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Efficacy of Passive Solar Heating on Hygienization of Enteropathogens Contaminated Biowaste during Anaerobic Digestion

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Abstract: The use of biowaste as feedstock in anaerobic digestion is a best strategy in managing biowaste. Energy recovery in the form of biogas and enhanced soil fertility through digestate which recycle plant nutrients back to soil ensures sustainable agriculture. Unfortunate the presence of potentially infectious pathogens in biowaste as a result of practices such as haphazardly dumping of waste fractions in comingled with infectious waste poses health risk to humans and environment. Other bad practices including human open defecation, poor handling of pets, animals and avian excreta in cities equally contribute the pathogens load. In that regards, there has been biosafety concern on biowaste use given the involving threat of infectious pathogens and risks of spreading diseases to digestion facility workers and other environmental components. In this lab-scale study, the efficacy of passive solar heating at effecting hygienization in biowaste and therefore curb the emerging risks of pathogens contamination was investigated. Potato peels artificially contaminated with *E. coli* and *S. senftenbergensis* was used to simulate enteropathogen contaminated biowaste. Within 15 days of drying, the temperature of 68.3˚C reached on top of the peels and 56.7˚C inside the peels heap resulted into dried potato peels with 8.1% moisture and fully pathogens inactivation above 6.0logCFU/g or (99.999%). These results conform well to the requirements in Regulation (EC) No. 208/2006. On reconstitution, the dried peels attributes were restored conforming to feedstock quality applicable to wet anaerobic digestion.

Keywords: solar drying, biowaste, biotreatment, hygienization, pathogens

1. Introduction

Despite the commendable role played by anaerobic digestion technique in management of biodegradable organic fraction of municipal solid wastes also known as biowaste [1-3], and the realizable benefits including providing renewable energy in the form of biogas [4, 5], providing digestion effluent (digestate) which is both biofertilizer and soil conditioner [6, 7] and an economic aspect of employment to biotreatment facility workers, the presence of potentially infectious pathogenic microorganisms (bacteria, virus, parasites, protozoa, fungi) and many other vectors in biowaste [8-11] greatly limit the potential to fully utilize biowaste as resource and feedstock for anaerobic digestion. This is due to the involving threats of pathogens contamination and possibility of spreading the pathogens to other environmental components [8]. Over the past years, there has been an increase in reports on outbreaks of food-borne illness and fatality cases in many parts of the world connected to human consumption of partially cooked or improperly blanched fresh farm and /or garden produce including leafy green vegetable and ready-to-eat vegetable that have been harvested from soil confirmed to have been preconditioned with digestate produced from anaerobic digestion of biowaste[12-14].

Application of unhygienized digestate on farmlands (crops and garden fields) as organic fertilizer after biological treatments is considered to be among the major routes of disseminating the pathogens into ecosystems. Most of the biowaste collected as part of municipal solid waste from cities and towns are known to contain pets' excreta which mostly contribute enteroviruses, protozoa, enteropathogens both coliforms (Klebsiella, Enterobacter, *Escherichia coli*, etc) and non-coliforms (Salmonella, Shigella, etc) [15]. Other pathways that introduce pathogens into biowaste include fecal matter from livestock such as cattle, goats, sheep and pigs, and droppings from avian (chicken, pigeons, guinea fowl, turkey, etc) kept under free-range system and/or sold at traditional open markets. Other contributors of enteric pathogens in MSW and consequently in the biowaste include septage and human open defecation, poorly managed wastewater, pit latrine emptying practice, septic tanks flooding out [16] and haphazardly dumping of residential generated solid waste in comingled form including kitchen waste and yard waste [17] with infectious medical waste, woman sanitary pads[18] and baby diapers[19].

