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Evaluation of Practical and Modern Composting Techniques in Kuwait

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Abstract
Kuwait imports more than 2 million sheep per year to feed its population. Moreover, Kuwait City produces 3.5 million kg of compostable municipal waste each day. Lastly, Desert Storm and Desert Shield has produced 120 oil lakes that need remediation. These three major environmental factors could be resolved by a simple solution, composting. This preliminary study focused on composting carcasses from the imported sheep and associated losses due to transportation. The unique climate conditions in Kuwait presented different challenges to composting. This study is the first step in addressing these environmental concerns.

This research was divided into 2 sections: 1) classic cement slab composting of dead sheep with microbiology assessment, and 2) Tri-Form Poly Ecodrum® composting of sheep with microbiology assessment. The classic method using the cement slab was modified by eliminating the turning step used to keep the process aerobic. Instead, air was introduced directly into the piles of compost. This modification did not shorten the time needed for composting but reduced the manual labor needed for the turning of piles. The Ecodrum® accelerated the composting process by at least five-fold; the four months processing by the traditional cement slab method was reduced to only 20 to 25 days using the Ecodrum®. This is the first report that investigates and details the comparison between classical and modern composting techniques to show their equivalency. The color and texture of the Ecodrum® and the cement slab products were similar. This report details the bacterial and fungal tests for the different compost products..

Keywords: Sheep, Composting, Ecodrum, Firmicutes, Proteobacteria, Ascomycota

Introduction

Kuwait imports more than 2 million sheep per year to feed its population. Disposal of deceased sheep is a problem. Moreover, Kuwait City produces 3.5 million kg of compostable municipal solid waste and garbage each day [1,2] (i.e. 1.55 kg/garbage/person/day, 4 million people, 58% of residue is food residue). In addition, Desert Storm and Desert Shield has produced 120 oil lakes that need remediation. These three major environmental problems could be resolved by a simple solution, ie., composting.

Although livestock production is an important industry in Kuwait, disposal of dead livestock is increasingly difficult. Managing dead animals in livestock growing situations is continually a challenge. As of 2005, the sheep population was over two million [1], and with a simple calculation assuming not less than a 5% mortality rate [3], then the dead carcasses would exceed 100,000 sheep/year. Dumping them in an empty space in the desert or on the backside of properties or farms has been difficult as well as illegal and unacceptable. Today's environmental climate calls for proper and environmentally sound methods of dead animal disposal; several disposal options are available. These methods include burial, burning, constructed disposal pits, composting, incineration, and daily pickup, rendering, landfilling [4], bio-reduction and aerobic or anaerobic digestion [5,6]. While there are many options for disposal of dead livestock, fear of the incomplete destruction of the pathogens limits the use of a number of these methods legally [5,7]. A multitude of limiting factors exist in the disposal of carcasses on and off-farm, including practicality and biosecurity. On-farm disposal methods are often the preference for carcasses due to the economic benefits of eliminating transportation costs. However, biosecurity would remain a concern. This gives composting and aerobic digestion priority in North American farms as methods of disposing the dead livestock [5,8]. The bio-reduction method has the disadvantage of generation of bio-aerosol and possible pathogen survival, ie., scrapie [9,10]. If scrapies is suspected, composting should not be used. Incineration is the only safe method to dispose of scrapie-infected sheep. Bacteria, such as Escherichia coli and Salmonella, are destroyed by heating over 600 C, and were not detected in finished compost[11].

From a broad definition, composting is a controlled, aerobic, biological decomposition of organic matter into humus like products [12,13]. It is similar to natural decomposition, except that it is accelerated by mixing organic waste with ingredients to optimize bacterial growth. The potential benefits for on-farm mortality composting include the following: 1) prevention of nuisance associated with flies, scavengers, and odors; 2) increased on-farm biosecurity to reduce risk of spreading pathogens; 3) recycling nutrients from mortalities; and 4) reduction of the risk of groundwater and surface water contamination [5,1014,15].

