H.E.A.T.
A New Sustainable Green Solution for Treating and Evaporating Hide Brine Wastewater

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Abstract

Salt curing of hides releases a significant amount of excess water that must be disposed. In larger abattoirs this can result in production of tens of thousands of gallons of saturated salt brine that is also contaminated with biological material from the hides. These brines are often stored in enclosed impoundments that ultimately fill and need replacement, or the facility must build multiple impoundments. The proprietary, biologically based, sustainable *Halophilic Evaporative Applications Technology (H.E.A.T.)* process has been developed as a method to accelerate the evaporation of salt saturated brines. The process has been tested for 18 months in a full-scale lagoon located at an operating American beef plant. The process successfully evaporated an additional 31.99 inches of brine (866,900 gal) acre^{-1} of concentrated hide brine in one year, nearly drying out the South lagoon. Ambient evaporation of the same brine in an identical control lagoon at the site was only 19.19 inches (520,000 gallons) acre^{-1} representing a 1.66× increase in brine evaporation from the *H.E.A.T.* microbes. During 2020, the plant produced 3,376,000 gallons of brine, meaning *H.E.A.T.* evaporated 100.4 % of plant production in one lagoon in its first year. This was accomplished without additional infrastructure, equipment, or external heating. During this test, Biochemical Oxygen Demand in the lagoons decreased over 98% with concomitant odor reduction. Beginning in October 2020, the lagoon began receiving all brine produced daily by the plant. This continued over the winter period, during which time the process evaporated over 34% of the inflow. Continued fertilization and microbial augmentation are essential for the continued healthy development of the system. Overall, the process and its essential microbial populations were stimulated by continuing inflow of fresh hide brines. The microbial process increases brine evaporation of concentrated salt brines, reduces odors and represents a new environmentally friendly mechanism for solving an industrial problem that has long plagued hide producers.

Introduction

The first description of a biological impact on evaporation rates actually occurred in 2500 BCE in China, where salt makers recognized that having their small pans turn red meant they would be able to harvest their salt within days rather than months.[1] This reddening is often seen in salt production situations[2] but its impact on the actual evaporation rate has been ignored prior to the Eastern Shore Microbes (ESM) developments described here. The H.E.A.T. process is based on developing custom culture and nutrient mixes for each class of brine to be treated or evaporated. The fertilizers and cultures are added to the lagoon to stimulate development of a huge population of microbes which can reach over 1 billion live cells in every milliliter (cc) regardless of the size of the lagoon. One likely mechanism for the process is that the actively metabolizing microbes give off excess metabolic energy as heat and increase the temperature of the brine much like typical compost piles. Alternatively, or even concomitantly, the dense biomass makes the lagoon red. This biomass reflects the hottest wavelengths of sunlight into the water increasing the brine temperatures even more. Neither of these mechanisms have been proven at this time but both are likely mechanisms for the process. There are additional possibilities ranging from the microbial movement causing microscopic disruption of the brine surfaces and/or microbial by-products that lower the surface tension resulting in more rapid evaporation or possibly the microbes themselves acting as foci for crystal formation causing the salt to precipitate more quickly than an uninoculated lagoon. Now that this acceleration has been documented these various mechanisms can be investigated.

Salt curing of hides prior to tanning has been used by the industry for many years. During this curing process, hides will release a significant amount of water into the raceways. While hide production was carried out in small operations, this excess brine was also a relatively small problem. As processing plants increased to ever greater head kill, the problem became more acute. Especially since the evaporation rate of water decreases as the concentration of salt increases evaporation may be retarded, as much as 0.71 for NaCl.[3] Due to multiple moving and opposing solid, liquid gas interfaces estimating evaporation in large open lagoon areas is difficult. As evaporation reduces the overall brine volume in a lagoon, it tends to force the brine surface lower in the lagoon. However, in saturated hide brines, evaporation causes salt precipitation, so that large scale salt deposition displaces the brine surface, pushing the air brine interface back upward. At the same time, large lagoons must be designed with sloping sides of different ratios depending upon

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the soil type surrounding the lagoon. This slope means that as the brine surface falls, there is a smaller overall evaporative surface and every cm (or inch) of drop represents a lower volume than the preceding cm (or inch). But since the salt layer is moving opposite to the surface, every cm (or in) of gain actually represents more volume displacement than the unit below. Finally, random but certain rainfall raises the water level, and may dissolve the underlying salt or it may actually form a low-density layer at the top of the brine. Once again, measuring this volume is not straightforward as lagoon liners are stretched above the lagoon berm directing rainfall into the lagoon from a surface area that may be significantly larger than the brine surface itself.

Consequently, following evaporation (and enhancement) requires monitoring all of these systems over time and with consistent measuring activities. This manuscript describes a study conducted on two large lagoons, filled with hide brines, during which these various parameters were followed over time. The data demonstrate that the proper microbial population does indeed significantly increase evaporation while simultaneously biodegrading the organic contaminants in the hide brine.

