A Microbial Antidote for Toxic Waste: The Algal-Bacterial Selenium Removal Process for Agricultural Runoff

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San Francisco Bay's population of Dungeness crab declined 79 per cent from 1961 to 1982 (Dahlstrom and Wild, 1983). The striped bass population fell between 50 and 73 per cent during approximately the same period (SWRCB, 1980). One possible minor contributor to these declines is the toxic element selenium (Greenberg and Kopec, 1985). Selenium, leached from the soil of the western San Joaquin Valley by irrigation water, is carried to the Bay by the San Joaquin River. Scientists are making efforts to develop water treatment plants to remove selenium from agricultural runoff before it reaches biologically important areas such as the Bay. One proposed selenium removal plant, called the Algal-Bacterial Selenium Removal System (ABSRS), would extract the selenium by mixing runoff water with a chemically reactive, fermented sludge of algae (Oswald *et al.*, 1988). The research described in this paper relates to the preparation of the algal sludge. The results will aid in designing the ABSRS. Such a plant could be a step toward detoxifying the waters flowing into San Francisco Bay.

An Altered Ecosystem

San Francisco Bay is a reaction chamber for life. Tidal waters flowing into the Bay through the Golden Gate meet and mix with the nutrient-rich San Joaquin and Sacramento River waters flowing into the Bay from the east. These waters, mixing together in the shallow Bay, provide the soup of nutrients, salinity and temperatures needed to support one of the richest ecosystems on earth. But in the late 1950s both crab and bass populations started experiencing declines that continue today. Chemical discharges have been implicated in the declines (Greenberg and Kopec, 1985; CDFG, 1983), and now pregnant women are cautioned against eating Bay seafood due to the heavy metals accumulated in the organisms' tissues (Jones, 1989). Selenium also accumulates in tissue. Waterfowl at the selenium-contaminated Kesterson Reservoir have produced large numbers of deformed embryos probably due to the high levels of selenium in the birds' tissues (Kilness and Simmons, 1985).

For 17 years under-field drain lines have collected selenium-laden irrigation water from 77,000 acres of western San Joaquin Valley farmland and dumped part of it into the San Joaquin River (SWRCB, 1984-1985) or reservoirs like Kesterson. Twenty-five hundred pounds of selenium are estimated to flow into the Bay through the Delta each year (Greenberg and Kopec, 1985). This selenium is mostly in the form of selenate (SeO_4^{-2}).

Past Research in Selenium Removal and Gas Production by Microbes

The Algal-Bacterial Selenium Removal System: A good explanation of the algal-bacterial treatment is found in Oswald *et al.* (1988). The following simplified explanation is drawn primarily from this source.

The algal-bacterial treatment consists of four main stages. The first two prepare the algal sludge, and the second two use the sludge to remove selenium from the runoff water. First selenium-laden water from a sub-field drain is pumped into a continuous canal designed to grow algae. The canal is stirred by a paddlewheel to keep the algae and nutrients mixed. Part of the effluent of the canal is drained off into a settling unit where algae are concentrated and removed from the water.

These concentrated algae are then fed into anaerobic digesters which are air-tight silos. There the algae are fermented to produce methane gas and the sludge needed to remove selenium from the drain water. The methane is burned to produce electricity to power the plant. An energy surplus may be available for other uses.

In the digesters, methane fermentation occurs in three stages (McCarty, 1964). First, microbes hydrolyze complex carbohydrates, proteins and fats into simple sugars and amino acids which, in the next step, are converted to aldehydes, alcohols, organic acids, ketones, carbon dioxide and hydrogen. In the last step, methane-forming bacteria reduce the above substances to methane and carbon dioxide. Reduction is the transformation of a substance due to the substance receiving electrons. Two reactions produce methane in digesters:
(With acetic acid as the methyl group donor.)

$$7\,CH_3COOH \rightarrow 7\,CH_4 + 7\,CO_2 \qquad \text{(eq. 1)}$$

$$3\,CO_2 + 12\,H_2 \rightarrow 3\,CH_4 + 6\,H_2O \qquad \text{(eq. 2)}$$

The reactions can be summarized:

$$7\,CH_3COOH \rightarrow 10\,CH_4 + 4\,CO_2 \qquad \text{(eq. 3)}$$

The acetic acid reaction accounts for about 70 per cent of the methane produced according to McCarty (1964). Thus, assuming no other significant gas-producing reactions occur, the ratio of methane to carbon dioxide produced should be, at most, 10:4 or 2.5:1.