Endowed with small investment capital (mainly do it yourself construction), independence from fuel supply and low running costs, passive solar heating (PSH) conventionally known as natural-circulation solar drying based on solar tunnel dryer stands to be commercially viable and ease to use method that can be used for inactivation of pathogens in biowaste during anaerobic digestion process. The most important raw materials for

this drying technique are solar radiation and air currents which are readily available in normal ambient environment. PSH produces an elevated temperature which directly induces pathogens die-off or evaporates water from the biowaste to a lower amount that is unavailable to the pathogens hence causing inactivation. The motivation behind carrying this research was therefore to apply and feasibly study the hygienization efficacy of PSH in biowaste hygienization and its contribution as an alternative hygienization options that could be used to curb the threats associated with spreading of infectious pathogens while dealing with biowaste for achieving total biosafety during anaerobic digestion and consequently safeguard the environment and public health at large.

2. Materials and Methods

2.1 Study samples

In this laboratory-scale investigation, potato (*Solanum tuberosum* L.) peels presenting varsity of biodegradable organic fractions from municipal solid waste streams. Potato tubers were bought from tradition open market at Stadthalle straβe 03046 Cottbus. The peeling was done manually for which 4kg of potato tubers gave approximately 1kg of the peels.

2.2 Enteropathogens simulation in biowaste and characterization

Figure 1 describes the formulation of the study sample. Two surrogate enteric microorganisms namely *Escherichia coli* (*E. coli*) and *Salmonella senftenbergensis* (*S. senftenbergensis*). *E. coli* was supplied from Leibniz Institute, Braunschweig while *S. senftenbergensis* was supplied from the Institute of Bioanalysis, Environmental Toxicology and Biotechnology, Halle in Germany. These two surrogate strains represent a wide range of enteric pathogens ranging from heat sensitive to most heat resistance. The two microbial pathogens were propagated in nutrient broth (Carl Roth GmbH). The enriched bacteria cells were filtered under 2.1mm Ø (Isopore Membrane Filters) and their viability status confirmed using Fluorescent Microscope (NiKON Eclipse LV 100) using 2 phase contrast at 20fold magnification. Before spiking, the bacteria cells were counted using NIS Element BR 3.1 software.

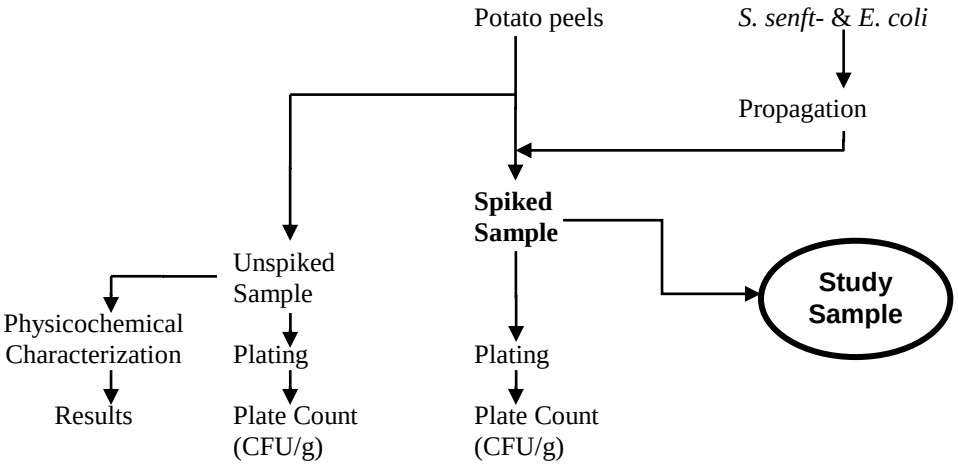

Figure 1: Layout of formulating and simulation of study sample

Based on the observed spatial distribution of live/dead bacteria cells, the final total bacteria cells liable for spiking was calculated according to the following equation:

$$\text{Cells/ mL} = \frac{\sum Cells}{\text{Segment/ Area}(mm^2)} * Filter(mm^2) \frac{1}{Sample(\text{mL})} * \frac{1}{Dilution} \quad (1)$$

Simulation of contaminated biowaste to be used in the experiment was done through contaminating half of the peels portion with pre-propagated *E. coli* and *S. senftenbergensis* suspension menstruum giving an initial bacterial density of $2.3x10^7$CFU/g and $1.3x10^8$CFU/g *E. coli* and *S. senftenbergensis* respectively. The other portion of uncontaminated peels was further divided into two portions. One portion was used to study some important physicochemical attributes of the peels in connection with supporting the survival of the spiked pathogens. To the other remained portion of unspiked peels, bacterial count based on plate count technique was carried out so as to establish background bacterial contamination density in case the potatoes had contamination before the spiking process.