Goals of Hide Brine Treatment: In order for any treatment to be effective as a solution to problems of brine disposal, the process must achieve certain specific goals. These include: enhanced evaporation; allowing smaller (or fewer) lagoons; reduction of Biochemical Oxygen Demand (BOD) with concomitant odor reduction; and finally, creating an evaporative environment that can keep up with plant production.

Materials and Methods

Purpose of the Project

This project was originally designed as a full 1 year to 18-month demonstration study. However, after being started the project was interrupted for several months by the Covid-19 pandemic and by plant closures during that time. One of the primary goals of the project was to determine if the H.E.A.T. process evaporated brine during the colder months of the year. The test site was at an active hide production facility in Northeastern Colorado.

Hide Lagoons

The hide lagoons used were each 4.8 acres in surface area and designed to hold up to 15,000,000 million gallons of brine. At the time of this project, each lagoon had been in use for nearly 30 years and each had built up thick layers of salt. These lagoons were chosen because each was being prepared for repair or decommissioning.

One lagoon (designated South) was chosen as the test site and its companion (designated North) was chosen as the control. The H.E.A.T. process was initiated in the South lagoon using proprietary custom designed mixes of culture and matching nutrients in August 2019. North Lagoon received no microbial or nutrient treatments. North Lagoon therefore, provided a measure of the ambient evaporation rate of untreated hide brines. Following this first inoculation, both the North and South lagoons were taken out of service to allow all parties to evaluate the events and document the changes that the H.E.A.T. system would foster. Due to the need to build the proper biomass and fertilization conditions, no measurements were taken until the system was ready. Originally, due to having a volume of nearly 2 million gallons in the test lagoon and beginning the biological process so late in the year this was planned to require up to five months. However, following a second inoculation and fertilization of the South Lagoon in October 2019 the biomass development occurred sooner than expected so the measurements began in November 2019. To begin the formal test both lagoons received additional brine from another hide lagoon. This decision was made due to the fact that the South lagoon was already drying and all personnel involved wanted to collect more data. Each lagoon was filled with several days' pumping. The plant utilized the same pump/hose system and pumped for the same lengths of time which was done to provide each lagoon with an equal amount of fresh brine. Standardized sampling points were then established in each lagoon, using three PVC pipes driven into the underlying salt pack to provide those sampling points. The PVC pipes were placed roughly in a line running along a north to south transect of each lagoon. From that point on, all measurements were taken by boat next to those pipes. Depth measurements were taken using a weighted 8-inch Secchi disk to provide a flat measurement that was less impacted by the rough bottom of the lagoon. Brine samples were sent to the ESM home laboratory for analyses on a monthly basis. Field measurements were obtained from the boat and all brine samples were obtained by an on-site water treatment operator.

A third and final inoculation and fertilization of the South lagoon occurred in the final week of May 2020 in order to prepare the system for the coming summer. No additional fertilizations, inoculations, or brine additions occurred between this May date and September 2020. Pond volumes were still monitored during this period although once again the frequency was impacted by Covid-19 outbreaks.

A second short term extension of the demonstration was initiated for the period of October 2020 to March 2021. This portion of the demonstration focused on the potential of the lagoon to begin receiving all of the excess hide brine coming from the plant.

Evaporation Measurement

To facilitate these measurements, a Computer Aided Design (CAD) table was generated using lagoon blueprints. The table was accurate to 0.01-inch levels in the ponds. The brine surface position for each measurement date was obtained using daily staff gauge measurements in each lagoon. The position of the salt pack was determined from the depth measurements using the weighted Secchi Disk at the three locations within each lagoon. Since the salt pack proved to be dish shaped with a deeper center in each lagoon, these measurements were averaged for each data point. Precipitation measurements were taken from the daily staff gauge reports provided.

These various numbers were treated as follows: each daily staff gauge measurement was converted into a measure of decimal feet and the total pond volume for that point was obtained using the CAD table. Once the average brine depth was obtained from the Secchi readings, the position of the salt pack was determined by subtracting the average depth from the overall brine surface position. The displacement volume of the salt pack was then obtained using the CAD table. The overall volume of brine remaining in the lagoons was then determined by subtracting the salt displacement volume from the total predicted volume given by the staff gauge reading.

During the project an additional factor in estimating evaporation in the South lagoon arose as the accelerated rates in the lagoon caused the salt pack to be exposed in an ever larger "beach." This was noted in March of 2020 and was only occurring in the test lagoon, not the control. Therefore, the best approach was not to add additional brine to the lagoons (since the control lagoon did not have a beach) and attempt at times to measure the extent of this beach. This proved to be extremely difficult to accomplish on a consistent basis but some estimates were obtained. In March of 2020 the beach was found to be 12 feet wide. The beach grew to be (50 ft) on a side by July 2020 and 75 ft by September 2020. An estimate of the evaporative surface of the South Lagoon was performed using equation 1.

