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CHARACTERISTICS OF PHOTOTROPHIC AND NON–PHOTOTROPHIC LAGOONS FOR SWINE MANURE

T. Chen, D. D. Schulte, R. K. Koelsch, A. M. Parkhurst

ABSTRACT. *Odors are a major result of inadequately sized and mismanaged anaerobic lagoons. However, purple or pink colored lagoons, indicating the presence of phototrophic purple bacteria, are less likely to be an odor nuisance than are non–purple lagoons. Eight swine lagoons were studied to quantify critical parameters thought to allow purple lagoons to be a more reliable odor control alternative. Bacteriochlorophyll a (Bchl a), which indirectly measures the abundance of phototrophic bacteria, was greater in purple lagoons than in non–purple lagoons (P = 0.01). Oxidation–reduction potential (ORP) was less negative for purple lagoons than for non–purple lagoons in both spring (lagoon temperatures of 6.7°C to 8.8°C) and during summer (temperatures of 22°C to 25°C), indicating conditions favoring phototrophism (P = 0.04). Dissolved oxygen levels were near zero and light penetration was minimal in all lagoons. Average sulfide concentrations of all the lagoons were in the range of 1.6 to 6.5 mg/L, which is below the preferred range for purple sulfur bacteria (PSB) growth. Purple lagoons appeared to have lower concentrations of ammonia, alkalinity, chemical oxygen demand, and electrical conductivity among the lagoons studied. Copper and zinc concentrations of all lagoons were not in the range considered to be toxic for anaerobic bacteria. Calculated volatile solids loading rates did not explain differences in Bchl a levels in the lagoons.*

Keywords. *Anaerobic bacteria, Manure management, Odor control, Swine lagoon effluent.*

An important disadvantage of anaerobic lagoons is the potential emission of undesirable odors. However, purple or pink lagoons are less likely to be considered an odor nuisance than are non–purple lagoons (Green, 1966; Meredith and Pohland, 1970; van Lotringen and Gerrish, 1978; Gebriel et al., 1994; MWPS, 2001; Zahn et al., 2001). Purple sulfur bacteria (PSB) and purple nonsulfur bacteria (PNSB) give anaerobic lagoons a distinct purple or pink color (Cooper et al., 1965; Holm and Vennes, 1970; Sletten and Singer, 1971; Cooper et al., 1975; Merrill et al., 1998; Zahn et al., 2001) due to pigments from a variety of carotenoids and bacteriochlorophyll *a* and *b* present in these bacteria (Pfennig, 1978).

Purple sulfur bacteria (PSB) are photoautotrophic organisms of the order *Rhodospirillales* belonging to the *Chromatiaceae* family. They carry out photosynthesis anoxygenically (Imhoff, 1995). That is, they do not evolve oxygen during photosynthesis and rely solely on light energy for ATP synthesis and CO_2 fixation. PSB oxidize reduced sulfur compounds (e.g., H_2S, thiosulfide) to sulfate or zero–valent sulfur to provide electrons for CO_2 fixation. PSB can store zero–valent sulfur temporarily as globules inside the cell, while CO_2 and simple organic compounds are utilized as carbon sources in the photosynthetic process (Pfennig and Trüper, 1989). In addition, PSB remove amine compounds, produce anti–viral substances, and yield a high–protein biomass that, if harvested, could be a potential feed product (Gebriel et al., 1994). PSB are ubiquitous in nature, commonly being found in sulfur springs and stagnating aqueous environments where H_2S is readily available.

Purple non–sulfur bacteria (PNSB) are photoheterotrophic and anoxygenic, and may also exist in anaerobic lagoons. PNSB belong to the family *Rhodospirillaceae* (Imhoff, 1995). Unlike PSB, they lack the capacity to oxidize zero–valent sulfur to sulfate. Both PSB and PNSB operate only under anaerobic conditions, although there are variations in the tolerance of both families to oxidative conditions (Pfennig and Trüper, 1989). PSB have a competitive advantage over PNSB in sulfide–rich environments (Pfennig, 1978). Except in a study by Zahn et al. (2001), PSB have been the dominant organism when species of purple bacteria have specifically been identified in animal waste lagoons (McFarlane and Melcer, 1977 – *Thiocapsa roseopersicina*; van Lotringen and Gerrish, 1978 – *Thiocapsa roseopersicina*; Wenke and Vogt, 1981 – *Tiopedia rosea*; Freedman et al., 1983 – *Tiopedia rosea*; and Earle et al., 1984 – *Tiopedea rosea*). Zahn et al. (2001) found that a PNSB species of Rhodobacter dominated the microbial community in a number of photosynthetic swine lagoons. Because of the phototrophic nature of PSB and PNSB, bacteriochlorophyll *a* (Bchl *a*) has often been used as a surrogate measure for the abundance of purple bacteria in waste treatment systems (Wenke and Vogt, 1981; Earle et al., 1984; Gebriel et al., 1994; Gilley et al., 2000, Zahn et al., 2001).

