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Epidemiology in an Anaerobic Digester**

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STUDY OF RESOURCE RECOVERY AND EPIDEMIOLOGY IN AN ANAEROBIC DIGESTER

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Abstract

Three 4-liter packed-bed anaerobic digesters were fabricated and operated at 35°C, pH around 7, and hydraulic retention time (HRT) of 20, 10 and 5 days to study the resource recovery and epidemiology in a controlled ecological life support system (CELSS). A simulated wastewater, consisted of shower water, clothwash water, dishwash water, handwash water, and urine flush water was used as the feeding solution. Under steady-state operation, COD, TOC, pH, nitrogen, phosphorus, and potassium were monitored in the digester input and output solutions. The volume and the CH_4/CO_2 ratio in the biogas produced from the anaerobic digesters were measured. The results indicate about 90% of TOC is converted while only 5 - 8% of N-P-K are consumed in the digester. A multi-drug resistant strain of *Salmonella choleraesuis* was used as the indicator bacterium in the epidemiology study. The levels of *Salmonella choleraesuis* in the influent and effluent were determined and decimal decay rate constants, k_d , were estimated. The k_d values were greater at higher initial doses than lower doses for the same HRT, and greater for batch digestion (7.89/d) than for continuous digestion (4.28, 3.82 and 3.82/d for 20, 10, and 5 d HRT, respectively).

Introduction

Currently, spacecraft life support systems are simple and sufficiently reliable for human space-flight missions of relatively short duration with small crew sizes and limited power availability. However, life support technologies for the coming era of exploration must address longer-duration missions in which humans require substantial amounts of consumable materials to sustain life for long periods of time. If these consumable materials must be provided by re-supply flights from Earth, a substantial logistics infrastructure is

required. Consequently, supplying all these consumables from Earth is an extremely expensive proposition. As a result, one of the most important challenges associated with longer-duration manned space flights is the development of a Controlled Ecological Life Support System (CELSS). This includes the technologies of air revitalization, water recovery, waste processing, food production, and food processing, all of which are logistically and economically essential for the resource recovery in a CELSS.^{1,4,7,8}

The major elements in a CELSS are carbon (C), hydrogen (H), oxygen (O), and nitrogen (N); and the minor elements are phosphorus (P), potassium (K), sodium (Na), Calcium (Ca) etc. A simplified element flow-diagram of a CELSS is presented in Figure 1. This figure illustrates the fundamental flow of major elements through the system.

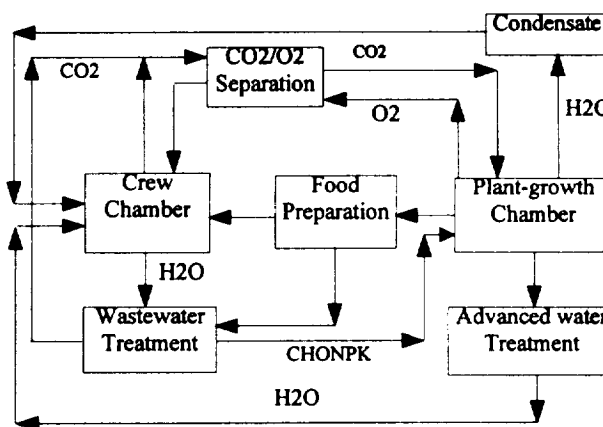


Fig 1. A simplified element flow-diagram of a CELSS.

In this example, crop plants are used to produce food for the crew. In addition to serving as the food production subsystem, the plants take up CO_2 produced by the crew, produce oxygen for the crew to breathe and

for oxidation of waste materials, and produce water vapor that can be condensed and collected to supply the crew's drinking and hygiene water. In the food processing subsystem, the foodstuffs produced by the crop plants are converted to a form palatable to the crew. Urine and feces, miscellaneous solid wastes, and waste biomass from the food processing subsystem are treated first in the wastewater treatment subsystem and then supplied to the plant growth chamber. The water out from the plant growth chamber is then further treated by an advanced water treatment subsystem. Any pure water produced from the advanced water treatment subsystem or from the condensate is supplied to the crew chamber.