(1) *Surface area of Brine* $= (459 - 0.95(D+L))^2$ where
D = estimated width of salt exposure
L = Exposed area of liner (calculated by subtracting staff gauge measures from design lagoon depth (12 ft).
459 = total length of the sides of the lagoon at the 12 ft interval.

Once again, this problem was not faced in the control North lagoon as the brine continued to remain in contact with the pond liners throughout the test period.

Bacterial Populations

Bacterial biomass was followed using standard surface spreads on CAS agar[4] supplemented with 20% (w/v) NaCl. All samples were decimal dilutions, with 0.1 ml of each dilution surface plated onto the CAS medium. Plates were incubated inside plastic bags (to increase humidity) at 35°C for at least two weeks to allow for the slower growth of cultures.

Biochemical Oxygen Demand (BOD)

BOD analyses were based on procedures described in USGS TWRI Book 9 Chapter A7[5] and Standard Methods for Water and Waste Water analysis 5210[6] with the following modifications to account for the high salinity of the brine. Diluent was prepared with 20% NaCl and sterilized. Dissolved Oxygen was measured using an RDO probe system, corrected for salinity and calibrated after each dilution and sample. Proprietary culture mix HS 001 was used as a seed culture. While BOD is an important measure of waste water treatments, this has proven to be one of the most difficult assays in these brines. This appears to be largely due to the heterogeneity of the materials. The brines contain significant amounts of dirt, blood and other coagulated materials that contribute to the overall BOD and do not evenly distribute in the BOD diluent. Therefore, one bottle of a replicate may show measurable BOD while a second bottle with the same sample volume may, following incubation, have no oxygen remaining. In order to provide some measure of stability for this assay we adhered strictly to the normal analytical criteria. After 5 days of incubation at 20°C, data is acceptable if all of the following criteria are met: 1) bottles must have at least 1.0 mg/L Oxygen remaining; 2) the second reading of dissolved Oxygen must be at least 2.0 mg/L below the first reading [i.e. a loss of at least 2.0 mg O_2/L]; 3) No apparent toxicity in more concentrated samples; 4) no data anomalies in a set of replicates [i.e. one bottle with no Oxygen loss and one with total oxygen loss]. Throughout the study, and given the high organic levels in the brine Rules 1 & 2 proved the most difficult to meet consistently.

Loss on Ignition

Loss on Ignition (LOI) is a bulk measure of the organic fraction of a sample after it has been dried at 120°C then incinerated at over 600°C to convert all carbon- containing materials to CO_2 and H_2O or other gasses. Measurement of this parameter did not begin until May 2020 so there is somewhat limited information. Loss on ignition studies were conducted using the procedure described by Heiri et. al[7] using only 6 crucibles. No attempt was made to determine carbonate content in these brines.

Results

Evaporation

Photographic examination of the South Lagoon demonstrated significant evaporation in 9 months (Figure 1). Evaporation in the South lagoon exceeded that in the North by a considerable amount. As shown in Figure 1, at the start of the case study, brine in the South lagoon was in contact with the lagoon liner. A similar situation was seen in the North Lagoon. By March 2020, the South Lagoon brine had receded from the liner and a "salt beach" several yards wide had appeared around the lagoon. Brine in the North lagoon was still in contact with the liner (image not shown). By the end of this first trial the South Lagoon showed a beach nearly 75 ft wide. The North Lagoon did not have a visible beach.

This result led to a decision to conduct a short follow-on study, using the drying South lagoon for active brine disposal. The goal of this portion of the testing being to determine if the single South Lagoon could evaporate fast enough to keep up with plant hide brine