2.3 Solar heating experimental design and working mechanism

The heating of enteropathogens contaminated potato peels was carried out using a model solar drying tunnel of the type *"Hohenheim"* having dimension size of 2mx1mx0.06m (length, width and height). Figure 2 describes the equipment. The dryer is made of flat wood half painted in black colour which save as a collector of solar incident rays while the other half is used as a drying point. Heat generated on the sunlight collecting area is blown by a fan to the sample placed on drying area at the other end of the wood to effect the drying process.

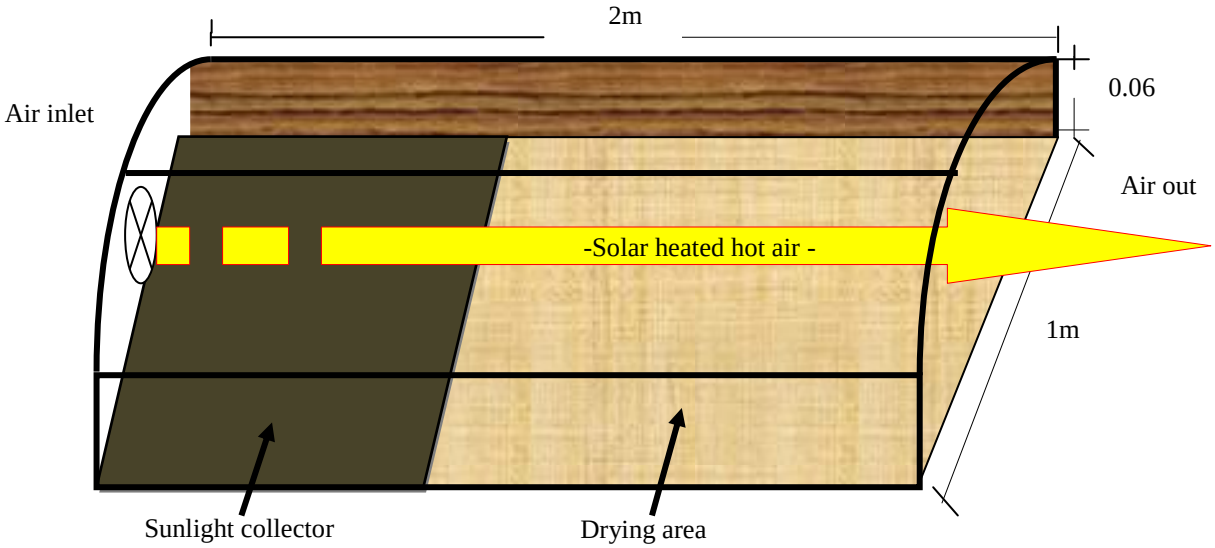

Figure 2: Passive solar heating model based on solar drying tunnel

As stated elsewhere [20], an automatic fan create higher air flow that takes evaporated moisture and volatile matter outside during the active drying period of the sunny day and during the night, cold air is blown inside the drying chamber to avoid condensation. Moisture evaporation from the material is a function of volumetric flow rate of inlet hot air blown using an automatic fan to the drying material. Figure 3 describes the solar drying process.