Sludge from the digesters is pumped into a reduction tank where the sludge is mixed with deoxygenated water from the canal. The mixture is held for a day or two in the highly reduced

environment created by the sludge. In this environment, selenate is reduced to one of its less soluble forms, such as selenite, elemental selenium, or organic selenium according to these simplistic theoretical reactions:

$$\text{Reduced Products} \rightarrow CO_2 + H_2O + 4\,e^- \quad \text{(eq. 4)}$$

$$+ \quad 4\,e^- + 2\,SeO_4^{-2} \rightarrow 2\,SeO_3^{-2} + O_2 \quad \text{(eq. 5)}$$

$$= \text{Reduced Products} + 2\,SeO_4^{-2} \rightarrow CO_2 + H_2O + O_2 + 2\,SeO_3^{-2} \quad \text{(eq. 6)}$$

The reduced selenium will become incorporated either in precipitated solids or the biomass of the algal-bacterial sludge. The selenium-laden by-products will be filtered from the water and sent to a hazardous waste site.

The California State Water Resources Control Board selenium standard is 5 µg Se/L. This level has been reached a few times in laboratory experiments with the ABSRS (Oswald *et al.*, 1988).

Research on the anaerobic digestion of microalgae has been conducted by Gerhardt (1989, pers. comm.), Chen (1987), Benemann *et al.* (1978), and McCarty (1964).

Methodology

The goal of the present research was to determine how the methane-producing and selenium-reducing capability of fermented algal sludge changes with the residence time, that is, the amount of time the fresh algae were given to ferment. For the greatest effectiveness of the plant, a residence time that maximizes the selenium reduction and methane production must be determined. Laboratory digesters and reducing chambers were used for this purpose. Inflow and outflow of mass from the digester systems were followed in order to confirm the theoretical operation of the digesters.

Digester setup: Three digesters with nitrogen atmospheres were assembled. The digesters were stoppered, two-liter flasks that were seeded with 1 L of anaerobic sludge from another digester and then kept at 25°C ± 3° in an incubator for 70 days (Figure 1). The optimal temperature for digestion is 40°C (Chen, 1987), but the ABSRS digesters will not be heated and are estimated to operate at 25°C. Algal feed was introduced and removed with a syringe through one hole of the stopper, and gas was collected through the other hole.

Digester feeding: Each digester was fed at a different rate, and therefore, each had a different residence time. One held the algae for 40 days, one 20 days and one 10 days. The feeding rate

was determined by dividing the 1000 ml volume of the digesting material by the desired residence time:

1000 mL/residence time = mL of sludge to feed per day

The digesters were fed on Mondays, Wednesdays and Fridays. It was assumed that this schedule would replicate the feeding regime of digesters of the treatment plant. The amount fed at each feeding was the feeding rate times the number of days until the next feeding. For example, the feeding rate of the 10-day residence time digester was 100 mL/day. Therefore, on Fridays the digester was fed:

(3 days)(100 mL/day) = 300 mL to be fed

After the algae were injected into the digester flask, the flask was swirled by hand for 15 s, and then sludge was withdrawn from the digester until the digester volume equaled 1 L again. Most of the time the amount withdrawn was equal to that which was injected into the digester, but occasionally less was withdrawn in order to replace sludge that had been volatilized by microbes.

The algae fed to the digesters were collected from the dissolved air flotation device of the Sunnyvale Wastewater Treatment Plant. Before feeding, the algae were heated to 60°C for two hours. Such treatment has increased the methane production of digesters in past experiments (Chen, 1987), probably by breaking algal cells and making their contents more readily available to the bacteria of the digesters.

After the heat treatment the cooked algae were either diluted or decanted to a level of 30 g of volatile solids/liter (gVS/L) and stored at 5°C. Grams VS/L is a measure of the feed's algal concentration. Volatile solids are the portion of the algal feed that can be burned away. Grams VS/L was measured using standard procedure (APHA, 1985). The feed injected into the digesters was between 5°C and 12°C. The feed had a concentration of 28.5 to 40.0 gVS/L, but because the same feed was injected into each digester, the variations in gVS/L were the same for each digester.

Gas Collection and Analysis: The gas generated in the digesters passed through 8-mm diameter plastic tubing to a condensation flask and then to a manometer. The manometer consisted of two four-liter jugs connected by a siphon. Jug 1, which was connected to the condensation flask, was stoppered and filled entirely with a solution of salt-saturated sulfuric acid. This solution prevented gas from dissolving into the liquid.

As gas pressure built up in the system (Figure 1), the solution in jug 1 was forced through the siphon into jug 2. The volume of liquid in jug 2 was a function of the gas produced. The amount of gas in the manometer is calculated with formula "u" given in the Appendix.

