resulting from different fuels for biomedical waste collection, transport and treatment between 2009 and 2019. The amount of diesel fuel used by the biomedical waste collection and transport vehicles was used to calculate the GHG emissions from these activities. On the other hand, natural gas and electricity were only consumed at the sterilization plant for different purposes such as lighting, heating and running the autoclaves. The highest GHG emissions were observed from the natural gas use at the sterilization plant. Yearly GHG emissions from diesel combustion varied from 177 tCO_2-e to 312 tCO_2-e during the study period. However, GHG emissions from electricity consumption at the sterilization plant was much lower compared to that of diesel fuel combustion and natural gas use.

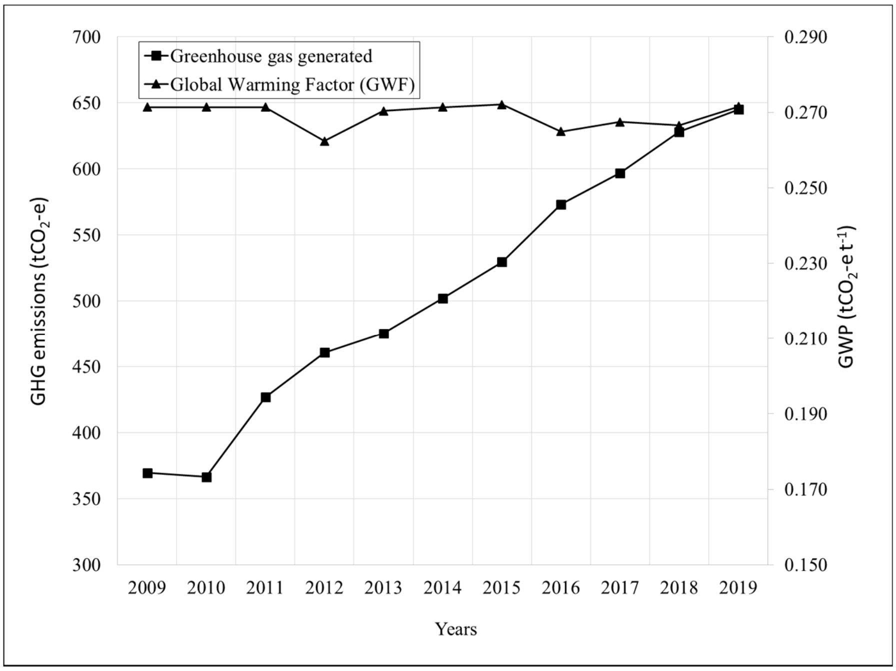

Figure 3. The amounts of greenhouse gases (GHG) versus the global warming factor (GWF) generated during the transportation and treatment of biomedical waste from 2009 to 2019.