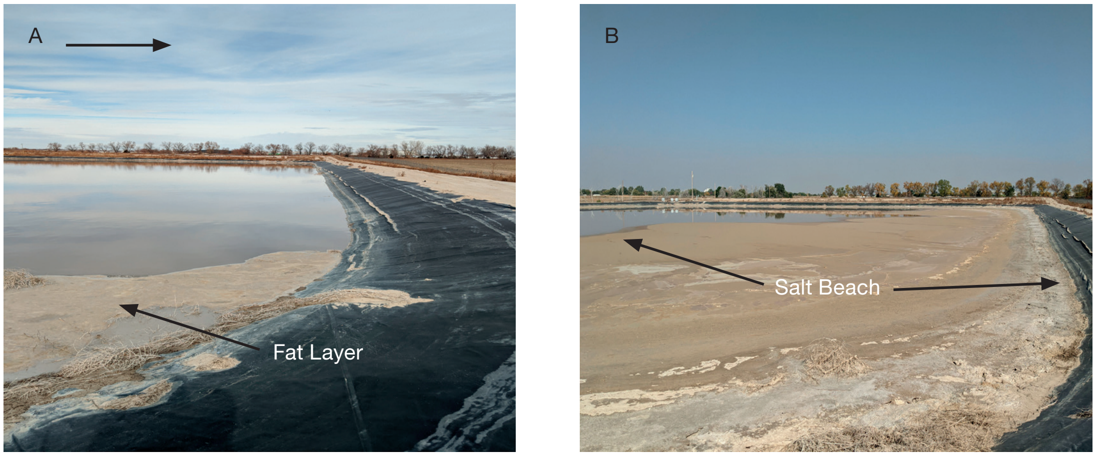

Figure 1. Evaporation development in South Lagoon over the initial 11-month study. **(A)** Left image November 2019; **(B)** right image September 2020. Black arrow in left figure shows North for both images

production. Unfortunately, due to the Covid-19 pandemic, this testing could not begin until October 2020 and was supported through March 2021 when funding terminated. At the point of termination, the brine depth of the South Lagoon was 9.83 ft on the installed staff gauge. This level, was identical to the brine readings for South Lagoon on 9 December 2019. At this level, the South lagoon contained 3,653,070 gallons of liquid. In March 2021 this level was reached with only 2,074,019 gallons (Table I). The difference in these volumes was caused by salt accumulation from evaporation in the 2019/2020 test.

Images of the progression of evaporation in the South lagoon showed a striking development of a salt "beach" that began to develop within 3 months of inoculation of the lagoon. Figure 1A shows the condition of the lagoon at the beginning of the demonstration project. Brine was touching the pond liner at all points, interrupted only by layers of accumulated fat (lower left in 1A). Within two months of inoculation, this fat layer was degraded and odors emanating from the brine were noticeably reduced. By March of 2020, a 12 ft wide salt "beach" that extended around the entire lagoon had developed. By September of 2020, this "beach" area extended out nearly 75 ft around the lagoon (Figure 1B).

Calculations based on brine depths indicated that the South Lagoon had evaporated 31.99 inches of brine from November 2019 – October 2020. Much of this evaporation occurred after the growing salt beach began to reduce the evaporative surface of the South Lagoon. During this project, the surface area of the South Lagoon decreased from the initial 4.8 acres to 3.4 acres (average 3.84 acres). More importantly the evaporation rate per acre actually increased even with the smaller surface area (Table I). By contrast, the North lagoon evaporated only 19.19 inches of brine in a larger and constant surface area over the same time period (Table II).

By September 2020, the South Lagoon reached an evaporation rate of over 6,000 gallons of brine (acre^{-1} day^{-1}). Comparison of this level with the 105 - year average fresh water evaporation for this area

Table I

Evaporation in treated South lagoon over first 9 months of 2020.

Date	Surface area of brine (acres)	Evaporation (gal/acre)	Acre inches evaporated	Sampling interval (days)	Evaporation (gal/acre/day)	FW evaporation (Month[1])
12/9/19	4.8					
1/13/20	4.8	64,583	2.38	36	1794	0.00
2/14/20[2]	4.8					0.00
3/4/20	4.6	93,478	3.45	20	4674	1.75
5/21/20	4.3	83,721	3.09	98	854	6.96[(3)]
6/16/20	4.2	119,048	4.40	27	4409	4.42[(3)]
7/10/20	4	82,500	3.04	25	3300	4.84
9/17/20	3.4	423,529	15.63	70	6050	7.57[(4)]
Final total		866,859	31.99		3,514 (Avg)	28.73

1: Monthly Fresh Water (FW) evaporation in the local area over 105 years. These values have been corrected from Class A Evaporation pan readings by multiplying pan readings by 0.7 as described (8); 2: Depth measurements indicated a gain of brine in this lagoon for this date but no corresponding gain was detected in the adjoining lagoon. Therefore, these lines have been omitted; 3: Values for any month with two sampling dates have been combined; 4: Evaporation for multiple months have been added for these samples.

Table II

Evaporation in untreated North lagoon over first 9 months of 2020

Date	Surface area of brine (acres)	Evaporation (gal/acre)	Acre inches evaporated[1]	Sampling interval (days)	Evaporation (gal/acre/day)	FW evaporation (Month[1])
12/9/19	4.8					
1/13/20	4.8	0	0	36	0	0.00
2/14/20	4.8	18,750	0.69	32	586	0.00
3/4/20[2]	4.8					1.75
5/21/20	4.8	77,083	2.85	98	787	6.96[3]
6/16/20	4.8	107,708	4.38	27	3989	4.42
7/10/20	4.8	60,416	2.23	25	2417	4.84
9/17/20	4.8	256,250	9.45	70	3661	7.57[4]
Final total		520,270	19.19		1,907 (avg)	28.3