Technologies for wastewater treatment include physical, chemical and biological methods. In general it is believed that a hybrid treatment system performs better than a single system. A combination process of anaerobic digester with a plant growth chamber, and an advanced water treatment unit was proposed for resource recovery and epidemiology study in a CELLS.⁵ A conceptual flow diagram of this process is shown in Figure 2. Advantages for anaerobic bio-process are 1) methane gas is produced, 2) less biomass is generated than aerobic process, and 3) the effluent is rich in inorganic nutrient for plant growth. Combining plant-growth with anaerobic bacterial systems provides distinct advantages. For an example, the efficiency of removal of ammonium and nitrate nitrogen can be increased during plant growth when compared to bacterial systems without plant growth.⁹

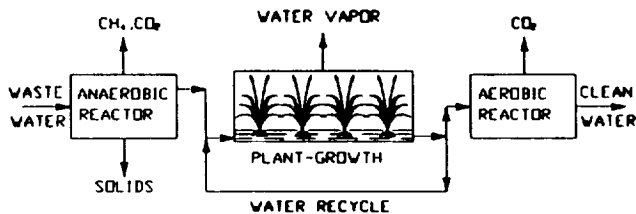


Fig. 2. Flow-diagram of an anaerobic/plant-growth/aerobic process

Wastewater containing human feces can present biological hazards when the intestinal flora consists of pathogenic species of bacteria belonging to genera such as *Salmonella*, *Shigella*, and *Vibrio*. They can cause illness if given the proper environmental conditions that enhance their growth and their transmission. Thus, the

risk of transmission of infectious diseases must be a consideration in the treatment of wastewater. In this study, three 4-liter packed-bed anaerobic reactors were built and used to test the resource recovery and epidemiology. Information obtained from this study will be very helpful for the development of a hybrid wastewater treatment system in combining an anaerobic process with a plant growth chamber.

Experiment

The experimental setup of each digester is shown in Figure 3. The digester is a 4-liter glass reaction kettle (ACE Glass 6505) packed with 5/8" size of polypropylene pall rings. Two perforated plexiglass with 1/8" thickness plates were used to hold the packing material inside the reactor. Characteristics of the packing materials are listed in Table 1. A magnetic drive pump was used to circulate the solution and to keep the liquid phase uniform after the injection of the feed solution. The reactor was placed in 35°C incubator to obtain mesophilic condition. Biogas was collected by a gas collection system which also can be seen from Figure 3.

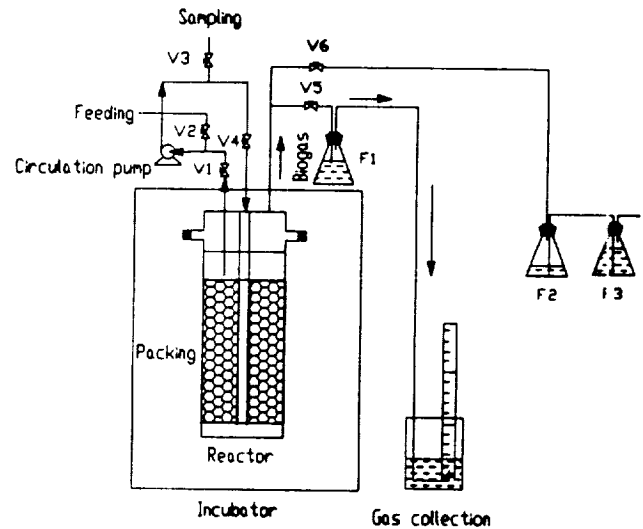


Fig. 3. Experimental setup of an anaerobic digester.