In the process of composting, microorganisms break down organic matter and produce carbon dioxide, water, heat, and humus, the relatively stable organic end product. Under optimal conditions, composting proceeds through three phases (see figure 1). First, there is the mesophilic, or moderate-temperature phase, which persists for approximately two days; second, there is the thermophilic, or high-temperature phase, which can last from a few days to several months. And finally, there is a several-month cooling and maturation phase [16,17]. Different communities of microorganisms predominate during the various composting phases. Initial decomposition is carried out by mesophilic microorganisms, which rapidly break down the soluble, readily degradable compounds. The heat they produce causes the compost temperature to rapidly rise. As the temperature rises above to about 40°C, the mesophilic microorganisms become less competitive and are replaced by others that are thermophilic or heat-loving. At temperatures of 55°C and above, many microorganisms that are human or plant pathogens are destroyed. Because temperatures over about 65°C kill many forms of microbes and limit the rate of decomposition, compost managers use aeration and mixing and addition of water to keep the temperature below this point [18,19]. During the thermophilic phase, high temperatures accelerate the breakdown of proteins, fats, and complex carbohydrates like cellulose and hemicellulose, the major structural molecules in plants. As the supply of these high-energy compounds becomes exhausted, the compost temperature gradually decreases and mesophilic microorganisms once again take over for the final phase of curing or maturation of the remaining organic matter" [6].

The decomposition is due to these microorganisms which produce enzymes that are released into the compost [17]. The enzymes are proteins ie.,cellulases, etc., and these proteins do the first stage of decomposition. There is a thin film of water around the organic particle and the coarse material holds an air space in the mix [16,17], allowing oxygen to react with bacteria, fungi, or actinomycetes [6]. Then, these enzymes from these microorganisms digest the microscopic bits of organic matter breaking apart the carbon bonds. The breaking of these carbon bonds results in the release of energy. This energy and associated carbons are used for cell growth and nutrients as well as being released as heat. As the heat accumulates, populations of thermophilic ("heat-loving") microbes become more active and take over the process of sterilizing the pile [20] and breaking down pathogens as well as the excess carbon which becomes carbon dioxide. The carbon dioxide is released into the air along with water vapor, and then into the atmosphere. This is what forms the stable organic material called compost or humus.

In the decomposition of organic matter, multiple reactions, both biological and chemical are occurring at the same time. When enzymes break down the organic matter, many intermediate compounds are formed, such as organic acids and ammonia. These intermediate compounds eventually become food for the microorganisms or get locked up in the humus-like material. Humus may form through the partial breakdown of complex compounds such as lignin, cellulose, fats, and proteins, which are able to associate with each other and form these complex, super molecule structures called humus. Humus occurs partially through the polymerization process that turns simple molecules into complex ones.

The important step in composting for biosecurity is that the pile reaches at least 48.9-65.6°C (thermophilic conversion) in order to destroy pathogens [10,18,21], and fly larvae, and weed seeds (Dahlquist et al 2007). This ensures the degradation of pathogens and reduces the risk of disease and breach of biosecurity. Figure 1 illustrates the compost temperature ranges [22].

In this study, classical composting methods will be compared to composting in the Ecodrum®, a composting machine that can take daily additions of animal carcasses. This allows for the use of continuous composting, increasing efficiency of carcass disposal as well as minimizing costs. The Ecodrum® has the capacity to compost approximately 3000 kg/wk when using six drums connected together and maintains temperatures up to 70°C, thusly, killing most pathogens. The Ecodrum® will be automated to revolve once every six hours with daily additions of carbon matter and organic matter to achieve optimal composting by capitalizing on the continuous capabilities of The Ecodrum®.

This report covers a two part study of composing sheep and the quality of the resultant compost. Part 1 is the cement slab method and the microbiology associated with the degradation process.; Part 2 is the Ecodrum® method of composting sheep and associated microbiology. This process involves putting down a cement slab with at least four inches of organic material on top followed by an animal carcass with an additional 4-12 in of organic material with sufficient water. However, it is by far, the cheapest and most commonly used in the United States [7].