1: Monthly Fresh Water (FW) evaporation in the local area over 105 years. These values have been corrected from Class A Evaporation pan readings by multiplying pan readings by 0.7 as described (8); 2: Depth measurements indicated a gain of brine in this lagoon for this date but no corresponding gain was detected in the adjoining lagoon. Therefore, these lines have been omitted; 3: Values for any month with two sampling dates have been combined; 4: Evaporation for multiple months have been added for these samples.

showed that the H.E.A.T. treated lagoon consistently exceeded fresh water evaporation for most of the measured months (Table I) once the measured pan rates were converted (0.7× pan measurements) to account for the larger lagoon size. The data also indicated that the microbially treated lagoon evaporated brine through December and January when fresh water evaporation is nil. During this period both lagoons received 131,500 gallons of precipitation per acre. This volume was not added to these calculations simply because both lagoons would have received the same amounts. Precipitation was added to the calculations for the second part of this case study because it focused only on the South Lagoon.

Table II reflects the same data points for the control North Lagoon. These data illustrate the expected effects of increasing salt concentrations in decreasing brine evaporation which is the main problem the H.E.A.T. process is designed to solve. Over the same time period, this identical lagoon evaporated only 19.19 inches of brine. Some surface area decrease did occur due to the wall and liner slope however conversion of the calculated square feet (using equation 1) to acres did not fall below the 4.8-acre value. More importantly, evaporation from the North Lagoon only exceeded a corrected fresh water rate on a single occasion, during summer 2020. But, as with Table I, this occurred during one of the two longest sampling intervals. This makes it difficult to determine if the measurements might have been caused by movement of the sampling boat, or measurement in a depression in the bottom. Overall however, evaporation in this lagoon never exceeded that of the South Lagoon, for any sampling point or any of the data calculations. Compared to fresh water the decrease in evaporation rate due to the concentrated salt was nearly 30%. Unlike South Lagoon; the North lagoon did not evaporate any brine during the winter period.

Given the data from the earliest part of the trial (Table I), indicating that the H.E.A.T. treatment did evaporate brine during the winter period, the test was extended to determine if this evaporation would continue with brine waste addition and to measure the extent of winter evaporation. At this time a drone with photographic capacity became available. Drone flights provided an overview of both lagoons. Waste brine was added only to the South Lagoon, so all measurement of North was stopped. The results of this demonstration and the responses of the H.E.A.T. cultures are shown in Figures 2 & 3 and in Table III. Daily brine addition began on 20 September 2020 and continued to the end of March 2021 when the demonstration ended.

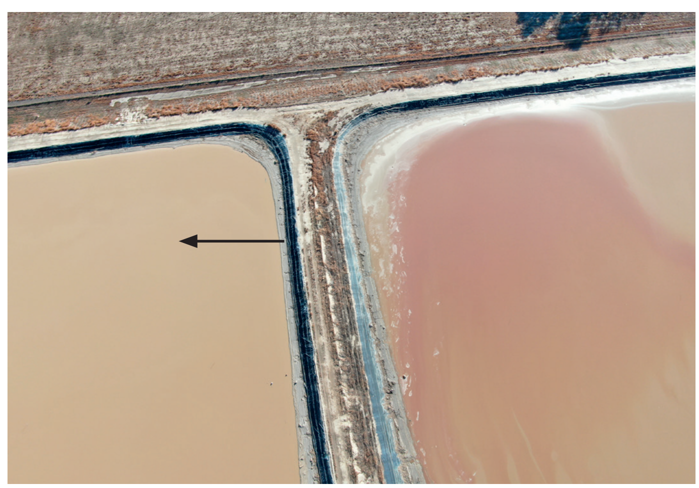

Figure 2. Aerial view showing both control and the test lagoon (North left; South right) on 29 October, 2020. The red plume in the right-hand image shows the bacterial bloom occurring as the microbes began to attack the inflowing brines one month after fertilization and addition of H.E.A.T. cultures. The white area along the top of the South lagoon is the remains of the beach as new brine fills the lagoon. Arrow indicates North.

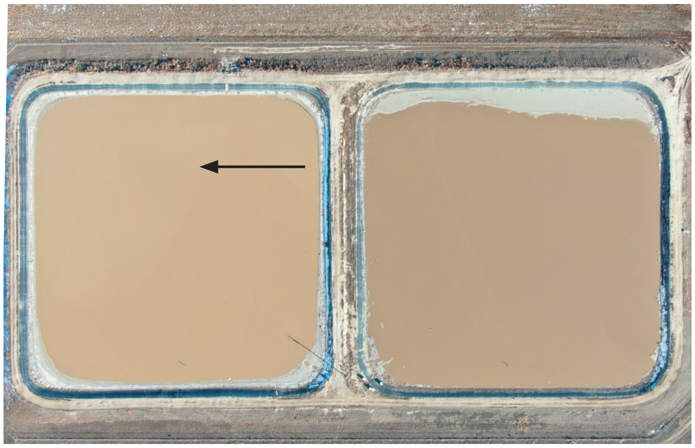

Figure 3. Aerial view (4 December 2020) of North (left) and South (right) lagoons. The red microbial plume seen in Figure 2 has now spread throughout the South Lagoon (hence the darker color of the brine). The white material at the Eastern edge of the South lagoon is the remains of the salt beach on that edge since brine enters the lagoon from the lower left corner of South Lagoon. Arrow indicates North.