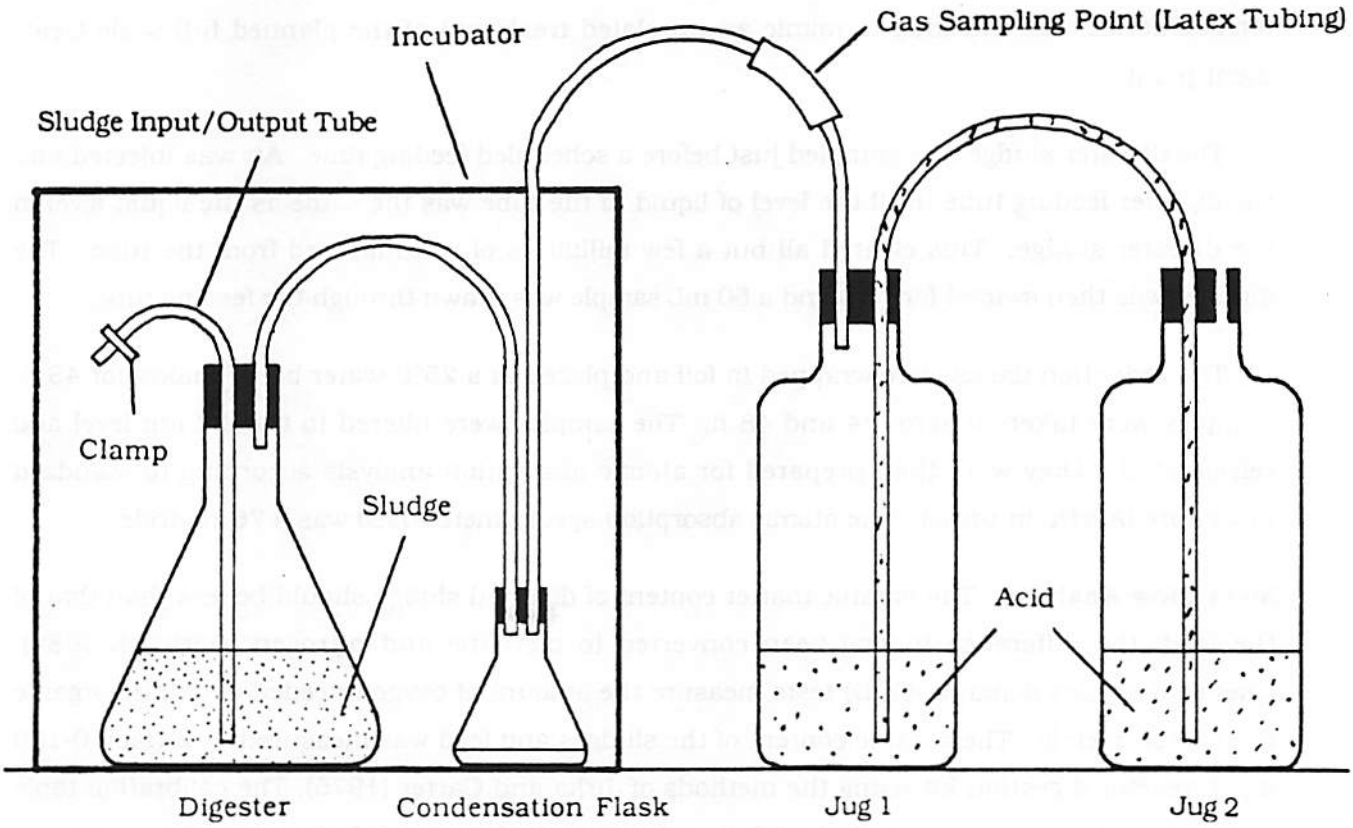

Figure 1. Digester Setup

Samples of the gas from each digester were analyzed by a Carle AGC series 100 gas chromatograph and the per cent methane, carbon dioxide, oxygen, nitrogen and carbon monoxide were determined. The methane production in liters per gVS (L/gVS) was calculated using formula "y" in the Appendix.

Selenium Removal Analysis: Three 275 mL flasks were prepared for the selenium removal test, one for each digester. The reduction flasks, like the digesters, were stoppered and equipped with a fluid input/output tube and a gas release tube. The flasks were flushed free of oxygen with nitrogen and injected with a mixture of 100 mL of subsurface drain water (449 mg Se/L) from the Murrieta Farms of Mendota, Calif.; 70 mL of digester sludge; 20 mL of effluent from a previous selenium reduction test; 10 mL of algal feed and 1 mL $FeCl_3$ (4 mg Fe/L). The feed had

been frozen for 21 days, thawed, diluted to 30 gVS/L, and refrigerated for eight days before the test. This storage may affect the feed's degradability.