The benefits of phototrophic activity in anaerobic waste treatment lagoons have been known for some time. For

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example, Holm and Vennes (1970) described the elimination of sulfides due to PSB in municipal waste lagoons. Sletten and Singer (1971) noted that PSB reduce odors resulting from hydrogen sulfide and remove simple volatile organic compounds from anaerobic lagoons. Cooper et al. (1975) reported reduced concentrations of sulfides in industrial organic waste due to “red” sulfur bacteria. Earle et al. (1984) used PSB to reduce soluble BOD, phosphorus, and ammonia in swine lagoon effluent by approximately 90%, 50%, and 30%, respectively, while obtaining a protein yield of 1.8 g L^{-1}. In a study of 29 swine lagoons in Iowa, Oklahoma, and North Carolina, Zahn et al. (2001) classified ten as phototrophic based on cluster analyses of lagoon total sulfur and phosphorus concentrations, and methane, odor, and volatile organic compound emissions. The ten phototrophic lagoons had significantly lower odor intensities and volatile organic compound emission rates than the other lagoons when sulfur and phosphorus levels were less than 20 and 100 mg/L, respectively. One report, McGahan et al. (2001) in Australia, contradicts the literature by indicating a lack of correlation between PSB indicators and odor reduction in swine lagoons. However, only one of the six lagoons in that study was judged to be truly purple, and it was noted that high levels of pH (7.4 to 8.0), electrical conductivity (6.4 to 9.2 dS m^{-1}), and iron may have compromised the PSB’s ability to perform.

Limited design and management information is available to assist planners or operators of anaerobic lagoon systems on how to enhance and maintain populations of phototrophic purple bacteria. The American Society of Agricultural Engineers (*ASAE Standards*, 2001) and the Natural Resources Conservation Service (NRCS, 1996) do not mention phototrophic lagoons in their design procedures for anaerobic lagoons. Instead, odor control in lagoon design involves reduced volatile solids loading rates (VSLR) compared to that for simple storage and treatment. However, some design and management information for enhancing purple bacteria presence has been published. In the study by Zahn et al. (2001), average VSLRs ranged from 0.07 to 79 kg_{VS} m^{-3} d^{-1}, with the lower rates associated with phototrophic lagoons. In another study, van Lotringen and Gerrish (1978) developed a model for predicting the role of PSB in H_2S removal from anaerobic lagoons. They also provided a case study in which it was observed that inoculating a swine waste lagoon with PSB resulted in a large PSB presence and that proper manipulation of pumpdown and loading strategies also fostered PSB growth.

Other studies show that oxidation reduction potential (ORP), electrical conductivity (EC), and diet provide insight as to management of lagoons to foster PSB growth. For example, Schulz and Barnes (1990) indicated that swine waste lagoons appeared to have low odors as long as the ORP of the surface was more positive than –76 mV. Bakke et al. (1999) used a laboratory study to show that high loading rates (256 g_{VS} m^{-3} d^{-1}) could be maintained while achieving an ORP of –7.5 mV and high levels of Bchl *a*. Electrical conductivity integrates factors such as rainfall, evaporation, diet, and drinking water quality with management factors such as loading rate and pumpdown cycles in lagoons. Fulhage (1995) reported that odor levels increase when EC levels exceed about 10 dS m^{-1}. Schulte and Koelsch (1998) analyzed results from 37 lagoons and found that Bchl *a* levels were greatly diminished when EC levels exceeded approximately 6 dS m^{-1}. A threshold of 2500 to 5000 mg L^{-1} salts is suggested as an indicator of the need to dewater or dilute lagoons (*ASAE Standards*, 2001). Depending on the individual salts present in a lagoon, 2500 to 5000 mg L^{-1} could result in EC ranging from approximately 2.5 to 11 dS m^{-1}. Choice of heavy metals used in animal diets may also play a role in successful management of lagoons containing PSB. Gilley et al. (2000) concluded that, to encourage high levels of Bchl *a* for odor reduction from anaerobic swine lagoons, dietary zinc rather than copper was preferable as a supplement for weanling pigs.

From the literature cited in the previous paragraphs, there is a lack of statistically based data sets with which to distinguish between the characteristics of phototrophic and non–phototrophic swine lagoons for manure management. The objectives of the research reported in this article were to: (1) compare and contrast the characteristics of phototrophic (purple) and non–phototrophic swine manure lagoons, and (2) report management data that may improve the understanding of such lagoons.

MATERIALS AND METHODS

LAGOON DESCRIPTION

The criteria for selection of lagoons involved locating several lagoons that were reported to turn purple and several that never turned purple. The criteria also involved producers’ interest and willingness to participate by sharing design and management information and allowing sampling of their lagoons. Eight swine lagoons were chosen, all located in southeast and south–central Nebraska. Five of the eight lagoons were selected based on a history of turning purple, while the three remaining lagoons were non–purple lagoons. The lagoons received manure from facilities that had a variety of swine production phases (table 1).

DATA COLLECTION

Lagoon Management and Site Survey

Management and site surveys of the eight facilities were conducted prior to lagoon sampling. A personal interview was conducted with each producer, resulting in the following information:

Table 1. Lagoon identification and conditions.