This report is the first study that defines composting in the unique desert conditions seen in Kuwait. The goal of this study is to create a means of managing dead animals from livestock production in an environmentally sound manner that meets Kuwaiti standards and regulations as well as produce a compost that can serve as an acceptable agricultural amendment. Recuperation of associated losses due to the sheep importation is necessary and essential for future studies on waste disposal of the 3.5 million kg per day of compostable municipal solid waste produced by Kuwait City. This preliminary study focuses firstly on efficient carcass composting methodologies.

Materials and Methods

Classic Cement Slab Method

Eighteen dead sheep with a weight not exceeding 100 kg (the upper limit weight of imported sheep in Kuwait), were used in this pilot experiment (three carcasses in each pile) on the cement slab. Six piles were used with two piles each for fine, coarse, and medium shavings/chips as a carbon source. Each slab measured 3 m in diameter and 1.5 m in height. The animals were laid out on a tarmac and covered with 40 cm of used chips. In a classical way of composting large animals, three types of shredded wood can be used as a carbon source. Each pile contained three sheep, with each sheep having average weight of 40 kilograms (120 KG total per cement slab). This classic technique is often used with large animals in North America [7], wherein a cement slab is covered by shavings of different sizes and allowed time to compost. In this study, this technique was minorly modified to reduce physical labor associated with turning of the piles by using a network of piping underneath the compost piles that contained holes down the length of the tubing to ensure proper aeration. Using the described classic cement slab method, carcass compost piles contained three dead sheep within the medium, coarse and fine chips in the pile. This was the carbon source and included materials such as wood chips and straw with a mixture of manure. A controlled amount of water was added and the pile heated up due to the activity of the aerobic bacteria. The initial nitrogen was provided by the urine and manure in the straw.. It is important to maintain a roughly 2:1:1 ratio of manure to sawdust/wood chips to sheep [11,19-22], which maintains the required 25:1 ratio of carbon to nitrogen as measured by a Leco1000 Combustion Furnace Analyzer.

Monitoring of moisture and temperature of the pit is required to verify that controlled parameters are sufficient to maintain optimal composting conditions. Moisture was verified via a Mütec HUMY 3000, and temperature monitored using a 0.5 m stainless-steel thermometer. The C:N ratios were estimated at the beginning of the study when designing the components to be placed on the slab or in the Ecodrum® to ensure the optimal ratio between 25:1 and 30:1 is achieved [23].

Temperature of the slab was measured daily; ambient temperature was on average between 35 to 40°C. The temperature inside the slab increased within the first week and reached 70°C before decreasing to ambient temperature towards the end of the four month period. Temperature of the Ecodrum® was measured daily using the integrated temperature gauge. Similar patterns to the slabs were seen with the Ecodrum®; by the fifth day, temperature had reached 60°C. If the Ecodrum® was not given carbon and nitrogen sources every 17 days, temperatures would decrease to the ambient temperature.

Moisture was measured weekly on the slabs. Moisture data was not recorded during experimentation for the Ecodrum® due to the nature of the system being closed and therefore near impossible to regularly check the moisture content.

Ecodrum® Method

One Ecodrum® (Tri-Form Poly, North Dakota) comprised of six units was fed daily with the same volume of dead sheep (averaging 40 kg each) and sawdust/wood chips making this a continuous process. Medium wood chips were used in the Ecodrum® based on the assumption that in such a short period of time (less than one month), the size of the wood chips was negligible on composting times.

The Ecodrum® was used for more than one ton of organic matter (dead sheep) and carbon source in a controlled recipe over a 21 days period. The end product was received and had an excellent phenotypic and physical characteristics. The Ecodrum® was set to revolve once every six hours using the automated system. 1.765 metrictonnes of dead carcass were added followed by 1.926 metric tonnes of shaved wood as a carbon source for a total of 3.740 metric tonnes of compost matter. The product of the Ecodrum® after 21 days of aerobic composting was sieved and separated from any remaining bones.