The feed and the effluent from a previous test were included in the mixture to inoculate and provide nutrients for any microbial cultures that might mediate the reduction reactions. The ferric chloride was included to mimic an unrelated treatment of the planned full-scale treatment plant.

The digester sludge was sampled just before a scheduled feeding time. Air was injected into the digester feeding tube until the level of liquid in the tube was the same as the liquid level in the digester sludge. This cleared all but a few milliliters of residual feed from the tube. The digester was then swirled for 15 s and a 50 mL sample was drawn through the feeding tube.

The reduction flasks were wrapped in foil and placed in a 25°C water bath/shaker for 48 h. Samples were taken at zero, 24 and 48 h. The samples were filtered to the .22 µm level and refrigerated. They were then prepared for atomic absorption analysis according to standard procedure (APHA, in press). The atomic absorption spectrometer used was a 76 Hydride.

Mass Flow Analysis: The organic matter content of digested sludge should be less than that of the feed, the difference having been converted to methane and nitrogen (Gerhardt,1989). Chemical oxygen demand (COD) tests measure the amount of oxygen needed to oxidize organic matter completely. The organic content of the sludges and feed was measured by a Hach 0-150 mg/L reactor digestion kit using the methods of Jirka and Carter (1975). The calibration table of Jirka and Carter was verified with a potassium hydrogen phthalate standard, using a Bausch and Lomb 20 spectrometer. The digester sludge samples were taken in the same manner as those for the selenium test. The sludge was diluted to 1:250 and 1:500, and the feed was diluted to 1:750. Two trials of each sludge sample were tested.

Two moles of oxygen are needed to oxidize one mole of methane:

$$CH_4 + 2\,O_2 \rightarrow CO_2 + 2\,H_2O. \qquad \text{(eq. 7)}$$

The COD of the methane produced by the digestion of one L of feed is:

$$\frac{1\ \text{mole}\ CH_4}{22.4\ \text{L}\ CH_4} \times \frac{2\ \text{mole}\ O_2}{1\ \text{mole}\ CH_4} \times \frac{32\ \text{g}\ O_2}{\text{mole}\ O_2} \times 28.2\ \frac{\text{gVS}}{\text{L Feed}} \times A\ \frac{\text{L}\ CH_4}{\text{gVS}} = 80.6\,A\ \frac{\text{g}\ O_2}{\text{L Feed}} \qquad \text{(eq. 8)}$$

where A = the methane production/gVS of the digester in question, and 28.2 gVS/L is the average feed concentration in the 10 days before the sludge samples were taken.

Each flask contained some oxygen and nitrogen. It was assumed that the oxygen was due to an air leak. Since air has a known N_2 to O_2 ratio, the nitrogen present due to air leaks could be calculated. Any nitrogen in excess of the air component was considered nitrogen production by the digester. Ammonium oxidizes according to the reaction:

$$8\,NH_4^+ + 6\,O_2 \rightarrow 4\,N_2 + 12\,H_2O + 12\,H^+.$$

The COD of the nitrogen produced was calculated in the same manner as the methane COD (eq. 8).

Results

Gas Production: Methane, carbon dioxide, and hydrogen sulfide were gases produced by the digesters, though hydrogen sulfide existed only in trace amounts. Oxygen and nitrogen were present probably due to air leaks. The average portion of each gas in the digesters is listed in Table 1.

Residence time (days)	10	SD*	20	SD	40	SD
CH_4/CO_2	3.8		3.6		5.3	
CH_4	67.3%	3.9	69.1%	3.4	62.2%	1.2
CO_2	17.7	4.1	19.1	2.2	11.8	2.4
O_2	3.2	0.93	2.9	0.70	5.5	1.5
N_2	8.8	3.2	7.6	2.1	16.7	3.0
$\underline{L\,CH_4}$ † gVS	0.07	0.03	0.1	0.02	0.06	0.03

*SD = standard deviation.
†L CH_4/gVS is the mean value of the thrice weekly measurements of methane production from day 24 to day 70.

Table 1. Gas Production in Percentage of Total Gas Produced and Methane Production per gVS

The mean methane production/gVS is listed in Table 1 and represented in Figure 2. The difference between the mean methane productions could not be easily verified statistically due to the non-normal distribution of the measurements.