Lagoon	One–Time Capacity of Facility	Conditions
A	216 nursery pigs, 24 lactating sows.	Purple
B	169 nursery pigs, 275 growing pigs, 280 finishing pigs, 125 sows and gilts, 12 boars.	Purple
C	600 nursery pigs, 400 growing pigs, 260 sows and gilts, 16 boars.	Purple
D	1200 nursery pigs, 520 sows and gilts, 2 boars.	Transitional[a]
E	250 growing pigs, 250 finishing pigs.	Transitional[a]
F	1136 nursery pigs, 1600 growing pigs, 1800 finishing pigs, 525 sows and gilts, 26 boars.	Non–purple
G	230 nursery pigs, 130 sows and gilts, 8 boars.	Non–purple
H	1050 nursery pigs, 515 sows and gilts, 12 boars.	Non–purple

[a] Lagoons reported to turn purple in previous years, but failed to turn purple during year of sampling.

Table 2. Lagoon liquid dimensions in spring and [summer].

Lagoon	Depth (m)	Surface Area (ha)	Volume (m^3)
A	2.0 [2.0]	0.1 [0.1]	1020 [1 100]
B	3.0 [3.0]	0.2 [0.2]	2 100 [2 100]
C	3.8 [2.2]	0.4 [0.3]	10 200 [4 300]
D	5.5 [5.7]	0.9 [0.9]	28 300 [36 450]
E	2.2 [2.4]	0.2 [0.2]	2 590 [2 860]
F	1.7 [2.1]	0.6 [0.7]	10 100 [12 900]
G[a]	2.0 [0.5]	0.3 [0.2]	4 860 [1 000]
H	3.0 [2.7]	0.4 [0.4]	7 410 [7260]

[a] Pumped just prior to summer sampling.

- Swine herd inventory (type, number and weights of animals, schedules) for estimating manure production.
- An estimate of cleanup and flush water use.
- Lagoon loading procedures and effluent removal frequency and timing.
- Feeding programs (ingredients and consumption) with focus on copper, zinc, and antibiotics in feed.

Lagoon Characterization

The eight lagoons were sampled in early spring and again in mid–summer of 1996. The liquid volume of each lagoon on the day of sampling was obtained by measuring the liquid surface dimensions, slopes, and lagoon depths. Horizontal dimensions and slopes were determined using a measuring wheel and level; depths were obtained by lowering a weighted 20–cm Secchi disk (Wildco) until the disk met resistance. The Secchi disk was also used to determine light penetration. Information on lagoon liquid depth and the calculated surface areas and volumes are summarized in table 2.

The volume of each lagoon was divided into four approximately equal quadrants at each visit. Liquid samples were collected from each quadrant at the surface and at the 0.3, 0.6, 1.2, and 2.1 m depths using a 2.2 L horizontal water bottle sampler (Wildco). Water temperature, ORP, and pH determinations were made in situ at each depth. Dissolved oxygen (DO) readings were only taken at the surface and 0.3 m depth in spring, and at the surface, 0.3, and 0.6 m depths in summer. A YSI Model 58 meter (Yellow Springs Instrument Co.) was used for DO measurements. ORP and pH values were determined with a Hach Model E10 pH Meter (Hach Co.). A digital thermometer (Radio Shack) was used to measure lagoon water and air temperatures. Samples that were to be used for sulfide and chemical oxygen demand (COD) determinations in the laboratory were preserved on site according to Standard Methods (APHA, 1997). All samples collected for laboratory analysis were put on ice in the field to retard degradation.

Laboratory and Field Procedures

Table 3 lists the measurement location, season, and analytical protocols for the samples. Bchl *a* procedures were modified from those of Austin (1988) and Siefert et al. (1978) by centrifuging a 500 mL sample at 2400 g for 25 min, adding 10 mL boiling methanol to the pellet for a few seconds, and then adding 3 mL 0.05% NaCl (w/v) and 13 mL n–hexane to the methanol extract to obtain a two–phase extraction. Absorption in the hexane phase was measured at a wavelength of 768 nm.

Table 3. Sampling protocol.

Parameter	Where Measured	Spring	Summer	Method
Temp. (°C)	Field	[a]	[a]	Digital thermometer
DO (mg/L)	Field	[a]	[a]	YSI Model 58
ORP (mV)	Field	[a]	[a]	Hach Model E10
pH	Field	[a]	[a]	
Bchl *a* (µg/L)	Lab	[a]	[a]	Modified from Austin (1988) and Siefert et al. (1978)
Sulfide (mg/L)	Lab	[a]	[a]	Standard Methods (APHA, 1997)
Sulfate (mg/L)	Lab	[a]	[a]	
NH_4–N (mg/L)	Lab	[b]	[b]	Flow injection analysis (Keeney and Nelson, 1982)
TS (mg/L)	Lab	[b]	[b]	Standard Methods (APHA, 1997)
FS (mg/L)	Lab	[b]	[b]	
VS (mg/L)	Lab	[b]	[b]	
TSS (mg/L)	Lab	[b]	[b]	
Alkalinity (meq/L)	Lab	[c]	[b]	
EC (dS/m)	Lab	[c]	[b]	Orion Model 140 conductivity/salinity meter
COD (mg/L)	Lab	[c]	[b]	Standard Methods (APHA, 1997)
Cu (mg/L)	Lab	[d]	[b]	Atomic Absorption Method 968.08 (AOAC, 1990)
Zn (mg/L)	Lab	[d]	[b]	
TKN (mg/L)	Lab	[e]	[e]	Western States Laboratory (1977)

[a] Samples were taken at each depth in each quadrant, so there were depth and quadrant effects on statistics.