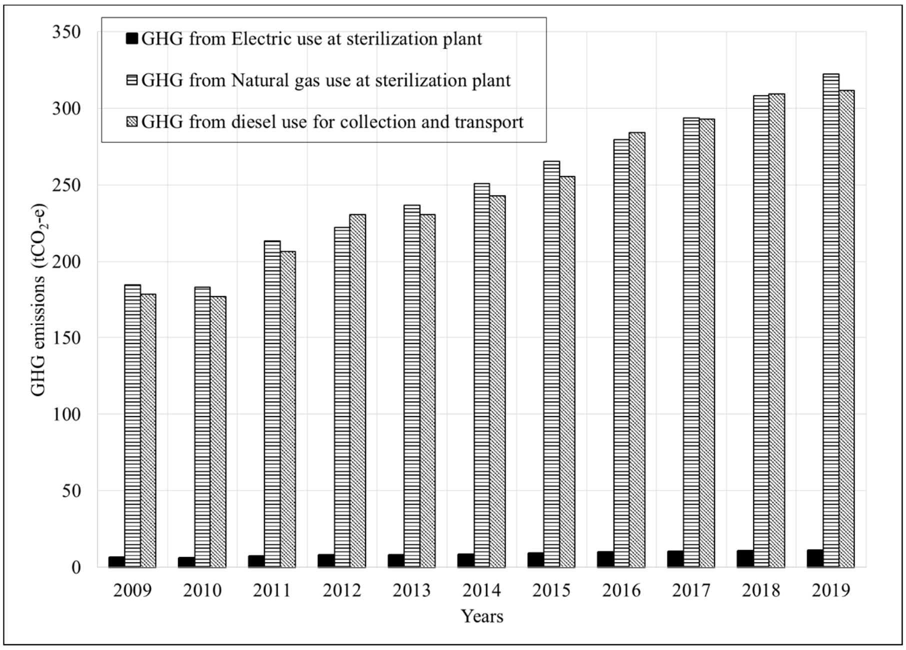

Figure 4. Amounts of GHG emissions from different fuels for biomedical waste collection, transport and treatment between 2009 and 2019.

4. Discussion

In this study, the average amount of biomedical waste generated per hospital bed was calculated as 1.19 kg.bed^{-1}.day^{-1}. In one study, Mato and Kassenga [24] reported biomedical waste amounts varying between 0.84 and 5.8 kg.bed^{-1}.day^{-1}. Abu-Qudais [25] found at five Jordanian hospitals that the average daily biomedical waste generation rates varied between 0.29 and 1.36 kg.bed^{-1}.day^{-1}. In a similar study conducted at Italian hospitals, the biomedical waste generation rates varied between 0.2 and 3.5 kg.bed^{-1}.day^{-1} [26].

Daily routine bioindicator tests have shown that the removal rates for *Bacillus stearothermophilus* were always higher than 6 $\log_{10}$ in this study. Sterilization effectiveness varies with many parameters that affect the heat transfer and vapor penetration, such as waste contents, waste density, moisture content, and container types [27,28]. In order to prevent any damage on the shredder, it was ensured in this study that the incoming waste was always free of metal objects. Sterilization efficiency is usually observed by giving a level of assurance at 10^{-3} or 10^{-6}, which indicates that there is a chance in thousands and millions, respectively. Essentially, this means that at least 3 or 6 $\log_{10}$ pathogen reductions should be maintained. This level of reduction is usually possible because steam sterilization autoclaves are generally operated at minimum standards (121 °C for 30 min or 134 °C for 5 min) [8,10]. The steam autoclave in this study performed at a contact time of 45 min, a temperature of 150 °C, and at a steam pressure of 5 bar. However, some studies claim that these parameters are not sufficient for the complete sterilization of all biomedical waste types [28,29]. For instance, the inclusion of a grinding system before sterilization allows better sterilization due to a larger waste surface area for steam. Shredding transforms waste into an unrecognizable form and provides a volume reduction of up to 80% [7]. The shredder system achieved approximately 70 to 80% volume reduction throughout this study. It should be noted, however, that the use of integrated shredders and autoclaves can cause repeated failures and high maintenance costs [30–32]. As stated by the World Health Organization (WHO), in order to select the best biomedical waste treatment technology, they must pose minimal human health impact, minimal environmental impact, and must be cost-effective and easily implemented [33].