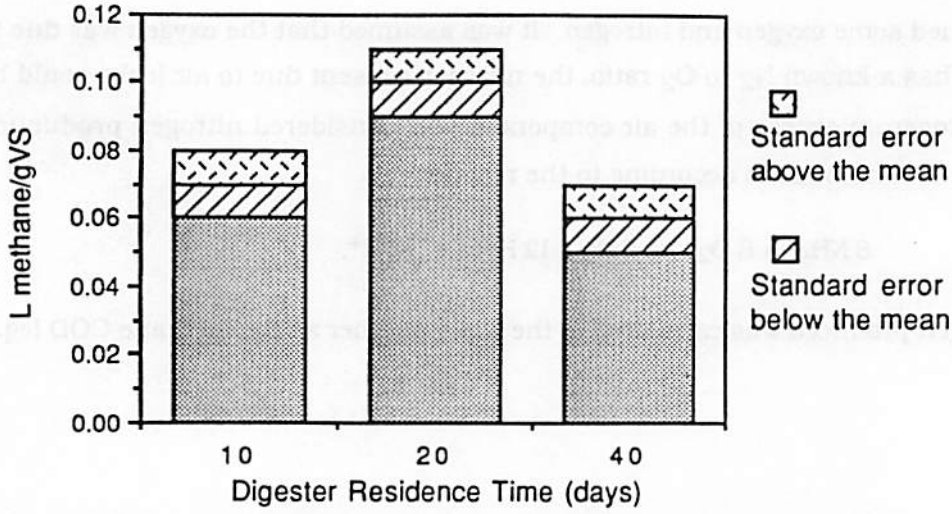

Figure 2. Methane Production

Mass Flow: Between 58 and 71 per cent of the organic matter of the feed remained in the sludges (Table 2, Figure 3). Using equation 8, the COD of the methane produced by each digester was determined. Theoretically, all the COD lost from the feed during digestion was converted to methane and carbon dioxide, and so mass was conserved. However, this did not occur. Only 24 to 45 per cent of the lost COD was converted to methane. Carbon dioxide does not count as having any COD because it is part of equation 7, which is the baseline oxidation level.

Substance tested	COD (gO_2/L)	% of feed	COD of CH_4 produced (gO_2/L)	% of feed	% of feed COD lost
Feed	48	—	—	—	—
10-day sludge	34	71	5.6	12	40
20-day sludge	30	63	8.1	17	45
40-day sludge	28	58	4.8	10	24

Table 2. Mass conservation measured by chemical oxygen demand

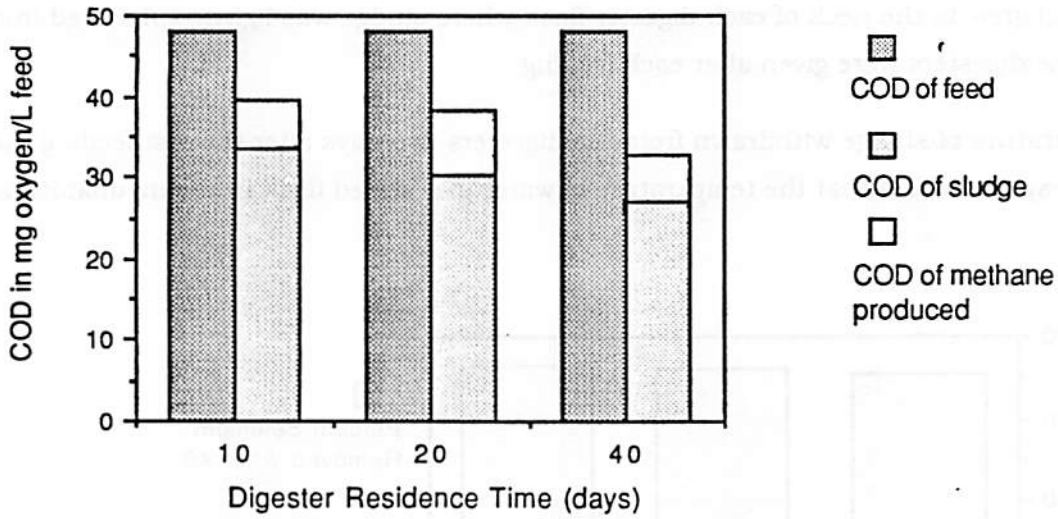

Figure 3. COD of Feed and Sludges

Selenium Removal: The initial concentration of selenium in the water from the under-field drain was 449 mg Se/L. After being mixed with digester sludge for 24 h, the selenium concentration was between 33 and 42 per cent lower. And after 48 h of mixing the selenium had been reduced by the sludge to about 90 per cent of the original concentration (Table 3, Figure 4).

Digester residence time	Selenium Concentration (mg Se/L) initial	24 hrs	% removal	48 hrs	% removal
10-day sludge	449	291	35	39	91
20-day sludge	449	260	42	35	92
40-day sludge	449	303	33	31	93

Table 3. Selenium reduction levels

Gases were produced during the 48 h mixing of the sludge and drain water. About nine per cent of the gas in the flask was carbon dioxide. The oxygen and methane portions were about four per cent. A trace was hydrogen sulfide. And nitrogen, which was used to flush the flasks of oxygen, made up the rest.