[b] Samples were composited over quadrants, so there was a depth effect on statistics.

[c] Two samples were taken: one was composited at surface over quadrants, and one was composited by quadrant over depths below the surface.

[d] Samples were not measured in spring.

[e] Samples were composited over all depths and quadrants so that each lagoon only has one datum.

Cu and Zn were determined using atomic absorption on the ash remaining from the volatile solids determination. The procedure consisted of adding 10 mL of 3 M HCl solution to the ash, boiling for 10 min to solubilize the ash, adding deionized water to bring the acidified solution to 100 mL, and filtering the same. Aliquots were then poured into glass tubes, and a Varian Model Spectra AA–30 atomic absorption spectrophotometer, with an air /acetylene flame, was used to determine concentrations.

STATISTICAL ANALYSIS

Table 3 also lists the statistical protocol by location (surface quadrant and depth) for each parameter. A split plot with 4–level repeated measure was used to statistically analyze the data (Littell et al., 1996). The whole plot was the farm (lagoon). The subplots were the quadrants in each lagoon, and the repeated measure for each lagoon was depth. Farms and quadrants were considered to be random, and the three impacts (purple, transitional, and non–purple lagoons) were considered as a fixed factor in the experimental design. Because both random and fixed effects were involved, the mixed model (Littell et al., 1996) was applied to the split–plot design. Depth was used as a covariant to model both linear and quadratic changes in the observed variables. Statistically significant effects were reported for alpha levels of 5% and below.

RESULTS AND DISCUSSION

The eight lagoons were divided into three groups: purple lagoons (A, B, and C), which turned purple every year; "transitional lagoons" (D and E), which were reported by the producer to have turned purple previously but failed to do so the year sampling occurred; and non–purple lagoons (F, G, and H), which never exhibited purple coloration.

BCHL a

Bchl *a*, an indicator of the presence of purple bacteria, was used in addition to visual observation to distinguish purple lagoons from the other lagoons. Because the vast majority of swine lagoon literature, in which purple bacteria have been specifically identified, has found the organisms to be of the family *Chromatiaceae* (i.e., PSB), Bchl *a* levels measured in this study are taken to be due to the presence of PSB. There is a possibility (Zahn et al., 2001) that some of the organisms could be of the family *Rhodospirillaceae* (PNSB).

Samples taken during the summer portion of this study had average Bchl *a* concentrations of 912 µg/L, near the middle of the range (0 to 2300 µg/L) reported for swine waste lagoons by Bakke et al. (1999), Freedman et al. (1988), Gebriel et al. (1994), Gilley et al. (2000), and McGahan et al. (2001). In this study, non–purple lagoons exhibited the lowest Bchl *a* levels (6 to 43 µg/L), while purple lagoons exhibited the highest levels in both spring (71 to 238 µg/L) and summer (524 to 1038 µg/L) (tables 4 and 5). Bchl *a* concentrations in the purple lagoons were significantly greater than in the non–purple lagoons (P = 0.01) and transitional lagoons (P = 0.05) in spring (table 4). Similar results were obtained in summer with P values of 0.02 and 0.08, respectively (table 5). All lagoons had higher Bchl *a* concentrations in summer than in spring. However, Bchl *a* increase was relatively greater in the purple lagoons than in the transitional and non–purple lagoons.

Tables 4 and 5 also show that Bchl *a* was distributed throughout the depth of all the lagoons, but at higher concentrations in the purple lagoons. However, light penetration, measured with a Secchi disk (Chen, 1997), ranged from only 2.5 to 11.4 cm in spring and 0.3 to 7.0 cm in summer, with no apparent differences between purple and non–purple lagoons. PSB themselves can cause extreme turbidity and may have been partially responsible for decreased light penetration in the summer. PSB, while undergoing photosynthesis near the surface, could be found throughout the lagoon depth, probably due to the motility of PSB (Pfennig, 1978) and because of mixing caused by thermal gradients and wind, or gas bubbles from anaerobic activity. DiSpirito et al. (1995) also found PSB distributed throughout the depth of a swine manure lagoon in Iowa.

TEMPERATURE

The optimum temperature for PSB growth ranges from 20°C to 35°C (Holt et al., 1994). None of the eight lagoons exhibited strong purple coloring, nor high Bchl *a* levels, during the sampling visit in spring, when liquid temperatures were in a range of 6.7°C to 8.8°C (table 4). By late July, lagoon temperatures ranged from 22°C to 25°C (table 5), and lagoons A, B, and C (having a history of turning purple) were again dark purple. Lagoons D and E, with a history of purple coloring (but failure to turn purple that year), did not exhibit this purple color and appeared more of a brown or gray color in spring and summer, thus the description "transitional." Lagoons F, H, and G were very dark brown or black in color

Table 4. Physical, chemical, and biological characteristics of lagoons in spring.