During this brine addition phase, the plant released all of its daily waste brine (avg 20,000 gal day^{-1}) into the South Lagoon. Within the first month of additions, the microbial population responded in a dramatic manner as is shown in Figure 2. The ESM red cultures bloomed to a point where the population was most visible in the Eastern portion of the lagoon and could be seen mixing with the inflow plume (Figure 2).

As the additions continued the population became more diffused through the South Lagoon causing the brine to darken as is evident in Figure 3 (righthand lagoon).

During the 6-month long trial, the South Lagoon received 2.21 million gallons of hide brine waste, and over 970,000 gallons of rainwater (based on a 5-acre runoff area). This continuous addition made measuring the evaporation rate even more difficult than the previous part of the demonstration. Considering that the South Lagoon is a terminal pond and since no leaks were detected in monitoring wells it was possible to assume that all losses of brine occurred via evaporation. Therefore, by calculating the total inflow, then comparing it to the measured gain it was possible to obtain at least a base estimate of brine losses through evaporation. These data are shown in Table III. These data show that at the beginning of the trial the South Lagoon biomass did evaporate over 57% of added brine. However, as the rainfall increased (Jan. – Mar. Table III) and brine volumes began to build, this evaporation effect appears to have tapered to only 6 % in March 2020. The data did reveal that during brine addition and the winter period, fertilization was an important component of this process. Table III shows the sampling points that roughly correspond to fertilization events. However, the fertilizations all occurred prior to the sampling dates. The project began with the addition of 550 gallons of fertilizer on 22 October 2020, over a month before the first sampling. The fertilization corresponding to the 4 Jan 2021 sampling also included 550 gallons of nutrients added on 23 Dec 2020 (nearly 2 weeks before the sampling). The final fertilization incorporated only 275 gallons of material and occurred on 25 Jan 2021, 21 days prior to sampling. In all cases the detected evaporation appeared to accelerate but was followed by a significant decrease in the rate as more brine (and rainwater) flowed into the lagoon diluting the added nutrients. The evaporation data in Table III indicates that while the ratio of nutrient to inflowing brine was around 0.05 : 0.06% (fertilizer : inflow) (Jan – Feb Table III) significant evaporation occurred despite the winter season. When this level dropped to only 0.03% in February 2021 a good evaporation rate was still detected however, this appeared to drop quickly to only 6% in March when no additional fertilization was provided.

Bacterial Populations

At the beginning of these trials the bacterial population in the brine for both lagoons was very low at 3.6×10^3 ml^{-1} total colony forming units (TCFU) and only 5.0×10^2 red colony forming units (RCFU) ml^{-1}. Once the treatments began these numbers began to increase. By March of 2020 the population in the South lagoon had increased over 1,000-fold to over 3.8×10^6 TCFU ml^{-1}, a major part of this total 1.2×10^6 RCFU ml^{-1} being the red cultures needed to accelerate evaporation rates. Due to warmer weather and the fresh brine added in December (2019) bacterial populations in the North lagoon increased to 1.7×10^5 TCFU ml^{-1} and 4×10^4 RCFU ml^{-1}. The project was placed on hold due to budgetary issues so continued fertilization and care of the cultures ceased. A May 2020 sample showed that the biomass had dropped significantly in the South lagoon (2.23×10^4 TCFU ml^{-1}; 1.27×10^4 RCFU ml^{-1}). Since negotiations were underway to continue the trial an additional fertilization was provided. North lagoon population dropped to pre-trial levels (1.1×10^3 total colonies/ml^{-1} and 3.7×10^2 red colonies/ml).

Treatment of the South lagoon began again in October 2020 for an additional 6 months at the request of the facility and to complete an 18-month total measurement period. After the first supplementation the population rebounded to 7.3×10^4 TCFU ml^{-1} and 2.9×10^4 ESM microbes RCFU ml^{-1}. By the end of December 2020 with continued nutrient and culture additions, the populations in South Lagoon continued to increase reaching 5.1×10^6 TCFU ml^{-1} and 3.0×10^5 RCFU ml^{-1}. This population reached levels that became visible to the naked eye (Figures 2 and 3) and was maintained there through the end of the trial.

Biochemical Oxygen Demand (BOD)

All early samples from these ponds showed extremely high levels of BOD with both lagoons running above 170,000 mg O_2/L. By May 2020 both lagoons showed a decrease in BOD (note neither lagoon was receiving fresh waste at this time but both had active native (North) and enhanced (South) microbial populations. North lagoon BOD showed 24,800 mg BOD/L while South reached 8,900 mg

Table III

South lagoon responses with all produced hide waste brines being added daily to the single lagoon. Some columns may not add up due to rounding errors while converting to decimal equivalents to reduce the size of the table. The full numbers are shown in the supplementary data files.