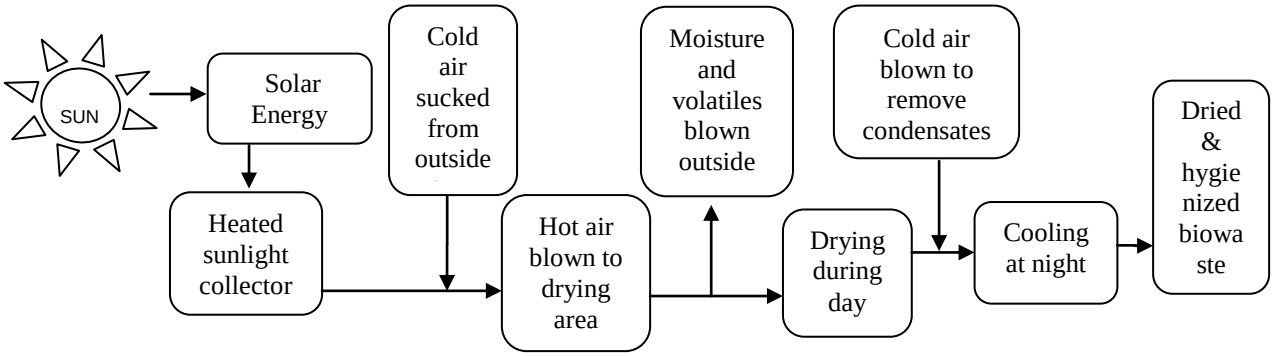

Figure 3: Layout of solar drying of pathogens contaminated potato peels

Depending on the sunlight strength, the photovoltaic cell produces more power to run the ventilator (solar driven fan) and therefore making not possible for the formation of condensate on top of drying materials. The sunlight passes through transparent plastic roof which also help to insulate the high temperature generated inside the solar tunnel from not escaping, resist UV radiation as well as protecting the material inside from dusts and other falling contaminants. The other end of the solar tunnel is mounted with wire meshed gable (mosquito net size) that prohibits insects and other vectors from entering the tunnel while at the ventilator; the wire mesh strainer is mounted for the same purpose. Air velocity entering the dryer and that leaving the dryer are to be calculated so as no pressure drop occurs in the drying chamber. The choice of ventilator therefore depends on the desired volumetric flow rate which is determined based on the size of the sunlight collector.

Using the relation that $1m^3/h = 2.8x10^4 m^3/s$ volumetric flow rate, the specified dryer base area used in this lab-scale study was estimated to be $2m^2$; with this size of the drying area, the recommended ideal air velocity at the dryer outlet is 0.2m/s. The cross section area of wire meshed gable for keeping pressure constant in the dryer during both active sun-shine and at night including cool days is therefore established using the followings equation:

$$Area(m^2) = (a \times h) + \frac{(H-h)}{6a}\left[3(H-h)^2 + 4a^2\right] \quad (2)$$

2.4 Temperature and Moisture monitoring

Oven drier (Haraeus 230V 50/60 Hz class II, Germany) set at 105˚C was used for moisture determination in the sample while total bacteria count technique was used for monitoring enteropathogens die-off. Temperature and sample moisture reduction were the two parameters monitored during solar drying experiments. The sensor probes for temperature reading were calibrated using LAUDA eco Re.104 thermostat for a confidence temperature interval of 25.0±0.4°C. Seven sensor knobs each 30cm long were used for automatic temperature recording at the interval of 15min. The data were automatically recorded in computer using HP VEE-Lysi ME 300-4-2015 hp VEE computer programme. Six out of the seven calibrated knobs were directly inserted inside the solar drying tunnel while the other two were for external temperature monitoring (room and the outside temperatures) for process control. To avoid bacterial contamination on the heating bed, the samples were placed in trays with a sample volume ratio of 1:3:6 (Height: Width: Length). In all experiments the sample height was maintained at 5-10cm. Temperature attained by sample was measured by two sensor probes in which one sensor was inserted inside the sample to monitor the in-sample temperature and the other sensor was placed at the top of the sample holding container to record the up-sample temperature (heat blown by the ventilator from the sun light collector).