	Purple Lagoons					Transitional Lagoons					Non–Purple Lagoons				
	Depth (m)					Depth (m)					Depth (m)				
Parameter	0.0	0.3	1.2	2.1	Mean[a]	0.0	0.3	1.2	2.1	Mean[a]	0.0	0.3	1.2	2.1	Mean[a]
Bchl *a* (µg/L)	103	71	238	106	117a	22	13	50	63	32b	14	6	12	14	11b
Temp. (°C)	6.9	6.7	7.1	6.8	6.9a	8.8	8.5	7.9	7.6	8.2a	8.0	8.2	8.0	7.6	8.0a
ORP (mV)	–54	–57	–25	–16	–36a	–130	–140	–141	–161	–120a,b	–288	–285	–273	–272	–280b
pH	7.7	7.6	7.7	7.7	7.6a	7.8	7.8	7.8	7.8	7.7a,b	7.7	7.7	7.8	7.7	7.8b
Sulfide (mg/L)	3.1	4.5	1.6	2.0	2.9a	3.3	3.0	2.9	3.4	3.0a	6.5	6.3	5.2	3.1	5.5a
SO_4–S (mg/L)	7.0	6.3	2.2	1.1	4.5a	7.9	6.7	6.8	5.9	5.7a	6.3	5.7	5.8	5.9	5.9a
NH_4–N (mg/L)	321	330	279	434	305a	471	439	465	495	433a	755	804	728	584	730a
TS (mg/L)	2170	1830	4200	13000	5050a	2500	2400	2550	2500	2470a	3870	4030	3900	3700	3890a
VS (mg/L)	1070	800	2070	6830	2600a	1350	1050	1350	1200	1270a	1740	1930	1800	1500	1760a
FS (mg/L)	1100	1030	2130	6170	2450a	1150	1350	1200	1300	1200a	2130	2100	2100	2200	2130a
TSS (mg/L)	390	370	326	10130	3890a	250	425	375	580	362a	650	550	470	350	520a
	Depth (m)					Depth (m)					Depth (m)				
	0.0	0.3 to 2.1			Mean	0.0	0.3 to 2.1			Mean	0.0	0.3 to 2.1			Mean
Alk. (meq/L)[b]	34	36			35	51	51			51	75	76			76
EC (dS/m)	3.3	3.3			3.3	4.4	4.4			4.4	6.4	6.4			6.4
COD (mg/L)	1380	1250			1320	3170	2720			2950	3650	4680			4170
TKN (mg/L)		490					315					835			

[a] Weighted (unequal number of samples at various depths and includes samples from 3.0 and 4.9 m depths in some lagoons). Means followed by the same letter are not significantly different at α = 0.05.

[b] As $CaCO_3$.

Table 5. Physical, chemical, and biological characteristics of lagoons in summer.

	Purple Lagoons					Transitional Lagoons					Non–Purple Lagoons				
	Depth (m)					Depth (m)					Depth (m)				
Parameter	0.0	0.3	1.2	2.1	Mean[a]	0.0	0.3	1.2	2.1	Mean[a]	0.0	0.3	1.2	2.1	Mean[a]
Bchl *a* (µg/L)	1018	980	1038	524	912a	161	164	173	72	140b	43	39	12	9	29b
Temp. (°C)	23	23	23	22	23a	24	24	24	24	24a	25	25	25	25	25a
ORP (mV)	–302	–282	–263	–301	–288a	–266	–282	–285	–273	–257a	–321	–326	–342	–345	–332b
pH	7.5	7.5	7.5	7.4	7.4a	7.8	7.8	7.7	7.7	7.8b	7.7	7.8	7.8	7.8	7.8b
Sulfide (mg/L)	3.4	2.7	2.3	2.8	2.8a	2.9	2.7	2.8	2.9	2.6a	3.9	3.9	4.9	5.7	4.4 a
SO_4–S (mg/L)	9.7	9.7	14.0	13.1	11.2a	4.0	2.4	2.6	3.6	2.6b	9.0	8.0	5.9	3.9	7.0a
NH_4–N (mg/L)	235	227	168	180	208a	339	363	374	352	341a	520	467	618	679	556b
TS (mg/L)	1800	2070	1850	5400	2610a	1650	1750	1800	5150	2350a	2870	3270	3700	6050	3790a
VS (mg/L)	800	870	850	2750	1220a	650	600	600	2400	940a	900	1140	1250	2400	1340a
FS (mg/L)	1000	1200	1000	2650	1390a	1000	1150	1200	2750	1410a	1970	2130	2450	3650	2450a
TSS (mg/L)	370	370	350	3890	1067a	250	175	250	5800	1330a	500	570	350	2480	1130a
Alk. (meq/L)[b]	33	35	28	30	32a	46	45	46	46	46a	64	61	96	82	73a
EC (dS/m)	2.7	2.7	2.8	2.9	2.7a	3.9	3.9	3.9	3.9	3.9a	5.6	5.6	7.3	7.3	6.3a
COD (mg/L)	480	600	610	680	582a	960	1201	1401	1201	1110a	990	910	1230	2360	1280a
Cu (mg/L)	0.2	0.3	0.2	2.3	0.7a	0.1	0.1	0.2	1.0	0.3a	0.3	0.3	0.5	1.7	0.6a
Zn (mg/L)	0.6	0.7	0.5	17.5	4.0a	0.4	0.5	0.7	12.6	2.9a	2.2	1.6	2.3	11.9	4.0a
TKN (mg/L)			657					590					890		

[a] Weighted (unequal number of samples at various depths and includes samples from 3.0 and 4.9 m depths in some lagoons). Means followed by the same letter are not significantly different at $\alpha = 0.05$.