While temperature and moisture were read daily for both the slab methods and Ecodrum® method, C/N ratios were only measured upon the completion of the Ecodrum® experimentation due to the closed nature of the system.

Microbial Analysis

The aim was to determine the number of cultural heterotrophic bacteria and total fungi present in 1 gram of samples from each pile at completion and the residue of Ecodrum®. This is equivalent to final phase of substrate depletion.

Serial dilution plate count method was adopted to determine the number of culturable microbes present in the sample (Benson, 2002). Nutrient agar was used for the enumeration of heterotrophic bacteria, whereas Potato Dextrose Agar with 50 µg/ml of streptomycin and 25 µg/ml of chloramphenicol was used for the enumeration of total fungi.

Serial Dilution: 1 g of sample was added to 9 ml of sterile saline mixed well in order to make the sample evenly distributed. This sample was marked as the original dilution. From this 10 ml of original mixture, 1 ml solution were transferred to another test tube containing 9 ml of sterile saline and mixed well. This tube was marked as 10-1 dilution. The aforementioned dilution process was repeated until a 10-6 dilution was achieved.

Spread Plate: The agar plates were marked as 10-2, 10-4, and 10-6. This represents the dilutions of the samples. 0.1 ml of the sample was aseptically transferred from the test tube marked as 10-2 to the middle of the agar plate. Then the sample was spread evenly on the surface of the agar plate using the flame sterilized glass spreader. The process was repeated for the other dilutions in triplicates. Prepared plates in triplicates with 0.1 ml of sterile saline water was used for serial dilution. This was a control sample to monitor the contamination. The nutrient agar plates were incubated at 30°C, whereas the PDA plates were incubated at 25°C.

Microbial Count and Morphology: The number of colony forming unit (CFU) appeared on nutrient agar plates at 24th and 48th hour were counted using colony counter. The plates with CFUs between 30 and 300 were within the countable range and were recorded. The plates with CFUs below and above the countable range were discarded. The number of colony forming unit (CFU) appeared on potato dextrose agar plates at 48th and 72th hour were counted using colony counter. 10-4 and 10-2 dilution was selected to observe different types of bacterial and fungal morphotypes respectively.

Statistics

The chemical analysis was completed utilizing ANOVA, using the general linear model procedure described by S.A.S.Institute [24]. Tukey's multiple range comparison tests were used to separate the means; a value of $p < 0.05$ was considered statistically significant. Temperature and moisture content analysis is shown in Results and Discussions with error bars representative of standard error of mean over triplicates ran for the fine, medium, and coarse sawdust/wood chip cement slab compost piles.

Results and Discussion

There was complete decomposition of the sheep in both the classical slab method versus theEcodrum® method as determined by the sieving of the final product. However, there was a significant difference (p <.05) in the time needed for these processes. The slab method took an average of 4 months in contrast to the Ecodrum® method which completed the process in 21 days. This produced the humus or compost that was desired. The compost for both slab and ecodrum methods were shown to have similar color and texture at the completion of composting to that of the traditional method, showing that these modern composting techniques are equivalent to their classical counterpart in efficacy.

The average temperatures in the slabs are seen in Figure 2. Temperatures of the cement slab compost piles were relatively consistent across fine, medium, and coarse sawdust/chip piles with no statistically significant differences recorded. The temperature within the Ecodrum® compost pile followed a similar pattern to that of the cement slabs but occurred over a 21-day period rather than a 120-day period. Temperatures reached above 65 degrees C which would have eliminated most of the pathological microorganisms [25].

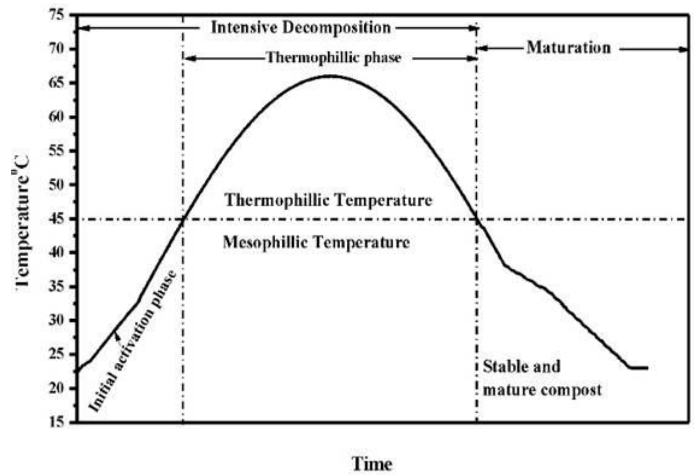

Figure 1: Compost temperature ranges (adapted from Salama et al., 2017).