2.4 Monitoring Pathogens inactivation after solar treatment

Both Endo agar and modified BGA were used for culturing and enumeration of bacteria colony units. As described in Regulation (EC) No. 208/2006, about 25g of solar dried contaminated potato peels was collected and monitored for enteropathogens existence and survival at every 24hrs interval of sample drying process. Sample portions drawn from the solar drying tunnel were suspended in sterilized water in the ratio of 1g sample: 10mL water. The mixture was gently agitated at room temperature 23±2°C at 130rpm (Janke and Kunkel Ika labortechnik Ks 501 digital, Deutschland) for 15min which is just below both *E. coli* and *S. senftenbergensis* specified doubling time [21, 22]. The resulting slurry was serially decimal diluted 10^{-1} to 10^{-6} and a volume equal to 100µL of the appropriate dilutions made was surface spread on two petri-plates one with Endo Agar for *E. coli* confirmation and the other with modified brilliant green agar (BGA) for confirming *S. senftenbergensis*. Viable bacterial colony enumerations and visual identification on the plate was done after incubation (Incubator Model 600, Memmert) of the petri-plates at 36°C for 24 hrs. The number of bacteria colony units enumerated was expressed as colony forming unit per gram (CFU/g). The original sample was also plated for assurance of both absence of background bacteria contaminated the peels and complete inactivation of the pathogens. Verification of mould growth in the samples of solar dried potato peels was also conducted. In this experiment, a three drying-wetting cycles were conducted in which a sample of solar dried peels was rewetting at approximately 30-50% moisture, stored in black plastic backs to maintain dark condition. The vessels were maintained at room temperature (18-23˚C) for 24h.

3. Results and Discussion

3.1 Hygienization efficiency of passive solar heating on potato peels

The use of solar drying tunnel targeted firstly reduction in sample moisture below the minimum requirement of the pathogens (Figure 4) and secondly raising temperature higher above the pathogens surviving ranges which theoretically are reported to be 46˚C and 65˚C for *E. coli* and *S. senftenbergensis* respectively.

Figure 4: Sensory quality of solar dried potato peels

3.2 Characterization of contaminated potato peels

The selected physicochemical and microbiological attributes of potato peels after simulation experiment and the monitoring results for temperature rise during passive solar drying of the peels are shown in Table 1**.** Potato peels are among the high moisture content biowaste. The recorded moisture of above 75%w/w presents an extreme moisture content explain the existing challenge faced during collection, handling, storage, transportation of biowaste and their attraction to both vectors and infectious pathogens thus limiting their use as feedstock in most biotreatment options. The spiked *E. coli* and *S. senftenbergensis* were in a high enough population loads above most reported environmental levels of contamination [23].

Table 1: Selected physicochemical and microbiological level of simulated biowaste

Parameter	*Results*	*Unit*
Moisture	77.3±0.6	% w/w
Temperature	26.0±0.5	˚C
E. coli	$2.3x10^7$	CFU/g
S. senftenbergensis	$1.3x10^8$	CFU/g

3.3 Temperature development during PSH and monitoring

Drying process and thus pathogens inactivation in potato peels took a total of 16 days (see Figure 5). Within these days of drying, the maximum recorded temperature inside the solar drying tunnel was 79.1˚C on the days with good sunshine while the minimum was 44.3˚C in cloudy and foggy days because of poor sunshine. The average maximum temperature from collecting zone that effected drying and bacteria die-off therefore was 68.3°C while the average temperature inside the drying potato peels was recorded to be 56.7°C.