[b] As $CaCO_3$.

in the spring. In the summer, lagoons F and H remained black, but lagoon G had just been pumped down and was green due to an algal bloom that occurred just prior to sampling.

DISSOLVED OXYGEN AND ORP

Dissolved oxygen (DO) levels in the lagoons at the surface ranged from 0.2 to 0.3 mg/L in the spring sampling period, and with the exception of lagoon G, were approximately 0.1 mg/L in the summer. The algal bloom in lagoon G caused elevated DO levels (1.9 mg/L) to occur at the surface. DO levels below the surface (0.3 m and deeper) were approximately zero both in the spring and summer in all lagoons.

Oxidation reduction potential (ORP) has been used in anaerobic systems to measure the intensity of reducing conditions. Converse et al. (1971) reported that an ORP of –250 mV indicates highly anaerobic conditions in wastewater. Tables 4 and 5 indicate that, although all lagoons were anaerobic, there were noticeable differences from spring to summer and from purple to non–purple lagoons. While the non–purple lagoons were highly anaerobic in spring (ORP ranged from –272 to –280 mV), the purple and transitional lagoons had ORPs ranging from –16 to –161 mV, with the transitional lagoons clearly mid–range between the extremes. As shown in table 4, purple lagoons had significantly greater (positive) ORPs than the non–purple lagoons (P = 0.04) in spring. This indicates that the non–purple lagoons may have been overloaded in winter, resulting in the possible release of odors during springtime recovery of those lagoons. The data (table 5) indicate that the ORPs for purple lagoons in summer were again significantly more positive than those for non–purple lagoons (P = 0.006), although all were highly anaerobic. Although anaerobic conditions are required for PSB growth, the results of this study indicate that the purple lagoons had a lesser reducing environment than did the non–purple lagoons, especially in the spring.

pH

The average pH values for purple, transitional, and non–purple lagoons were 7.6, 7.7, and 7.8, respectively, in spring and 7.4, 7.8, and 7.8, respectively, in summer (tables 4 and 5). Statistical analysis showed that the pH differences between purple and non–purple lagoons were significant (P = 0.005) in both spring and summer (tables 4 and 5). According to Holt et al. (1994), the preferred pH range for the growth of PSB is 6.8 to 7.5. However, Pfennig (1978) found that the optimum pH for the growth of PSB is in the range of 7.0 to 8.5. Thus, the pH values (7.4 to 7.8) of all the lagoons appeared to be near or within the range for survival of PSB.

SULFIDES AND SULFATES

Sulfide, sulfate, and Bchl *a* concentrations in purple anaerobic lagoons are highly interdependent, and the relationships are influenced by temperature and light intensity (van Lotringen, 1978; Gebriel et al., 1994). The data in tables 4 and 5 were from samples taken between approximately 10:00 a.m. and 3:00 p.m., when photosynthesis would have been near maximum, enabling much of the sulfide to have been oxidized to zero–valent sulfur or sulfate. Thus, the relatively low levels of sulfide and correspondingly higher levels of sulfate reported in tables 4 and 5 are not surprising. Bchl *a* production is a function of sulfide availability, peaking at approximately 8 mg S/L in the study by Gebriel et al. (1994). Earle et al. (1984) and van Lotringen (1978) reported that PSB grew well at sulfide concentrations of 7 to 28 mg/L and 13 to 19 mg/L, respectively, both in purple lagoons receiving swine manure. In general, sulfide concentrations were in the range of 1.6 to 6.5 mg/L, with 2.9 and 2.8 mg/L measured in the purple lagoons and 5.5 and 4.4 mg/L in the non–purple lagoons during spring and summer, respectively (tables 4 and 5). Although the sulfide levels appeared lower in the purple lagoons, the variability and number of samples prevented the differences from being statistically significant at the 5% level (tables 4 and 5). The observed concentrations reported in tables 4 and 5, with the exceptions of lagoons B and F in spring, appear to be below the range previously reported for purple lagoons.