The ratio of CH_4/CO_2 in the biogas was measured by gas chromatography (GOW-MAC 350) with thermal conductivity detector using a 6 feet Haysep-Q packed column. The total organic carbon (TOC) in the aqueous solution was determined by a TOC analyzer (TOC-5000,

Shimadza Scientific Instrument, Inc.). The chemical oxygen demand (COD), total nitrogen (N), total phosphorus (P), and potassium (K) were detected by using a HACH spectrophotometer (Model 2000).

Table 1. Characteristics of Pall rings.

material	polypropylene
size	16 x 16 mm
surface area	0.00342 m ² /g
specific area	3.412 cm ⁻¹
porosity	0.877

The inorganic nutrients used in this study are listed in Table 2. This formula supply the necessary major and micro nutrients, reducing agents to remove the oxygen in the solution, and buffer solution.

Table 2. List of inorganic nutrients

chemicals	concentration (mg/l)
CaCl ₂ .H ₂ O	6.25
NaPO ₃	0.25
(NH ₄) ₂ HPO ₄	96.50
L-cystein	2.50
CoCl ₂ .6H ₂ O	10.00
FeCl ₂ .4H ₂ O	20.00
Na ₂ S.9H ₂ O	75.00
MgCl ₂ .6H ₂ O	266.75
NH ₄ Cl	369.00
KCl	100.00
KI	.63
NaHCO ₃	pH adjustment

All the chemicals used in this study were ordered

from Fisher Scientific and Aldrich with ACS grade chemical pure. The simulated wastewater consists of clothwash water, dishwash water, handwash water, shower water, fresh urine, and urine flush. The formula of the simulated wastewater was specified by NASA-JSC and is shown in Table 3. Deionized water was used to prepare the simulated wastewater which was prepared freshly prior to each feeding. The soap used in this experiment was provided by NASA-JSC.

Table 3. Formulation of NASA simulated wastewater.

Item	l/person/day
shower water (4uses /d, 12 g soap/use)	5.32
hand wash (16 uses/d, 2g soap/use)	4.07
clothes wash (30 g soap)	12.44
urine (16 uses/d)	1.51
urine flush	0.49
dish wash	9.07
total	32.90

A multi-drug resistant strain (RS) of *Salmonella choleraesuis*, subspecies: *choleraesuis*, serotype: *typhi*, antigenic formula: 9, 12, Vi:d was obtained from American Type Culture Collection (ATCC No. 19214) and was used as the indicator bacterium in this study. This strain is resistant to chloramphenicol, tetracycline, streptomycin, and sulfanilamide. It is not known to be part of the indigenous flora of animals nor is it known to occur in nature. A wild strain of *Salmonella choleraesuis*, non-resistant to the above antibiotics (NRS), was utilized in the experiment as the control. It was routinely streaked on the antibiotic-containing agar plates to check the inhibitory action of the medium.

Nutrient agar and nutrient broth (Fisher, Pittsburgh) were used as growth and storage media for the RS and NRS bacteria. MacConkey agar CS (Difco, Detroit) was used as a bacterial quantification medium. It was supplemented, after sterilization, with chloramphenicol, streptomycin, tetracycline, and

sulfanilamide (Sigma, St. Louis) at concentrations that inhibited growth of NRS but not the RS bacteria. This medium was therefore referred to as MacConkey-antibiotic agar.

The experiment started by immobilization and acclimation of the bacteria in the digester. Seeding solution (3.5 liter) was obtained from a local municipal wastewater treatment plant and was added to each anaerobic digester. Initially, local municipal wastewater was used as the feed solution. However, after biogas production was observed, the feed solution was changed gradually to NASA simulated wastewater. Experimentation was begun when the digesters achieved a steady-state of gas production and pH.