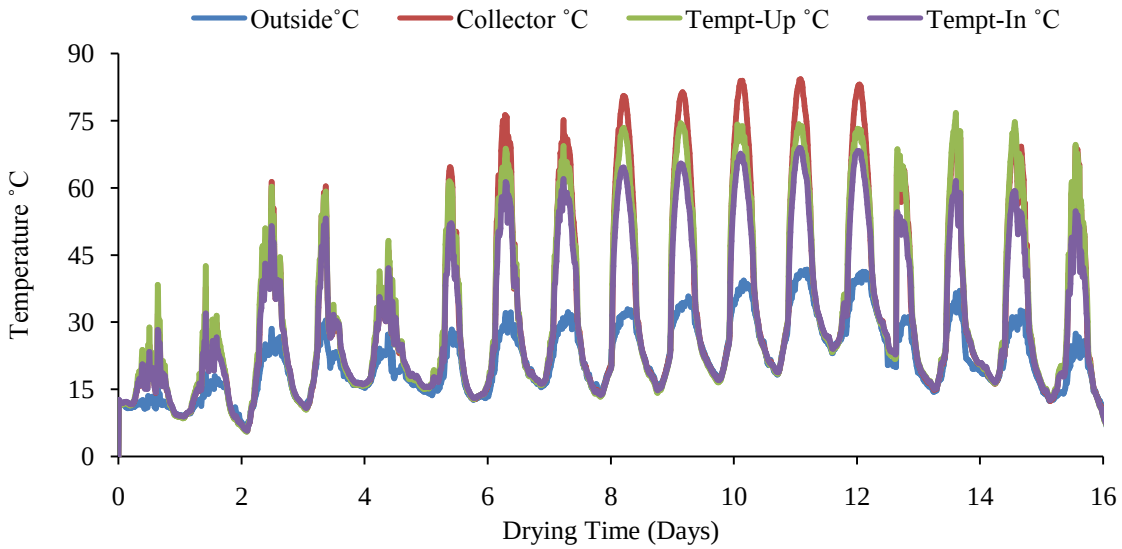

Figure 5: Temperature-Time patterns of passive solar heating of contaminated potato peels

The observed pathogen inactivation time during drying of the potato peels conforms to the time suggested in the Federal Biosolids Technical Regulations which recommends 55°C for 15 days. Denmark, The Netherlands specification and Germany biowaste Ordinance for compost pathogens equally recommend temperature-time combination of 55˚C for 2 week to be sufficient at achieving complete pathogens inactivation [24].

The derived collector area for the solar drying tunnel had 2.0m^2 and the total cross sectional area of the gable was 0.148m^2 giving the required ventilation of 0.14m^2 that blow hot air used at hygienization of enteropathogens contaminated sample. Since the UV radiations from the sun estimated to be 550-1075W/m^2 [25, 26] are resisted by the transparent plastic roof, therefore pathogens reduction or die-off in solar drying tunnel could have caused by reduction in moisture and rise in temperature. Reduction in moisture content causes an increase in dry matter and mineral concentration which lower water activity a parameter that support bacteria cells. For instance, *Salmonella spp* optimally grow at A_w0.93 or higher [27] and *E. coli* requires A_w >0.950 [28]. Temperature higher above the pathogen cells survival ranges normally reported from 55˚C for *E. coli* [29] and above 65˚C for *S. senftenbergensis* [30] denature cell protein thus causing completely bacterial inactivation.

The pathogens die-off in both bacterial strains had similar patterns suggesting that moisture reduction and temperature were both affecting the pathogens in the similar way (see Figure 6). Bacteria need water as a media for transporting nutrients into their cells. In the first 3 and 4 days of solar heating, the population of *S. senftenbergensis* in the sample was surprisingly reduced at a higher rate than that of *E. coli* regardless of the proven heat resistance and the initial population of *S. senftenbergensis* spiked in the sample. This might be attributed to the presence of inhibitors such as protocatechuic acids, gallic, caffeine and chlorogenic which are bacteriostatic and bacteriocidal exhibited in potato peels as earlier on extracted and reported in Schieber and Saldaña [31] and Al-Weshahy and Rao [32]. In the second week, the generated heat during drying process induced stress to the microbial cells thus probably increased heat resistance especially to *S. senftenbergensis*. Also the reduced moisture content of the sample increased the mortality rate of the enteric pathogens as water requirement for their survive is estimated at 15% [33].