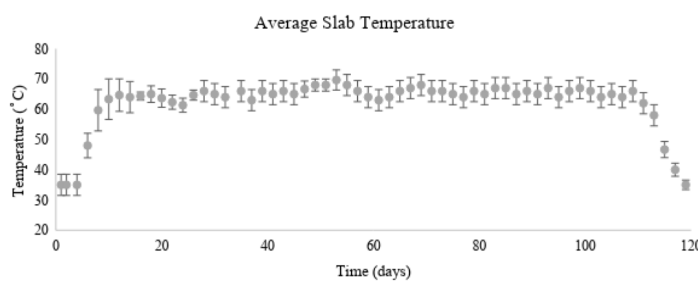

Figure 2: Average temperature per day of fine, medium, and coarse sawdust/wood chip slabs. Error bars represent standard error of the mean.

The moisture content in all slabs followed a similar pattern with no statistically significant differences recorded across the slabs (Figure 3). Water was added to the slabs as needed to keep the temperatures below a toxic level to the microorganisms. The temperature measurements of the Ecodrum® compost followed a similar pattern for moisture content as seen with the slab compost piles except having occurred over 21 days rather than 120 days. This indicates that the Ecodrum® was performing on par with the classic cement slab compost piles.

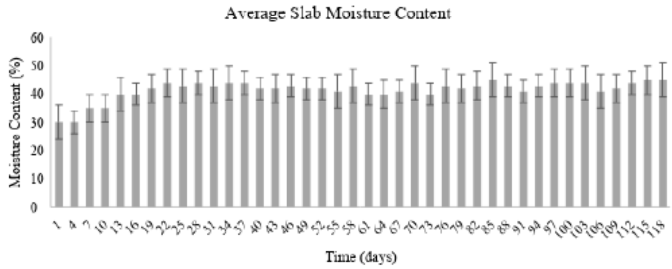

Figure 3: Average moisture content over the 120-day experimentation time across all slabs tested. Error bars represent standard error of the means.

The carbon to nitrogen ratio for optimal degradation is important to monitor throughout composting to ensure optimal microorganism degradation. This C:N ratio requires the correct portion of carbon for energy and nitrogen for protein production; this ratio should be around 25 - 30 parts carbon to one part nitrogen. This ratio was measured once a week in each pile throughout experimentation; these values were then averaged and are shown in Figure 4 below. The overall average was seen to be 26.4:1 across all slabs while the carbon:nitrogen ratio in the Ecodrum® was 25.2:1 on day 21, at the completion of the maturation

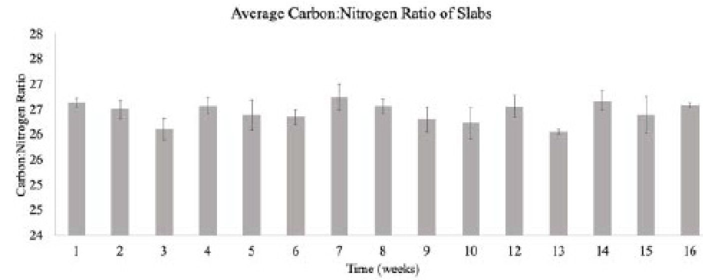

Figure 4: Average Carbon:Nitrogen ratio across the slabs throughout experimentation. Overall, the carbon:nitrogen ratio was 26.4:1. Error bars represent standard error of the mean.