A volume of 350 ml of the digester solution was withdrawn from the anaerobic digesters after steady-state had been achieved. Afterward, 3.5 ml of *Salmonella* bacterial suspension was injected into the digesters through the rubber tube of the input valve. Following injection, the bacterial suspension was flushed with 346.5 ml of simulated wastewater. This withdrawn-and-fed was done at an interval determined by the hydraulic retention time (HRT). The *Salmonella* population in the digester was measured from the 350 ml withdrawn solution. For the decay study of *Salmonella* bacteris in a batch digester, five ml of solution was withdrawn from the batch digester after 3, 6, 12, and 24 hours for pH measurements and colony counts. The excess solution was re-injected into the digester after each measurement.

Viable counts of the indicator bacteria were determined by preparing 10-fold serial dilutions of the solution in 0.1% peptone water. One-tenth ml volumes of the dilutions were spread with sterile glass L-rods over the MacConkey-antibiotic agar plates. Colony counts (CFU/ml) were determined after 24 hours of incubation at 35°C.² The biofilms on the pall rings were examined at the conclusion of the study for the presence of attached indicator bacteria. One pall ring was selected and was mixed with peptone water with the use of a votex mixer. The mixed suspension was then streaked on MacConkey-antibiotic plate, and the suspect colonies were identified by the Crystal Identification System after 24 hours incubation. No indicator bacteria were isolated on the MacConkey-antibiotic plate from seeding or simulated wastewater. However, *Pseudomonas aeruginosa* from the seeding solution was detected.

Result and Discussion

Resource Recovery

The performance of the anaerobic digester may be seen from Table 4. At the steady state operating condition, the TOC removal percent (87.13 ± 0.03 , 89.01 ± 0.03 , and 88.78 ± 0.02 for 20, 10, and 5 d HRT, respectively) seems independent from the HRT. This result is in consistent with the biogas produced from the digester. However, the COD removal percent (81.92 ± 5.25 , 74.61 ± 8.67 , and 68.18 ± 9.90 for 20, 10, and 5 d HRT, respectively) decreases as the HRT decreases as shown in the table. The standard deviation of COD values is quite high compared with that of TOC values. With this large standard deviation of COD, it may not be able to conclude that the decrease of COD with HRT is significant.

Table 4. Performance of the anaerobic digester.

HRT, day	20	10	5
TOC inf mg/ℓ	601.1	600.4	465.7
TOC,eff mg/ℓ	77.3	65.98	52.25
TOC load g/m ³ /d	29	58	106
COD, inf mg/ℓ	2336	2393	2401
COD, eff mg/ℓ	422.3	607.8	764.0
COD load g/m ³ /d	117	239	480
pH	7.65	6.91	7.11
biogas ml/d	81.2	152.8	299.8

Mass balances of carbon for 10 days HRT are shown in Table 5. The TOC in the influent has four fates: 1) converted into IC in the effluent, 2) converted into biogas, 3) adsorbed by the microbial cells, and 4) left as the residue TOC in the effluent. The amount of

carbon used in the biosynthesis of microbial cells may be too small to be considered in the mass balance. When the amount in the fates 1, 2, and 4 were measured, the amount of carbon adsorbed by the microbial cells could be estimated from the overall carbon mass balance. The results, as shown in Table 5, indicate that 40% of the influent TOC is converted into IC, 25% into biogas, 25% is adsorbed, and 10% is left as TOC in the effluent. The amount of carbon absorbed by the microbial cells is either converted eventually or desorbed back to the solution. The desorption was observed when the TOC in the influent was reduced from 265.7 to 183.4 and then to 170.8. Under this condition, the mass balance indicates a negative adsorption as shown in Table 5.

Table 5. Mass balance of carbon* for 10 days HRT.

day	1	2	3	4	5	6
TC i	265.6	265.6	267.9	185.9	173.4	192
TOC i	263.4	263.4	265.7	183.4	170.8	189
IC i	2.2	2.2	2.1	2.5	2.6	2.8
TCe	127.2	134.2	130.3	123.1	135.6	102
TOC e	23.9	35.9	17.1	18.3	21.8	22.8
ICe	103.2	98.4	113.6	104.8	113.9	65.1
bio-gas	66.5	74.1	87.4	72.2	61.7	71.2
adsorbed	71.9	57.3	50.2	-9.4	-33.5	28.2

* The unit of the carbon here is mg.
i = influent
e = effluent

The mass balances of nitrogen, phosphorus, and potassium are shown in Table 6. It can be seen from the influent and effluent of this table that the 95% of nitrogen and phosphorus and 92% of potassium are left in the effluent solution. This high N-P-K content solution may serve as a good nutrient for the plant growth.