The advantages and disadvantages of five biomedical treatment technologies are summarized as follows [34]: 1—Landfilling: it is one of the oldest methods for biomedical waste disposal in undeveloped countries. However, biomedical waste landfilling includes some serious disadvantages such as the contamination of soil and water, the spreading of pathogens, and high GHG emissions. The only advantage of the landfilling of biomedical waste is the low cost and easy operation; 2—Sterilization: this process is preferred in several applications because it offers many advantages such as excellent efficiency, short treatment times, lower cost, minimum GHG emissions and air pollutants, environment-friendly technology, and the availability of wide range of autoclave sizes. However, sterilization has some disadvantages, such as the odor problem, unsuitability for hazardous and pathological wastes, and shredder requirement; 3—Incineration: the incineration of biomedical waste is suitable for all waste types, provides a high volume reduction, has a potential for energy recovery, and provides complete sterilization. The disadvantages of incineration include the following: a—the equipment is more costly to operate than the other alternatives, b—the process must meet the stringent regulatory requirements of air pollution control, c—heavy metals are usually found in the ash, d—it produces high GHG emissions, and e—dioxins and furans can be generated; 4—Microwaving: microwaving is another technology that can be used for biomedical waste treatment. The advantages of microwaving technology include the following, a—it is an environment-friendly technology, b—it offers high volume reduction, c—it produces no liquid waste, and d—it generates minimum air pollutants. The disadvantages of microwaving include; high cost, not suitable for all waste types, odor problems, and high GHG emissions; and 5—Plasma pyrolysis: this process has the following advantages, a—it is suitable for all types of wastes, b—it occupies less space, c—it is environmentally sound, d—it does not require a chimney, e—toxic residuals are minimum, f—it does not require segregation, g—energy recovery is possible, and h—it can reduce the waste volume by over 90%.

The collection and transport of biomedical waste would likely result in GHG emissions similar to the GHG emissions from municipal solid waste (MSW) collection and transport activities. However, treatment systems for MSW and biomedical waste are completely different, except for incineration. Therefore, GHG emissions from these different treatment systems for MSW and biomedical waste would also be different compared to each other. For instance, a net GWF of −0.274 tCO_2-e.ton^{-1} was reported in the literature in a landfill gas (LFG) combustion unit, which indicated that 1 ton of MSW landfilled in order to generate electricity by burning LFG eliminated 0.274 tCO_2 of GHG emission [19]. In a similar LFG to energy study, Malakahmad, Abualqumboz [35] reported an average GHG emission of 0.291 tCO_2-e.ton^{-1}. Yaman [19] reported GWF of −0.94 tCO_2-e.ton^{-1} from the combustion of MSW (waste to energy), which indicated that the incineration process eliminates more GHG emissions than it generates. This of course takes into account the energy generated from the incineration process that would offset the additional GHG emissions arising from different energy generation systems. Similar to four different studies, GWFs of −0.01, −0.12, −0.2385, and −1.019 tCO_2-e.ton^{-1} were also reported, respectively [36–39]. Khan, Khan [40] and Ali, Wang [41] conducted case studies and reported that the treatment and disposal of biomedical wastes can also be assessed according to their greenhouse gas emission rates.

The current waste management practices of countries can effectively reduce greenhouse gas emissions from the waste sector. For example, a wide variety of mature, environmentally friendly technologies are available to reduce emissions and provide common benefits for public health, environmental protection and sustainable development. Collectively, these technologies can directly reduce greenhouse gas emissions or prevent significant greenhouse gas generation. It also represents an important and growing potential for the indirect reduction of greenhouse gas emissions by minimizing, recycling and reusing waste, the conservation of raw materials, improved energy and resource efficiency, and the prevention of fossil fuel use. It should also be emphasized that there are high uncertainties regarding global waste greenhouse gas emissions resulting from national and regional differences in definitions, data collection and statistical analysis. Reducing greenhouse gas emissions from waste should be addressed in the context of integrated waste management. For instance, life cycle assessment (LCA) is an important tool to consider both the direct and indirect effects of waste management technologies and policies [42–44].

5. Conclusions

In this study, the collection, transport and steam sterilization of biomedical waste and the associated GHG emissions from these processes were investigated. The results of the steam sterilization system performed effective treatment for biomedical wastes containing needles, syringes and other non-hazardous and non-pathological infectious wastes. *Bacillus stearothermophilus* bacteria were investigated for the effectiveness of steam on bacteria during the sterilization process. It was observed during this study that the steam autoclave performed most effectively at a contact time of 45 min, a temperature of 150 °C, and at a steam pressure of 5 bar, to inactivate *Bacillus stearothermophilus*. Under these operational conditions, daily bioindicator tests showed that the removal rates for *Bacillus stearothermophilus* were always greater than $6\log_{10}$.