To understand the density of bacteria at the end stages of composting, one-gram samples of each final compost pile were analyzed for total culturable number of heterotrophic bacteria (CFU) at 48 hours. Table 1 shows the results of the bacteria isolated from the completed compost from the slab piles and Ecodrum®.

Table 1: Bacteria cultured from samples of mature compost piles upon completion of experimentation. Beyond those listed, species of bacteria were found in each treatment that fell into a generalized “other” category that were unable to be further defined with the analytical techniques used here.

Treatment	CFU/g sample at 48 hrs	Phylum	Class	Gram +/-
Fine	2.7 x 105	Proteobacteria	Bacillus sp.	-
		Proteobacteria	Bacillus sp.	-
		Firmicutes	Coccus sp.	+
		Firmicutes	Streptococci sp.	+
Medium	2.5 x 105	Proteobacteria	Bacillus sp.	-
Coarse	1.3 x 105	Proteobacteria	Bacillus sp.	-
		Firmicutes	Coccus sp.	+
		Firmicutes	Streptococci sp.	+
Ecodrum®	1.6 x 104	Proteobacteria	Bacillus sp.	-
		Firmicutes	Coccus sp.	+

There were no differences in CFU/g between fine and medium shaving/wood chips, but the CFU/g sample from the coarse shavings/wood chips was less. These results can be seen in Figure 4; fine and medium sawdust/wood chips were shown to have no statistically significant difference between CFU at 48 hours, and the same correlation was seen between coarse and Ecodrum® compost piles. Interestingly, the average of each of those two groups were shown to have a statistically significant difference ($p = 0.006 < 0.05$). This implies that the cement slab compost piles using either fine or medium sawdust/wood chips saw significantly higher numbers of bacteria at 48 hours compared to the coarse and Ecodrum® piles. While this would indicate that a difference could be seen upon completion of composting (fully matured compost piles), analysis of mature piles showed that all composting piles had similar microbial communities across fungi and bacteria.

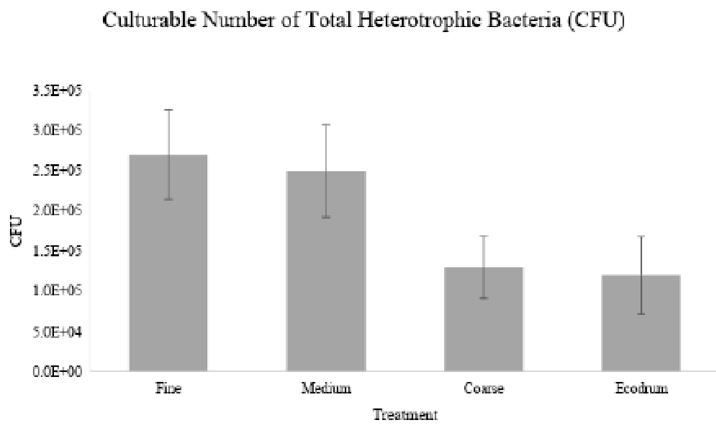

Figure 4: Culturable number of total heterotrophic bacteria (CFU) at 48 hours into composting after inoculation on plate. Error bars represent standard error of the mean. *represents statistically significant difference (p = 0.006).

Bacteria cultured from all compost pile samples at the end of the trial fit the mesophilic temperature range and maturity profile as outlined in Song et al., 2019, and Figure 1. Bacterial culture numbers were greater for fine and medium than they were for coarse and Ecodrum® (Figure 4). Mostly represented in the mature compost piles were Firmicutes and Proteobacteria(Table 1), which are well documented to contain a variety of mesophilic bacterium that are known to exist in mature compost piles. Galitskaya et al. have used 454 pyrosequencing to better define the species seen in compost piles with carbon to nitrogen ratios between 25:1 and 30:1 with a moisture content between 40% and 60%, which confirms the efficacy of the bacterial composting seen in this experiment. Further, this data is consistent with data presented previously [19]; Proteobacteria are consistently the highest occurring bacterial phylum in composting piles followed by phylum Firmicutes. It has shown that in the succession of bacteria and proteins that degrade compost components are mostly cellulose degraders. These phyla of bacteria are likely common in composting due to their high cellulase activity, indicating their likeliness to be found in compost piles that maintain sufficiently high carbon to nitrogen ratios.