Miscellaneous Observations: The sludge in the digesters stratified between feedings. The fresh algal cells floated to the top and partially digested algae settled. This left the middle layer of the digester mostly clear of cells.

White fungi grew in the neck of each digester flask where sludge was lightly splattered from the swirling the digesters were given after each feeding.

The temperature of sludge withdrawn from the digesters two days after the last feeding was 21° to 22°C despite the fact that the temperature of water in a sealed flask in the incubator was 25°C.

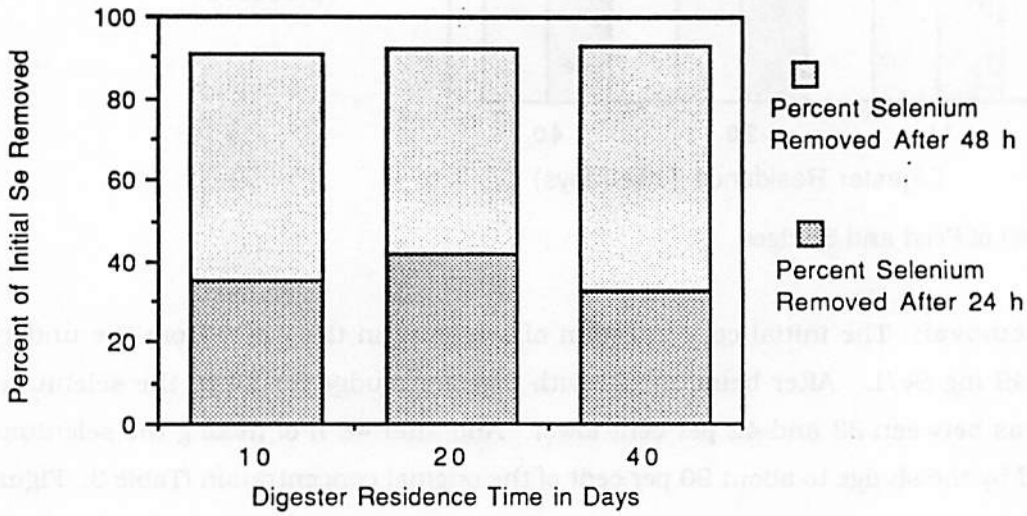

Figure 4. Selenium Removal

Discussion

Mass Flows: The COD of a sludge is a measure of its organic matter content. The difference between the sludge COD and the feed COD should theoretically have been converted to methane according to equations 1 and 2. This would have conserved the mass inflows and outflows of the system. However, using equation 8, at most 45 per cent of the organic matter removed from the feed was converted to methane.

In none of the digesters was enough nitrogen produced to account for a significant portion of the removed COD. This leaves a portion of the COD unaccounted for by methane or nitrogen production. The organic matter of the feed that was not used to produce methane or nitrogen might have been used by the fungi that grew in the neck of each digester flask or by some other digester reaction.

Gas Production and Residence Times: Chen (1987), running digesters at 38°C with daily mixing and feeding, found longer residence times lead to greater methane production. In this study, however, the 40-day residence time production was less than the 20-day production (Figure 5).