It was shown in this study that the biomedical waste collection, transport and sterilization processes generated a total of GHG emissions of 5573 tCO_2-e from 2009 to 2019. A large part of the GHG emissions generated in this study was from the combustion of diesel fuel by biomedical waste collection and transportation vehicles and the natural gas consumed at the sterilization plant. On the other hand, the use of electricity at the sterilization plant produced less GHG emissions than that of diesel and electricity use. Furthermore, the average GWF was calculated as 0.269 tCO_2-e per ton of biomedical waste collected, transported and sterilized. The biomedical waste treatment by steam sterilization seems to be a safe and cost-effective treatment method compared to incineration, which can release hazardous air pollutants and GHGs. However, precautions should be taken to reduce the amount of GHG emissions by, for example, using electricity-powered biomedical waste collection vehicles, using solar energy panels on the roofs of sterilization plants, and also using biodegradable biomedical waste collection bags.

Life cycle assessment (LCA) can provide decision-support tools. There are many combined mitigation strategies that can be implemented cost effectively by the public or private sector, using LCA and other decision-support tools. Therefore, as a future work, a complete and comprehensive study of LCA should be conducted to determine the complete GHG emissions from medical waste collection, transport and sterilization process.

Funding: This research received no external funding.

Acknowledgments: The author would like to thank Kocaeli Metropolitan Municipality (Turkey), IZMIT Waste and Residue Treatment Incineration and Utilization Corporation (IZAYDAS) for providing the data, and Imam Abdulrahman Bin Faisal University for academic support.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Mohee, R. Medical Wastes Characterization in Healthcare Institutions in Mauritius. *Waste Manag. (New York, NY)* **2005**, *25*, 575–581. [CrossRef] [PubMed]
2. Mbongwe, B.; Mmereki, B.T.; Magashula, A. Healthcare waste management: Current practices in selected healthcare facilities, Botswana. *Waste Manag.* **2008**, *28*, 226–233. [CrossRef] [PubMed]
3. Rutala, W.A.; Mayhall, C.G. Medical Waste. *Infect. Control Hosp. Epidemiol.* **1992**, *13*, 38–48. [CrossRef] [PubMed]
4. USEPA. Medical Waste. Available online: http://www.epa.gov/osw/nonhaz/industrial/medical/ (accessed on 2 February 2020).
5. TUIK. Turkish Statistical Institute. Available online: www.tuik.gov.tr/ (accessed on 3 February 2020).
6. Toktobaev, N.; Emmanuel, J.; Djumalieva, G.; Kravtsov, A.; Schuth, T. An innovative national health care waste management system in Kyrgyzstan. *Waste Manag. Res. J. Int. Solid Wastes Public Clean. Assoc. ISWA* **2015**, *33*, 130–138. [CrossRef]
7. Voudrias, E.; Graikos, A. Infectious Medical Waste Management System at the Regional Level. *J. Hazard. Toxic Radioact. Waste* **2014**, *18*, 04014020. [CrossRef]
8. Karagiannidis, A.; Papageorgiou, A.; Perkoulidis, G.; Sanida, G.; Samaras, P. A multi-criteria assessment of scenarios on thermal processing of infectious hospital wastes: A case study for Central Macedonia. *Waste Manag.* **2010**, *30*, 251–262. [CrossRef]
9. HCWH. *Non-Incineration Medical Waste Treatment Technologies in Europe*; Health Care without Harm Europe: Prague, Czech Republic, 2004.
10. WHO. *Safe Management of Waste from Health-Care Activities*; World Health Organization: Geneva, Switzerland, 1999.
11. Ananta, E.; Heinz, V.; Schlüter, O.; Knorr, D. Kinetic studies on high-pressure inactivation of Bacillus stearothermophilus spores suspended in food matrices. *Innov. Food Sci. Emerg. Technol.* **2001**, *2*, 261–272. [CrossRef]
12. Rajan, S.; Pandrangi, S.; Balasubramaniam, V.M.; Yousef, A.E. Inactivation of Bacillus stearothermophilus spores in egg patties by pressure-assisted thermal processing. *LWT—Food Sci. Technol.* **2006**, *39*, 844–851. [CrossRef]
13. Iciek, J.; Papiewska, A.; Molska, M. Inactivation of Bacillus stearothermophilus spores during thermal processing. *J. Food Eng.* **2006**, *77*, 406–410. [CrossRef]
14. IPCC. AR4 Climate Change 2007: Mitigation of Climate Change. Available online: https://www.ipcc.ch/report/ar4/wg3/ (accessed on 4 February 2020).
15. Yang, D.; Xu, L.; Gao, X.; Guo, Q.; Huang, N. Inventories and reduction scenarios of urban waste-related greenhouse gas emissions for management potential. *Sci. Total Environ.* **2018**, *626*, 727–736. [CrossRef]
16. UNFCC. National Greenhouse Gas Factors 2016. Available online: https://unfccc.int/process/transparency-and-reporting/reporting-and-review-under-the-convention/greenhouse-gas-inventoriesannex-i-parties/national-inventory-submissions-2018 (accessed on 15 March 2020).
17. REC. *Kocaeli Greenhouse Gas Inventory and Climate Change Action Plan*; REC: Kocaeli, Turkey, 2018.
18. IPCC. IPCC 6th Assessment Report. Available online: https://www.ipcc.ch/assessment-report/ar6/ (accessed on 5 February 2020).
19. Yaman, C. Investigation of greenhouse gas emissions and energy recovery potential from municipal solid waste management practices. *Environ. Dev.* **2020**, *33*, 100484. [CrossRef]
20. Korkut, E.N. Estimations and analysis of medical waste amounts in the city of Istanbul and proposing a new approach for the estimation of future medical waste amounts. *Waste Manag.* **2018**, *81*, 168–176. [CrossRef] [PubMed]