Date	Interval (days)	Precip. (mil. gal.)	Hide Brine added (mil. gal.)	Total water added (mil. gal.)	Lagoon Brine Vol. (mil. gal.)	Change in volume Between samplings (mil. gal.)[3]	Difference (actual - Theoretical) (mil. gal.)	% Evaporated (total vol. change/total added) × 100	Theoretical Staff Gauge (based on volume)	Staff Gauge (daily measure)
9/17/2020					0.75					9.00
11/3/2020	48	0.02	0.55	0.57	0.996	0.25	0.32	57[1]	9.38	9.00
11/25/2020	12	0.04	0.37	0.41	1.26	0.26	0.14	34	9.35	9.08
1/4/2021	41	0.37	0.39	0.76	1.72	0.47	0.29	38[1]	10.00	9.50
1/21/2021	17	0.03	0.26	0.29	1.97	0.25	0.04	14	10.90	9.58
2/16/2021	27	0.22	0.30	0.52	2.07	0.10	0.43	82[1]	10.10	9.75
3/3/2021	16	0.03	0.18	0.21	2.45[2]	0.38[2]			9.97	9.83
3/18/2021	15	0.26	0.16	0.42	2.84	0.39	0.03	6	10.35	10.08
		0.97	2.211	3.18	2.09[4]			35 (of total added)		

1: fertilization events that occurred during the study; 550 gallons added prior to November and January sampling; 275 gallons added prior to Feb sampling. 2: measurement showing lagoon volume gain in excess of total added. 3: Calculated by subtracting each measured volume from the previous measurement and rounding to hundredths (i.e. 0.01); 4: this number reflects the total volume gained over the test. The original 0.75 million gallons was subtracted from the total 2.84 in the final cell. The total evaporation (35%) was calculated by dividing these two volumes.

BOD/L. While both are a significant reduction South lagoon clearly has much less BOD. In October 2020 South lagoon began receiving daily influx of brine from the Hide Plant yet the BOD continued to drop as the microbial population remained active. By December 2020, South BOD had dropped to only 3,000 mg/L while North (which still received no fresh waste) still registered 21,200 mg/L. BOD testing was halted in January due to the increasing ratios of fresh brine entering the lagoon. This brine would bring additional high BOD into the lagoon causing this parameter to rise.

Loss on Ignition

Initial LOI experiments showed 25.7 and 26.7% total dissolved and suspended solids in the South Lagoon and North Lagoon respectively. Of that fraction, 2.5% was organic (South) with 2.1% organic in the North. This difference was likely due to the ESM biomass in the South Lagoon. The LOI in the South Lagoon remained at 2.5% organic fraction, into December 2020.

Discussion

This case study demonstrated that the H.E.A.T. process does accelerate the evaporation rates of hide brines in large and working storage lagoons. The data show that the microbial population increases dramatically as long as supplemental nutrients and culture augmentations are being provided. This became more apparent as the population dropped dramatically from March 2020 to May 2020 when additions stopped for budgeting reasons and due to the coronavirus pandemic. The combination of additional nutrients in May and presumably, the warmer summer weather helped maintain the microbes until treatments resumed, but questions remain about how much more evaporation could have occurred with proper maintenance throughout the year. While the microbial population rebounded dramatically once regular maintenance was re-started, the overall condition of the test lagoons (old, nearly filled with salt and with deteriorating liners) precluded a longer-term effort in this case. It also indicates that the process may well be better in the long run if the treated lagoons are receiving fresh brines plus the proper culture and nutrient supplements to compensate for materials being diluted by the in-flowing fresh brines. This would occur because in a static lagoon the microbes attack the residual organic fractions, reducing it to gasses and some biomass. This was evident from the extensive BOD reduction (172,000 to 3,800 mg O_2 /L that occurred during the test period). BOD levels in the 3,000+ range are still extremely high for a normal waste stream discharge: but considering the starting level this represents a 98% reduction, and would include a corresponding reduction in odors. Since the North lagoon received no treatment but was allowed to remain fallow some degradation also occurred in the North Lagoon but the remaining BOD was still significantly higher (21,200 mg O_2/L) than that in the South Lagoon. Having a large biomass did not however negatively impact the crystals being produced in the lagoon, as it added only 0.4% to the total organic fraction of the precipitating crystals. This was evident from the LOI results showing that the lagoon being treated produced crystals with an organic fraction of 2.5% compared with 2.1% in the North Lagoon. While no attempt was made to further characterize the respective organic fractions, it is likely that the material in the North Lagoon came mostly from the original hides (probably complex proteins and fats); while that in the South was more microbial biomass from the BOD breakdown.