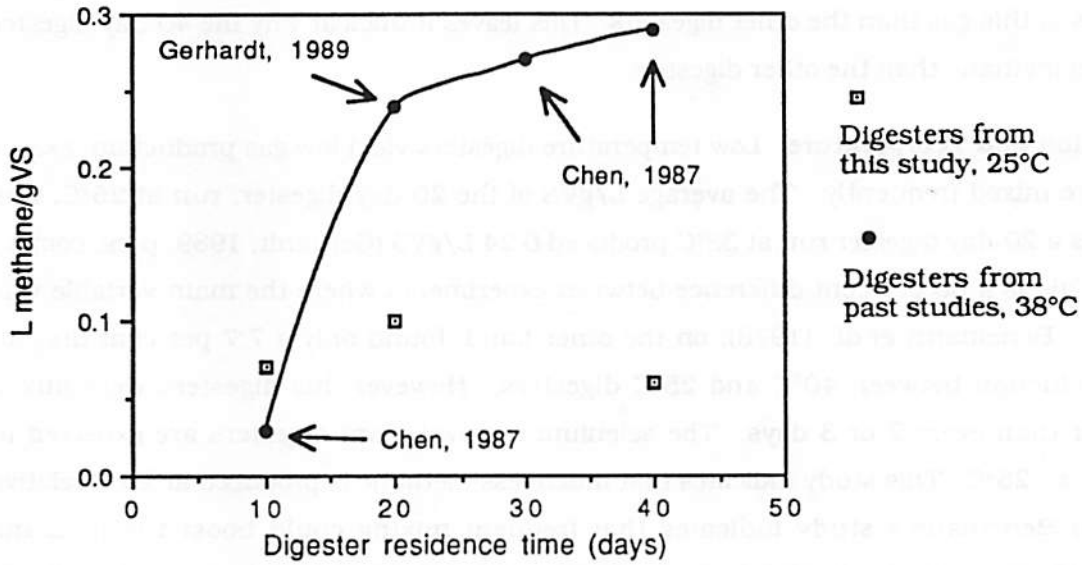

Figure 5. Temperature and Residence Time Effects on Methane Production

One explanation for the lower production by the 40-day digester in this experiment is a gas leak in the collection system. An escape of gas was unlikely, though. In the 40-day digester apparatus, the liquid level in jug 1 was almost always higher than that of jug 2. Because this condition created a negative pressure in the gas lines, gas should have tended to leak into the system rather than out of it. Indeed, more N_2 and O_2 were measured in the 40-day digester manometer than in the manometers of the other digesters which had widely varying liquid levels.

Another possible explanation is that the bacterial culture of the 40-day digester was stressed due to the low flow of nutrients and low temperature. Thus, even though the feed was exposed to microbes longer than in the 20-day digester, the 40-day culture was not able to produce as much methane as the 20-day digester. If this is the case, the ABSRS digesters should be run at residence times shorter than 40 days. This study indicates that a 20-day residence time yields the most methane.

A final explanation for this lower methane production might be that enough oxygen leaked into the 40-day digester to kill off some the anaerobic methanogens and thereby reduced gas production.

However, the algae in the 40-day digester *were* being degraded. The feed lost more COD in the 40-day digester than in the other digesters. Apparently, the COD of the 40-day sludge was converted to something other than methane or nitrogen. Aerobic digestion might account for

the degradation. A by-product of aerobic digestion is carbon dioxide, but the 40-day digester produced less of this gas than the other digesters. This leaves it unclear why the 40-day digester produced less methane than the other digesters.

Gas Production and Temperature: Low temperature digesters yield low gas production, except when they are mixed frequently. The average L/gVS of the 20-day digester, run at 25°C, was 0.10, whereas a 20-day digester run at 38°C produced 0.24 L/gVS (Gerhardt, 1989, pers. comm.) (Figure 5). This is a 58 per cent difference between experiments where the main variable was temperature. Benemann *et al.* (1978), on the other hand, found only a 7.7 per cent drop in methane production between 40°C and 25°C digesters. However, his digesters were mixed hourly rather than every 2 or 3 days. The selenium removal plant digesters are expected to operate at about 25°C. This study indicates that much less methane is produced at 25°C relative to 38°C, and Benemann's study indicates that frequent mixing could boost the methane production. However, mixing is an expensive process. The additional methane produced may not be worth the expense.

Chen (1987) was not able to cultivate a very successful methanogenic population at 38°C with a 10-day residence time (Figure 5) even though he mixed his digesters daily. His 10-day digester yielded .03 L/gVS, 86 per cent less than his 20-day digester. In this study, on the other hand, the 10-day digester produced .07 L/gVS, only a 30 per cent drop in production vis-a-vis the 20-day digester. The low temperature, non-daily mixing and/or pretreatment of the feed may be responsible for the better methane production of this study's 10-day digester.

Gas Production Variability: In the present study, L/gVS varied widely from day to day. The standard deviation of the production ranged from 0.03 to 0.02 L/gVS (Table 1). Perhaps the variation was due to variations in the feed. The volatile solids of the feed ranged from 40.0 to 28.5 gVS/L during the period of time which was used to calculate the gas production, and some feed was frozen for up to 21 days and then refrigerated for up to 10 days before being injected into the digesters. The temperature changes may affect the feed's degradability.

Methane to Carbon Dioxide Ratio: Table 1 shows that the mean ratio in all the digesters was much higher than that of McCarty's study (eq. 1 and 2). Equation 1, with its CO_2 product, appears to have accounted for less than 70 per cent of the methane forming reactions. Also, the relative importance of equation 1 and 2 varies considerably, as indicated by the standard deviations of the carbon dioxide and methane percentages (Table 1).

Selenium Removal and Temperature: Lower digestion temperatures had little effect on the selenium-reducing capacity of the sludge. The typical selenium-reducing capacity of Sunnyvale algae, digested at 38°C with 20-day residence time, has been >90 per cent removal after 48 h of mixing (Gerhardt, 1989, pers. comm.). The algae digested at 25°C and residence time 20 days removed 92 per cent of the selenium after 48 hours.