VOLATILE SOLIDS LOADING RATES AND DILUTION

Producers sometimes have been encouraged to construct lagoons larger than recommended by ASAE, MWPS, or NRCS to reduce potential odor problems. While relatively large lagoons may reduce odors, the survival of PSB may not depend entirely on low volatile solids loading rates (VSLR) (Bakke et al., 1999; McGahan et al., 2001). This is because the VSLR, a typical design parameter for sizing lagoons, dictates only the treatment volume. The total design volume of a lagoon consists of the treatment volume, a sludge layer, a storage volume (manure plus wash and flush water), and capacity for net precipitation plus a 25–year, 24–hour rainstorm (MWPS, 2001; *ASAE Standards*, 2001). Producers in this study were unable to provide the design data needed to determine the treatment volume. However, data for estimating the total liquid volume of the lagoon, the volatile solids production rate, and cleanup and flush water use were obtained. Therefore a "modified VSLR" was calculated based on the liquid volume in the lagoon (instead of on the treatment volume) at the time of the visit. Purple lagoon B experienced the highest modified VSLR (130 g VS $m^{-3}d^{-1}$) observed for all lagoons in this study (table 6). It was approximately 20% greater than recommended treatment volume VSLR (110 g VS m^{-3} d^{-1}) for odor control in southeastern Nebraska (MWPS, 2001). It would appear that volatile solids loading rate may be less critical than previously anticipated for allowing PSB to prosper. This is consistent with laboratory results of Bakke et al. (1999), in which Bchl *a* levels of 310 μg/L were achieved in spite of a VSLR of 256 g VS m^{-3} d^{-1}, and field results (McGahan et al., 2001) in which a pink lagoon (Bchl *a* = 695 μg/L) had a 178 g VS m^{-3} d^{-1} VSLR, which was 200% of the Australian design recommendation.

Management factors, such as the amount of flush and cleanup water, may also play a role in encouraging purple lagoons. Since phototrophic bacteria require sunlight, dilution of the manure with flush and cleanup water may foster sunlight penetration and bacterial growth. In order to compare the amounts of dilution used by the operators of each lagoon, the calculated rate of volatile solids production by the herd was divided by the reported use of flush and cleanup water (table 6). On this basis, the purple lagoons had an average dilution rate of 0.11 m^3/kg VS (range 0.08 to 0.14 m^3/kg VS). This was in contrast to an average dilution rate of 0.05 m^3/kg VS for the transitional and non–purple lagoons (range 0.003 to 0.19 m^3/kg VS).

Table 6. Reported and calculated operating characteristics.

Lagoon	Condition	Calculated Volatile Solids Production[a] (kg/day)	Estimated Flush and Cleanup Water (m^3/yr)	Calculated Dilution Rate[b] (m^3/kg VS)	Modified Loading Rate[c] $\left(\frac{\text{g VS}}{m^3 \cdot \text{day}}\right)$
A	Purple	57	2820	0.14	61 [53]
B	Purple	274	11800	0.12	130 [130]
C	Purple	258	8000	0.08	22 [61]
D	Transitional	424	800	0.005	13 [12]
E	Transitional	158	2900	0.05	61 [55]
F	Non–purple	1570	1500	0.0003	122 [156]
G	Non–purple	114	7800	0.19	53 [114]
H	Non–purple	386	1800	0.03	51 [53]

[a] Calculated from estimated herd weight and NRCS (1996) values.

[b] Flush water plus cleanup water divided by calculated volatile solids production rate.

[c] Calculated by dividing the estimated VS added per day by the total liquid volume of the lagoon (table 2) at the time of sampling. Values in [brackets] are calculated rates during summer. Volatile solids loading rate for the permanent pool as recommended by NRCS (1996) is 70 g m^{-3} d^{-1} for Lincoln, Nebraska.

Although interesting, the modified VSLR and dilution rates do not provide conclusive evidence that purple lagoons are a sole consequence of loading or dilution.

AMMONIA, SOLIDS, ALKALINITY, CONDUCTIVITY, AND COD

PSB are known to consume simple organic acids and ammonia and to reduce BOD in anaerobic lagoon effluents (Holm and Vennes, 1970; McFarlane and Melcer, 1977; Earle et al., 1984). In tables 4 and 5, it would appear that solids, alkalinity, EC, and COD concentrations were lower in the purple lagoons than in the non–purple lagoons; however, statistical comparison of the means does not confirm that observation. Only ammonium concentrations were statistically lower (in the summer) for purple than for non–purple lagoons (P = 0.01) and transitional vs. non–purple lagoons (P = 0.0005) (table 5). Variability and the compositing strategy for sampling (table 3) may have prevented the differences in solids, alkalinity, EC, and COD from being statistically significant. Analysis of the variability in the data indicated that an additional one to three lagoons in the summer were likely needed to detect statistically different levels of alkalinity, COD, and EC at the 0.05 probability level. A total of 18 lagoons would possibly have been needed to show like differences in alkalinity, EC, and ammonium in the spring. Solids levels (spring and summer) and COD (spring) were extremely variable, and estimation of the additional number of lagoons needed for those parameters was not attempted.