21. Fruergaard, T.; Astrup, T.; Ekvall, T. Energy use and recovery in waste management and implications for accounting of greenhouse gases and global warming contributions. *Waste Manag. Res. J. Int. Solid Wastes Public Clean. Assoc. ISWA* **2009**, *27*, 724–737. [CrossRef] [PubMed]
22. IEA. *Energy Policies of International Energy Agency Countries 2016 Review Turkey*; IEA: Paris, France, 2016.
23. DOE. *Energy Consumption of Die Casting Operations*; US Department of Energy: Washington, DC, USA, 2003.
24. Mato, R.R.A.M.; Kassenga, G.R. A study on problems of management of medical solid wastes in Dar es Salaam and their remedial measures. *Resour. Conserv. Recycl.* **1997**, *21*, 1–16. [CrossRef]
25. Abu-Qudais, H. Techno-economic assessment of municipal solid waste management in Jordan. *Waste Manag.* **2007**, *27*, 1666–1672. [CrossRef] [PubMed]
26. Liberti, L. Optimization of Infectious Hospital Waste Management in Italy: Part II. Waste Characterization by Origin. *Waste Manag. Res. J. Int. Solid Wastes Public Clean. Assoc. ISWA* **1996**, *14*, 417–431. [CrossRef]
27. Maamari, O.; Mouaffak, L.; Kamel, R.; Brandam, C.; Lteif, R.; Salameh, D. Comparison of steam sterilization conditions efficiency in the treatment of Infectious Health Care Waste. *Waste Manag.* **2016**, *49*, 462–468. [CrossRef]
28. Lemieux, P.; Sieber, R.; Osborne, A.; Woodard, A. Destruction of spores on building decontamination residue in a commercial autoclave. *Appl. Environ. Microbiol.* **2006**, *72*, 7687–7693. [CrossRef]
29. Tiller, T.; Linscott, A. Evaluation of a Steam Autoclave for Sterilizing Medical Waste at a University Health Center. *Am. J. Infect. Control* **2004**, *32*, E9. [CrossRef]
30. Tsakona, M.; Anagnostopoulou, E.; Gidarakos, E. Hospital waste management and toxicity evaluation: A case study. *Waste Manag.* **2007**, *27*, 912–920. [CrossRef]
31. Ferreira, V.; Teixeira, M.R. Healthcare waste management practices and risk perceptions: Findings from hospitals in the Algarve region, Portugal. *Waste Manag.* **2010**, *30*, 2657–2663. [CrossRef] [PubMed]
32. Graikos, A.; Voudrias, E.; Papazachariou, A.; Iosifidis, N.; Kalpakidou, M. Composition and production rate of medical waste from a small producer in Greece. *Waste Manag.* **2010**, *30*, 1683–1689. [CrossRef] [PubMed]
33. Hossain, M.S.; Santhanam, A.; Nik Norulaini, N.A.; Omar, A.K. Clinical solid waste management practices and its impact on human health and environment—A review. *Waste Manag.* **2011**, *31*, 754–766. [CrossRef] [PubMed]