Table 6. Mass balance of N-P-K for 10 days HRT.

element	N	P	K
inf, mg/l	496.5	47.4	142.0
eff, mg/l	470.0	44.5	130.0
consumed	26.5	2.9	12.0

Epidemiology

The declines of viable counts of *Salmonella choleraesuis* during continuous (withdrawn-and-fed) mesophilic anaerobic digestion at different HRT are illustrated in Figure 4. This figure indicates that the viable counts of *Salmonella choleraesuis* at the three HRT declined rapidly within the first 4 days after inoculation.

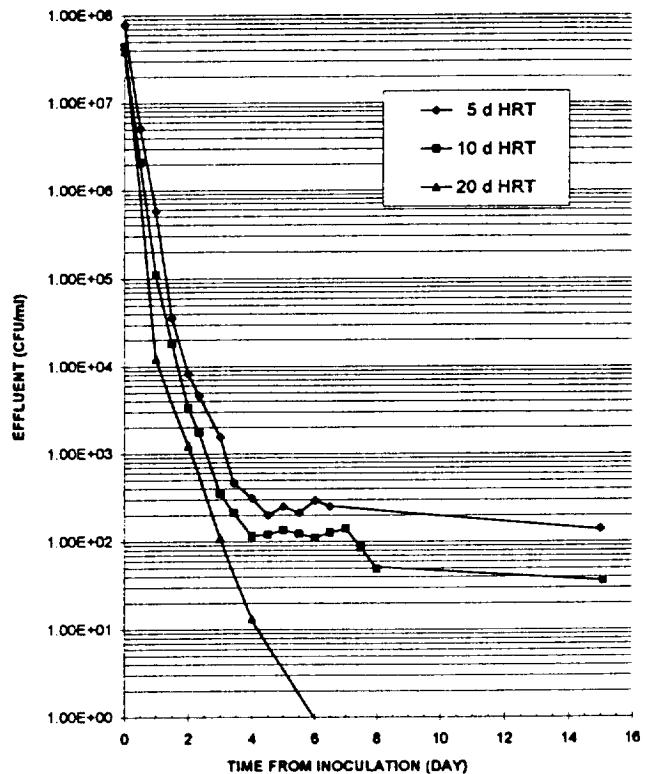


Fig. 4. Survival of *Salmonella Choleraesuis* in the single dose continuous anaerobic digestion (pH = 7.12).

The rapid declines of viable count of *Salmonella choleraesuis* were followed by a period of equilibrium for both 5 and 10 d HRT. However, for the 20 d HRT the viable count drops continuously to a level below the detectable limit. This phenomena could be explained by a lack of nutrient at the 20 d HRT operation.

The decimal decay rate (k_d) for the continuous digestion studies was calculated using the modified formula by Ginnivan.³

$$k_d = -\frac{1}{t} \ln\left(\frac{P}{P_0}\right) - \frac{v}{V} \quad (1)$$

where, P is the colony count of indicator bacteria in the withdrawn effluent, P_0 is the initial viable count in the digester (CFU/ml), v is the liquid volume of the effluent removed per day (ml/day), V is the liquid volume in the digester (ml), and t is the time interval (day). For a batch digestion system, the flow rate, v, is set to zero. The decimal decay rate (k_d) for the single-dose continuous digestion and batch digestion studies were determined from the slope of a plot of (P/P_0) versus t on a semi-logarithmic scale as shown in Figure 5 for 5 d HRT. Linear regression methods were used to estimate the slope and only those data within 4 days were used.