If the concentrations of these parameters were indeed lower in purple lagoons, and if the calculated organic loading rate was as high or higher in the purple than in the non–purple lagoons (table 6), then the impact of the high loading rates may have been offset by greater biological activity or by dilution. Electrical conductivity (which indirectly measures salinity) and fixed solids (FS) would be less influenced by biological activity than would ammonium, volatile solids, and COD, but could be affected by dilution, evaporation, feed constituents, and drinking water quality. Although not statistically different, the EC and FS levels of the purple lagoons were approximately half that of the non–purple lagoons. Evaporation in excess of dilution, failure to remove salinity via periodic pumpdown, and high salt levels in the feed or drinking water could contribute to excessive salinity in lagoons. The non–purple lagoons in spring and summer had total dissolved solids levels (TDS = TS – TSS in tables 4 and 5) of 3370 and 2660 mg/L, both within the salt range (2500 to 5000 mg/L) suggested by ASAE (*ASAE Standards*, 2001) for dewatering or dilution. The purple and transitional lagoons had TDS levels (range 1110 to 1890 mg/L) well below the suggested dewatering/dilution threshold. On an EC basis, the purple and transitional lagoons characterized in this study had a range of 2.7 to 4.4 dS/m, while the non–purple lagoons had mean levels of approximately 6.3 dS/m. This is consistent with the observation of Schulte and Koelsch (1998), in which 6.0 dS/m also appeared to be the dividing line between purple and non–purple lagoons.

COPPER AND ZINC

Copper, zinc, and antibiotics in manure may affect phototrophic purple bacteria survival. Research by Kugelman and Chin (1971) on the effects of heavy metals such as Cu and Zn on anaerobic treatment indicates that a high tolerance to heavy metals is possible due to their precipitation by sulfides and sequestration by ammonia and reactive groups of organic material. Their literature review (Kugelman and Chin, 1971) indicated that 5 to 10 mg/L of Cu and 10 mg/L of Zn were the highest concentrations for which anaerobic digestion proceeded satisfactorily. Brumm and Sutton (1979) found that Cu had a bactericidal effect on biological decomposition of stored swine manure at concentrations of 30 and 40 mg/L. In a study directly related to that reported here, laboratory reactors receiving manure from swine diets having high levels of copper decreased Bchl *a* levels and significantly increased sulfide levels, creating conditions apparently toxic to PSB (Gilley et al., 2000).

In this study, Cu and Zn were measured only in summer. There were no significant differences (P = 0.05) for Cu and Zn levels between purple and non–purple lagoons (table 5) during the summer. Cu and Zn concentrations ranged from 0.2 to 0.5 and from 0.4 to 2.3 mg/L, respectively, in the surface to 1.2 m depths of all the lagoons. This was above the levels (0.2 mg/L Cu and 0.1 mg/L Zn) found in phototrophic lagoons by Zahn et al. (2001) but below levels (0.5 to 0.7 mg/L Cu and 5 to 9 mg/L Zn) in the supernatant of the laboratory reactors used by Gilley et al. (2000). However, at the 2.1 m depth, which in some cases bordered the sludge layer, Cu and Zn levels ranged from 1.0 to 2.3 and from 11.9 to 17.5 mg/L, respectively. Samples were not taken from the sludge layer, where concentrations likely would have been greater due to the influence of settling. Lagoon E, which had a history of turning purple, failed to do so during the summer when sampling occurred. Higher than normal Zn additions in the feed (30 times greater ZnO levels than prior use was reported by the operator) over a four–month period the previous winter may have led to this failure. However, the producer reported that this lagoon turned purple later that fall. Gilley et al. (2000) indicated that, unlike Cu, normal levels of Zn were not detrimental to PSB and, in fact, that Zn may stimulate PSB growth.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study showed that:

- Purple lagoons had significantly higher concentrations of Bchl *a* than did non–purple lagoons in both spring and summer. All lagoons had higher Bchl *a* levels in summer than in spring. Bchl *a* is often used as an indicator of the presence of phototrophic purple bacteria.
- Calculated volatile solids loading and dilution rates did not conclusively explain differences in Bchl *a* concentrations between purple and non–purple lagoons.
- The ORP levels of all lagoons were significantly more negative in summer than in spring and were less negative for purple lagoons than for non–purple lagoons in both spring and summer.
- Purple lagoons had sulfide concentrations ranging from 1.6 to 6.5 mg/L as S, which was below the reportedly preferred range for PSB growth.
- Purple lagoons had comparatively lower concentrations of alkalinity, electrical conductivity, COD, and ammonia than non–purple lagoons. However, only the summertime ammonia concentrations were statistically lower at the 5% level of significance. Salt levels (as indicated by electrical conductivity) of the non–purple lagoons were above the recommended range suggested by ASAE.
- No significant differences in Cu and Zn levels were observed among the three groups of lagoons. The concentrations of Cu and Zn in the surface level of all lagoons were below the toxic range reported for anaerobic bacteria.

Several recommendations for further research are evident as a result of this study. For example, this study determined the characteristics of wastewater samples of purple and non–purple lagoons from just above the sludge layer to the surface. The influence of the sludge on pH, sulfide, and EC levels in the lagoons was unknown in this study. To get a better understanding of the differences between purple and non–purple lagoons, further work on the characteristics of lagoon sludge samples is needed. More work on anti–microbial feed additives on the survival of purple sulfur bacteria is also needed. The effect of seeding a lagoon with supernatant from a purple lagoon to enhance establishment of purple sulfur bacteria population in a newly constructed lagoon may prove valuable but is poorly documented and deserves further attention. Finally, because the levels of a number of measured parameters (e.g., alkalinity, EC, and COD) appeared to be numerically lower in purple and non–purple lagoons, but were not statistically different at the $P \leq 0.05$ level, it is recommended that additional lagoons be included in subsequent studies.

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