34. Khadem Ghasemi, M.; Mohd Yusuff, R. Advantages and Disadvantages of Healthcare Waste Treatment and Disposal Alternatives: Malaysian Scenario. *Pol. J. Environ. Stud.* **2016**, *25*, 17–25. [CrossRef]
35. Malakahmad, A.; Abualqumboz, M.S.; Kutty, S.R.M.; Abunama, T.J. Assessment of carbon footprint emissions and environmental concerns of solid waste treatment and disposal techniques; case study of Malaysia. *Waste Manag.* **2017**, *70*, 282–292. [CrossRef] [PubMed]
36. Smith, A.; Brown, K.; Ogilvie, S.; Rushton, K.; Bates, J. *Waste Management Options and Climate Change. Final Report to the European Commission DG Environment*; European Commission: Luxembourg, Luxembourg 2001.
37. USEPA. A Life-Cycle Assessment of Emission and Sinks. In *Solid Waste Management and Greenhouse Gas*; USEPA: Washington DC, USA, 2010.
38. Chen, T.-C.; Lin, C.-F. Greenhouse gases emissions from waste management practices using Life Cycle Inventory model. *J. Hazard. Mater.* **2008**, *155*, 23–31. [CrossRef]
39. Christensen, T.H.; Gentil, E.; Boldrin, A.; Larsen, A.W.; Weidema, B.P.; Hauschild, M. C balance, carbon dioxide emissions and global warming potentials in LCA-modelling of waste management systems. *Waste Manag. Res.* **2009**, *27*, 707–715. [CrossRef]
40. Khan, B.A.; Khan, A.A.; Ali, M.; Cheng, L. Greenhouse gas emission from small clinics solid waste management scenarios in an urban area of an underdeveloping country: A life cycle perspective. *J. Air Waste Manag. Assoc.* **2019**, *69*, 823–833. [CrossRef]
41. Ali, M.; Wang, W.; Chaudhry, N. Application of life cycle assessment for hospital solid waste management: A case study. *J. Air Waste Manag. Assoc.* **2016**, *66*, 1012–1018. [CrossRef]
42. Thorneloe, S.; Weitz, K.; Jambeck, J. Moving from Solid Waste Disposal to Materials Management in the United States. In Proceedings of the Tenth International Waste Management and Landfill Symposium, Cagliari, Italy, 3–7 October 2005.

43. Weitz, K.; Thorneloe, S.; Nishtala, S.; Yarkosky, S.; Zannes, M. The Impact of Municipal Solid Waste Management on Greenhouse Gas Emissions in the United States. *J. Air Waste Manag. Assoc. (1995)* **2002**, *52*, 1000–1011. [CrossRef]
44. WRAP. *Environmental Benefits of Recycling, an International Review of Life Cycle Comparisons for Key Materials in the UK Recycling Sector*; WRAP: Banbury, UK, 2006.