Fungi found were all of the Ascomycota phylum (Table 2), which are well known as cellulose degrading fungi [26]. This indicates that compost piles on the slab and in the Ecodrum® provided a sufficient environment for the desired fungal microbes as described by Song et al. [18], which validates the efficacy of fungal composting seen in this experiment. Fungal activity during the mesophilic and mature phases of composting is greatest, while cellulose degrading fungi are not conclusively active during the thermophilic phase [27-42]. This is to say most of the complex cellulitic compounds are degraded during the maturation phase, when active decomposition is taking place.

Table 2: Fungi cultured from samples of mature compost piles upon completion of experimentation

Treatment	CFU/g sample at 48 hrs	Phylum	Class
Fine	7.1 x 104	Ascomycota	Conidia sp.
		Ascomycota	Spherule sp.
Medium	5.5 x 104	Ascomycota	Saccharomycetes sp.
		Ascomycota	Spherule sp.
Coarse	5.5 x 104	Ascomycota	Spherule sp.
Ecodrum®	3.7 x 104	Ascomycota	Spherule sp.

As seen in Figure 5 below, there were no statistically significant differences between culturable number of fungi across all slabs and the Ecodrum®. This indicates that the modern technique of Ecodrum® composting has equivalent culturable numbers of fungi to the classical slab method of composting. Though the numbers of fungi are noticeably smaller, they contribute to most of the cellulose degradation that occurs in composting piles. As stated, this degradation occurs mostly during the mature phase of composting, when the temperature range is better suited for fungal activity.

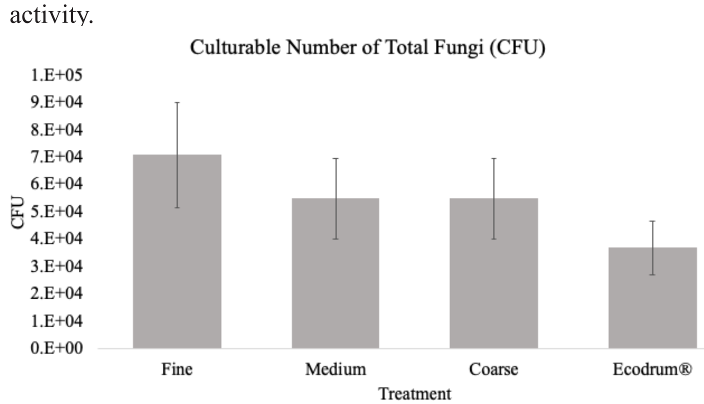

Figure 5: Culturable number of total fungi (CFU) at 48 hours into composting after inoculation on plate. Error bars represent standard error of the mean.

Conclusion

The results of this study clearly show that while classic composting methods and modern composting methods have similar efficacy in their ability to fully degrade carcasses, modern composting techniques are five times as fast and therefore much more efficient. Based on these results, it is believed that modern composting via the Ecodrum® should be implemented as the preferred method of composting over classic cement slab composting in Kuwait as an industry standard. This increased compost efficiency allows for recuperation of capitol lost in the importation of over 2 million sheep required to feed the people of Kuwait each year. Faster composting times via Ecodrum® composting units result in lower labor costs and rapid production of compost soil that can be used for the bioremediation of Kuwait's 120 oil lakes, as well as reduce the land space required to compost these sheep carcasses. These oil lakes will require huge quantities of composted soil which can be efficiently produced domestically in Kuwait using Ecodrum® composting in combination with classic cement slab composting to help the Kuwaiti economy and environment. This research acts as a pilot study that shows what can be done with composting municipal solid waste that can result in better soil condition of Kuwaiti's arid desert lands. Future studies will be performed on efficacy of Ecodrum® produced compost soil for the growth of leafy greens in greenhouses located in arid environments for further local uses of this valuable product.

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