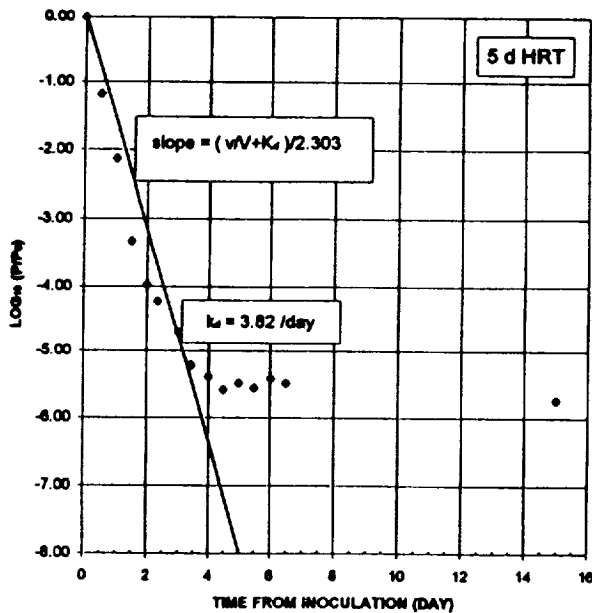


Fig. 5. Determination of k_d of *Salmonella choleraesuis* in the single dose continuous anaerobic digester for 5 d HRT.

Differences among the k_d at various HRT during continuous digestion were assessed by calculating upper and lower 95% confidence intervals. The k_d values were significantly different if their 95% confidence intervals did not overlap. The investigation of the effect of the various HRT on the survival time of *Salmonella choleraesuis* showed greater variations. Using an initial dose of approximately 10^7 CFU/ml for each HRT study, at an HRT of 20 days, the indicator bacteria were found to be below the level of detection by the end of the 6th day of the study. However, after a period of rapid decline in viable numbers, at HRT of 10 and 5 days, the indicator bacteria reached equilibrium (the viable numbers were not reduced by one logarithmic unit) by the end of the 15th day at approximately 40 CFU/ml for 10 d HRT and 1.4×10^2 CFU/ml for 5 d HRT.

The decimal decay rates (k_d) of the *Salmonella choleraesuis* during single-dose continuous mesophilic digestion operated at 20, 10 and 5 d HRT are calculated and listed in Table 7 with upper and lower 95% confidence intervals.⁶ The three k_d values were not significantly different because their 95% confidence intervals overlapped. The pH and biogas production during single-dose continuous digestion remained relatively stable throughout the digestion with a mean pH of 7.12 and mean biogas production of 183 ml/interval which contained a mean of 71% methane.

Table 7. Decimal decay rate constant, k_d , of *Salmonella Choleraesuis* during single dose continuous anaerobic digestion.

HRT, day	k_d , day^{-1}	lower limit k_d , day^{-1}	upper limit k_d , day^{-1}
20	4.28	3.07	5.48
10	3.82	2.88	4.76
5	3.82	3.36	4.28

The effect of bacterial feeding dose on the decimal decay rate (k_d) of *Salmonella choleraesuis* is shown in Figure 6. The correlation values (r) (0.62 at 20 d HRT; 0.87 at 10 d HRT; 0.90 at 5 d HRT) indicate that there is a strong positive relationship between the bacterial viable count of the initial dose and their k_d values. Biogas production and pH remained relatively stable throughout this digestion also with a mean pH of

7.48 and mean biogas production of 123 ml/interval which contained a mean of 71 % methane.