Regardless of the scientific and analytical results obtained during this project, the key bottom line is the evaporation increase. As is clearly shown in Figure 1 (left to right in the images) and the volume data in Tables I and II, H.E.A.T. treatment accelerated the evaporation rates during the project. The data obtained, indicate that the process increased the evaporation rate of brine in the South Lagoon by over 66% per acre (866,859 vs 520,270 gallons) but more importantly the rate achieved in the South Lagoon exceeded the local evaporation rate of fresh water which is a key comparative in designing evaporation lagoons. During the same period, plant records show that the plant produced the equivalent of 703,000 gallons (25.95 inches) per acre of hide brine meaning that evaporation from the H.E.A.T. process exceeded plant production in the first year. Therefore, based on the first year a single lagoon of less than 4.8 acres in size could actually evaporate the entire production from the plant. This was the focus of the final portion of the test as shown in Table III. Choosing the slowest period of evaporation to run this particular test was an unfortunate choice but reasoned on the basis that this would be the time of year most needing acceleration. At the same time all parties recognized that the pond would likely gain volume over the winter season but felt that this gain would be offset by added loss such that the lagoon would gain on the plant during the summer period. As Table III and Figures 2 and 3 show, this did indeed happen in the early portions of the test with the lagoon evaporating 34 - 82 % of the inflow at various points in the trial. Additional nutrients were not added after February for budgetary reasons and as shown in Table III the plant received significant late winter precipitation. In fact, based on a 5-acre run-off zone (including liner that extended over the berm in two months this rainfall equaled or exceeded (Jan, Feb, March) brine coming from the plant. This created a double problem for the process. First, the organisms used are very adapted to the extremely high salts and are inhibited or killed by fresh water (indeed the population dropped by a full log (to 10^5 TCFU and RCFU) between Feb and March 2021 despite receiving additional nutrient. While the fresh water will ultimately become saltier as the salt pack dissolves this lower density water will tend to float on top of the high-density brine further inhibiting brine evaporation, which will continue until natural mixing (by wind and/or temperature) mixes the lagoon. Once again, this is evident from the sudden drop off in evaporation in March 2021 (to only 6%). Two additional bits of information however, also point to the effectiveness of this process in that as Table III shows if the amounts of brine being added were not evaporated the pond staff gauge should have increased much more than was measured by plant personnel. These differences ranged from the measured readings being anywhere from 0.5 to over 1.0 ft. However, the March recordings were closer at 0.27 ft (3 inches). Finally, a comparison of the volume of brine present in the lagoon showed that in December 2019 with a gauge reading of 9.83 ft, South Lagoon held over 3.65 million gallons of brine. By March 2021, the same Staff Gauge measurement (9.83 ft) occurred when the lagoon contained only 2.45 million gallons (see supplemental tables). This meant that the salt pack had increased in size and now occupied an additional 1.2 million gallons of the pond. Given the solubility of salt (32% w/v) this would require nearly 3.6 million gallons of evaporation which is in excellent agreement with the overall measured evaporation.

Based upon all of the available data, images and measurements, the following conclusions are warranted. The H.E.A.T. process works and accelerates brine evaporation in an economically significant manner even in the first year of treatment. Especially in the first year of establishment, the microbial population and the overall treatment are very sensitive to continued nutrient supplementation. The process may well slow or backtrack if this treatment is interrupted. In addition to accelerating evaporation, the process also provides significant (98%) reduction in lagoon BOD. While the process and microbial populations needed, appear to be stimulated by continuing inflow, the system does require maintenance with ESM's supplemental recipes. During winter, even a treated lagoon will gain volume, but based on the first year's results, this gain should be compensated by increased summer evaporation. Further testing and continued measurement have not yet covered a full summer period. One collateral bit of information that must be considered in all of these systems, is that accelerating brine evaporation will cause treated lagoons to gain a larger salt pack. This always needs to be considered as it will somewhat shorten the design life of a specific lagoon. At the same time, the increased evaporation allows for fewer or smaller Lagoons or allows users to clean and maintain these lagoons well before the design life of the liners is compromised. Furthermore, while the salt produced in a hide lagoon should probably not be used for further hide curing, the material produced does appear to be of sufficient quality for some cost recovery by using it as a road salt or other uses where extreme purity is not required.

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Eastern Shore Microbes (ESM) is now introducing its proprietary biologically based sustainable *Halophilic Evaporative Applications Technology (H.E.A.T.)* process for use in the industry. The authors thank Mr. Charles Hall of MSC Engineering in Virginia Beach, Virginia for production of the CAD tables used in this work. They also are indebted to Dr. Anthony Nicastro for assistance verifying calculations, volumes and surface area data of the lagoons.

Statement of Financial Interest

The authors declare that they are owners of Eastern Shore Microbes and as such maintain a financial interest in the H.E.A.T. process.

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