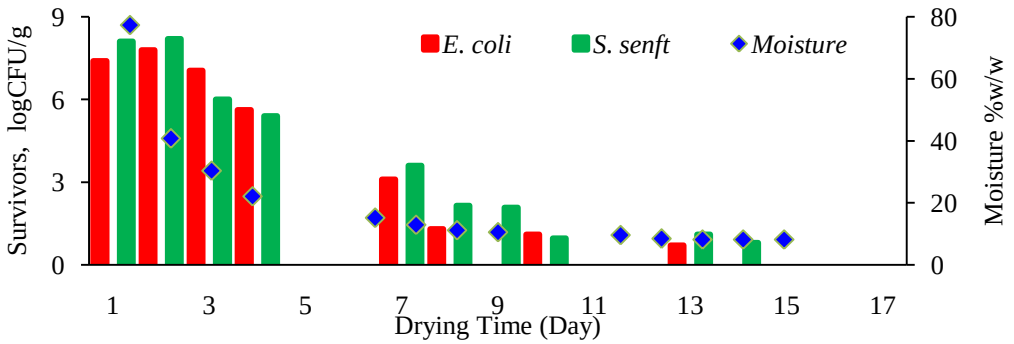

Figure 6: Pathogens inactivation and moisture reduction from potato peels during solar drying

The average heating temperature in the solar drying tunnel was 56.7°C. This temperature corresponds well to the one recommended in other studies including Wichuk and Mccartney[20] and Christensen et al.,[34] reported to effectively produce a complete inactivation of microbiological pathogens in the final products. This temperature range managed to reduce moisture content in potato peels from its initial amount of 77.3% w/w down to 8.1% w/w. This efficiency of moisture reduction to <10%w/w in PSH has been equally reported in other studies by Mohanraj and Chandrasekar [35]. In their study they observed that the rate of moisture reduction from chill was from 72% to 9.1%. Similarly, Sarsavadia [36] reduced moisture content from 86% to 7% in onion using a solar-assisted forced convection dryer. The drying time taken for inactivation of pathogen in this study compares well with the results reported in other investigations using different methods and matrix. For instance Déportes et al., [19] in their study, it took about 8-13days to eliminate *Salmonella spp* at temperature more than 55°C. In our case, *S. Senftenbergensis* being the most heat resistant, definitely more time was required to ensure its complete inactivation given the changing weather conditions which was the late spring period of the year. On the eighth-day of continual solar heating the moisture content in the sample dropped from 77.3%w/w to 12.9%w/w in which there was a maximum reduction in the enterobacteria. At this sample moisture content there was only 1.3log *E. coli* and 3.6log *S. senftenbergensis* demonstrating >6.0log reductions (99.999%) in both *E. coli* and *S. senftenbergensis*. There was no growth of either *E. coli* or *S. senftenbergensis* observed in solar dried potato peels in the sample tested on day 15, thus confirming the end of solar treatment with 8.1% moisture remaining in the sample.

3.4 Decimal reduction (D-value) of enteropathogens following solar drying

Figure 7 shows the D-values at 28-69˚C of the two enteropathogens strains following passive solar heating process. In this investigation, the D-values for achieving 1-log cycle reduction of *E. coli* during solar drying of contaminated potato peels waste was found to be 1.92 days while that of *S. senftenbergensis* was determined to be1.94 days. The D-values seem similar probably because two factors i.e. water stress and the heat both affected the pathogens survivor simultaneously hence effected their die-off equally and at the same rate.