Selenium Removal and Residence Time: The residence time of the digester feed does not appear to make a significant difference in the amount of selenium that the sludge can reduce in 48 h of sludge-drain water contact. However, after 24 h of contact the 20-day digester sludge appears to have reduced more selenium than the other two sludges. The fact that carbon dioxide was produced during the selenium reduction supports the first step of the simple theoretical reaction for selenium reduction (eq. 4).

The selenium removal plant operators will be able to adjust the digester's residence time without fear of losing reducing potential in the sludge. This will be important if the amount of algae available from the canals varies with time.

Future Research

This study could be improved by maintaining a steadier feed concentration, running replicate digesters, repeating the COD and selenium removal tests, and using more air-tight apparatus.

The 10-day residence time digester in this study produced more methane than Chen's 10-day digester (1987). Further tests could be done to see if, at low residence times, low temperature fosters larger bacterial populations than medium temperature.

It would also be good to run experiments to find out what happened to the organic matter that was not used to make methane or nitrogen. In the ABSRS, this organic matter may be a lost resource. It is interesting to note that if all the COD removed from the feed by the 40-day digester had been converted to the methane, the methane production would have been 0.25 L CH_4/gVS. This is close to the methane production of Chen's 40-day digester at 38°C: 0.29 L CH_4/gVS. The same holds true for the 20-day digester. If all the COD removed in the 20-day digester had been converted to methane, the methane production would have been 0.22 which is close to the production of Gerhardt's 20-day, 38°C digester. Perhaps the temperature difference doesn't affect the amount of degradation but instead affects what the organic matter is converted to. Various experiments could be run to test this idea.

From this research it can be concluded that, without frequent mixing, the methane production of the 25°C ABSRS digesters will be far less than that of 38°C digesters. Studies should be done to determine if frequent digester mixing at 25°C or digester heating is a cost effective means of producing additional methane. The 90+ per cent selenium removals show that the ABSRS is very promising. But lower selenium concentrations still need to be reached. Tests should be run to see if selenium levels might be brought down to the State standard by mixing the selenium water with sludge several times in series.

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Appendix

The following is the Lotus 123™ code for the calculation of various gas data. Variables without formulas following them are data that were collected rather than calculated. The code is for Lotus row #1. Each variable has its own column.

Variable name	Variable
Month	a
Day	b
Volume of sludge removed from digester	c
Volume of feed fed digester	d
Volatile solids of feed in mg/L	e
Difference in jug 1 and jug 2 liquid height	f
Air temperature	g
Air pressure, cm of Hg	h
24 hour time, hour	i
minute	j
Volume of liquid in jug 2	k
V. removed from jug 2 after readings taken	l
Jug 1 refilled with liquid, yes = 1, no = 0	m
Time since last feeding in days	n

```
@(a1=0, 1, @date(m$3, a1, b1)
- (@date(m$3, a0, b0) + ((j1-j0)/60+i1-i0)/24)
```

Cumulative volume in jug 2 — o

```
(@sum(l$0..l0) + k1) / 1000
```

Base volume of jug 2 since last jug 1 refill — p

```
@if (m0=0, p0, o0)
```

Liquid leaving digester	c1/1000	q
Liquid entering digester	d1/1000	r
Change in sludge volume	r1-q1	s

Cumulative change in sludge volume — t

```
@if(m0=0, t0+s1, s1)
```

Variable name	Variable
Volume of gas in jug 1	u

```
(h1-(f1/11.204))
*(o1-p1-(t1*(g1+273.15)/310))/
0.27824*(g1 + 273.15))
```

or in a more readable form:

$$\frac{[P - (\Delta H/12.8)]\,[C - B - (\Delta V T/D)]}{(76\text{ mm Hg}/273^{\circ}\text{K at STP})(T)} = \text{L gas}$$

Where P = air pressure in cm of mercury
ΔH = the height of liquid in jug 1
- height of liquid in jug 2
C = cumulative volume of liquid in jug 2
B = volume in jug 2 since jug 1 last refilled
ΔV = change in volume of digester effluent
T = air temperature in Kelvins
D = digester temperature in Kelvins
12.8 = mercury density/ acid solution density

Gas production in L/day — v

```
@if(m0=0, (u1-u0)/n1, u1/n1)
```

Volatile solids fed, instantaneous — w

```
(e1*r1)/1000
```

Grams VS/day, average — w1/n2 — x

Methane production/gVS, instantaneous — y

```
+v1*@avg(z-6..z6)/(100*x0)
```

Per cent methane in gas sample — z