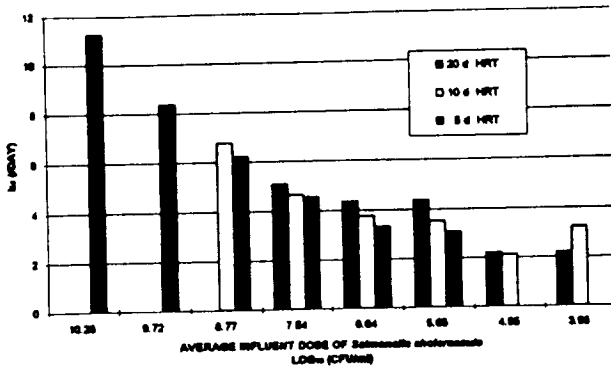


Fig. 6. Influent dose of *Salmonella Choleraesuis* and their k_d in the multi-dose study during continuous mesophilic anaerobic digestion under various HRT.

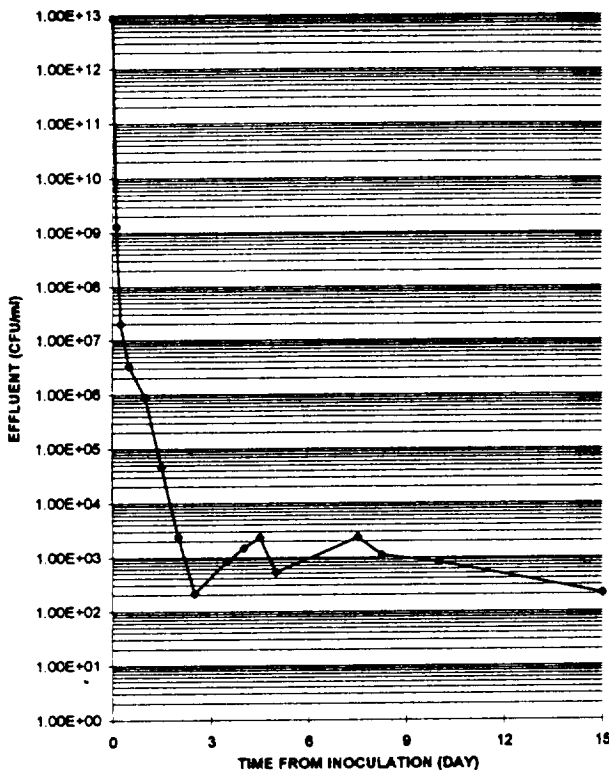


Fig. 7. The inactivation of *Salmonella choleraesuis* during batch mesophilic anaerobic digestion.

A rapid decline in the viable count of *Salmonella choleraesuis* during batch mesophilic anaerobic digestion is shown in Figure 7. The viable count of *Salmonella choleraesuis* declined rapidly within the first 2-4 days after inoculation. This rapid decline was followed by a period of equilibrium where the indicator bacteria remained at 10^2 CFU/ml until the 15th day from the inoculation. The decimal decay rate (k_d) of the *Salmonella choleraesuis* during batch mesophilic digestion is calculated by the same method used in the single-dose continuous process. The value of k_d calculated is 7.89 day^{-1} .

The viable counts of effluent correlated with the biogas suggesting a strong negative relationship ($r = -0.85$). A rapid decline in the viable population of the indicator bacteria was reflected in a large volume of biogas production; as biogas production declined, so did the viable population of the indicator bacteria in the digester. The pH values remained relatively stable throughout batch mesophilic digestion with a mean value of 6.90. The viable counts and the pH value did not show a relationship ($r = -0.07$).

Conclusion

Results indicate that the NASA simulated wastewater can be treated by anaerobic digestion. Mass balances indicate about 90% of the TOC is converted while only 5 to 8% of N-P-K are consumed in the digester. The maximum organic loading capacity was not reached indicating there is a possibility of increasing the loading rate. *Salmonella choleraesuis* survived at least 15 days from inoculation for 10 and 5 days HRT during continuous and batch digestion, but less than 6 days for 20 days HRT. The k_d values were greater at higher initial doses than lower doses for the same HRT, and greater for batch digestion (7.89 day^{-1}) than for continuous digestion ($4.28, 3.82$ and 3.82 day^{-1} for 20, 10 and 5 d HRT, respectively).

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