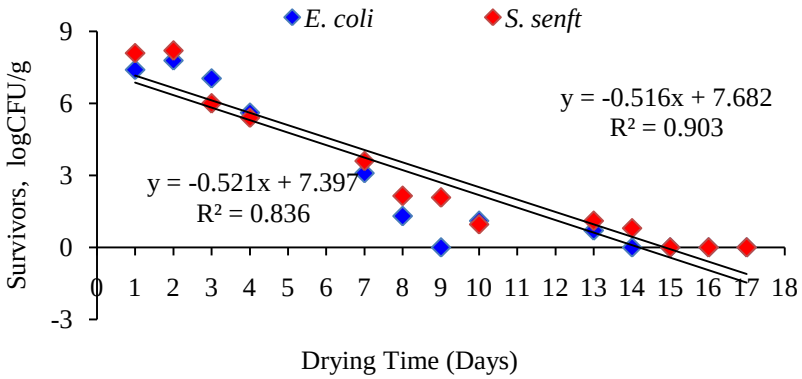

Figure 7: D-value of E. coli and S. senftenbergensis during solar drying

Since *E. coli* is not particularly heat-resistant compared to *S. senftenbergensis*, the similar D-values obtained suggest that the pathogens die-off during passive solar heating does not depend on heating only but rather on other factors including water stress. It is reported that reduction in water results into an increase in mineral salts content in the sample and affect water activity [28].

3.5 Post-solar drying attributes of enteropathogens spiked potato peels

The physical, chemical and microbiological quality of the solar dried potato peels after the solar drying experiment is shown in Table 2 below. Except for physical appearance and moisture content of the solar-dried potato peels, solar drying did not affect the quality of the peels. Moisture content was reduced for nearly 10-folds thus causing about 4-folds elevation in the sample total solids but with no significant changes in the organic total solids (oTS) calculated as percent of total solids (TS).

Table 1: Quality of solar dried pathogens contaminated potato peels

Parameter/ Units	*Before drying*	*After drying*
Moisture, %w/w	77.3±2.4	8.1±0.3
TS, %	22.7±1.7	91.9±0.2
oTS, %	94.7±2.0	94.3±1.2
pH	6.0±0.3	6.5±0.1*
C/N ratio	18:1	23:1
Conductivity, mS/cm	2.6	5.3*
Salinity, ‰	0.2	2.8*
E. coli (CFU/g)	7.4log	< LoQ
S. senftenbergensis (CFU/g)	8.1log	< LoQ

* Values determined after dispensing blended peels into water [(peel (1g)/ water (4mL)]
(± =uncertainty for 95% confidence)

As stated in Deublein and Steinhauser [37] and Weiland [38], the dried potato peels could be used for anaerobic digestion after water adjustment to 85-95% w/w for wet digestion. Upon water addition 1:4 (solar-dried peels to water), the peels showed improvement in pH 6.5, Salinity 2.8‰ and electrical conductivity of 5.3mS/cm. It was worth noting that, the solar drying of peels improved C/N ratio from the original value of 18:1 to 23:1. The observed units increase in the C/N ratio definitely comply to the loss of moisture together with some volatile compounds of nitrogen including ammonium–nitrogen (NH_4^+-N) and nitrate (NO_3^-) following high temperature heating days similar to the findings reported in O'Shaughnessy et al., [39]. Other physicochemical parameters including C/N ratio, pH, total solids (TS), Organic total solid as percentage of total solids (oTS (%TS) were within acceptable range for anaerobic digestion process while salinity and conductivity were below the limits for moderate inhibitory acceptable for anaerobic digestion. The pathogens level at below detection limits made the final produced dried potato peels conform to the Regulation (EC) No. 208/2006.

4. Conclusions

The efficacy of PSH applying solar tunnel dryer in artificially contaminated potato peels effected hygienization through both, rising in temperature and moisture reduction to a level that is not supportive the survival of pathogenic microorganism. Solar dried biowaste fetch good keeping quality with less deterioration and organic loss through self decomposition. This gives an assurance of safe feedstock to conventional anaerobic digestion systems whose levels of effecting hygienization to contaminated biowaste is confirmed to be inexistence or very low. Pathogen free biowaste signifying biosafety to biotreatment workers in the anaerobic digestion facilities whose daily works involve coming into contact with contaminated biowaste and therefore a motivation for subsequent biological treatments. Although potato peels was used as the only biomass substitute to biowaste, it is anticipated that the procedure and technical operation used in this study could be equally adapted to other types of biowaste and yield similar biosafety and quality on the final product. The only uncontrollable factor is the sunshine; with this method therefore the rule of the game is simply keeping both eyes on the weather with timing and laboratory confirmation for complete elimination of the pathogens being an input and output of the whole process.

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