METHODS AND MATERIALS

Experimental Facility

Site Location and Climate

The pilot plant was attached to the finishing wing of the Swine Research Center at Oregon State University about 3 km west of the campus. Corvallis, home of Oregon State University, is located in the mid-Willamette Valley just east of the Coast Range at a latitude of 44°38′ north, longitude of 123°12′ west, and a ground elevation of 70 m above mean sea level. The climate is strongly influenced by the Pacific Ocean 80 km to the west. The summers are warm and dry, and the winters cool and wet.

The average annual precipitation, almost totally in the form of rain, is 100 cm with an average of 18 cm (Table 9) during January and 0.8 cm during July. Nearly 70 percent of the annual total occurs during the five months from November through March while only 5 percent occurs during the summer months. On the average, there are only 3 to 4 days each year with measurable amounts of snow. Thunderstorms are rare and generally not severe.

Table 9. Climatological data for Corvallis, Oregon. Latitude: 44° 38′ north; longitude: 123° 12′ west; elevation: 70 m above mean sea level (Bates and Calhoun, 1976).

	Temperature								
	Daily[1]		Monthly[2]	Precipi-	Relative humidity[3]				
Month	Max.	Min.	Avg.	tation[2]	4AM	10AM	4PM	10PM	Irradiation[4]
	.....°C.....			cm	..... %				kcal/m^2/day
January	6.8	0	3.7	17.9	90	84	80	89	930
February	9.6	1.5	6.1	11.8	91	83	67	87	1480
March	12.1	2.6	7.4	10.7	89	72	59	83	2440
April	15.9	4.7	9.9	6.3	89	65	50	79	3420
May	19.6	7.4	13.0	4.5	92	55	41	78	4710
June	22.5	9.5	15.9	2.9	92	53	40	77	5190
July	27.1	10.8	18.6	0.8	92	45	32	75	5790
August	27.0	10.6	18.6	1.4	92	48	31	71	4740
September	24.1	9.0	16.5	3.3	84	57	37	70	3650
October	17.7	6.1	11.7	9.6	93	79	55	87	2090
November	11.1	2.9	7.3	15.3	93	85	81	91	1050
December	8.1	1.7	4.9	17.3	93	89	85	92	710
Year	16.8	5.5	11.2	100.8	91	68	55	82	3016

[1] 1931-1960 [2] 1941-1970 [3] 1967-1975 [4] 1966-1975

The seasonal differences in temperatures are relatively small. The difference in mean temperature between January, the coldest month, and July, the warmest, is less than 15 C. The average maximum temperature in January is 7.5 C and the average minimum temperature

for that month is 0 C. The corresponding temperatures for July are 28 C and 10 C respectively (Table 9).

Operational Components of Experimental Facility

Figure 6 shows a schematic diagram of the individual components of the pilot plant as they relate to each other in a self-contained unit with swine manure and heat as the major inputs and biogas and single cell protein (SCP) as the major outputs. The components, which are not all identified in Figure 6, include: (1) animal quarters with solid concrete floor and gutter, (2) flush tank to provide hydraulic transport of the manure, (3) sedimentation pit to collect manure and separate solids from liquids, (4) anaerobic digester to solubilize solids and to recover biogas, (5) nutrient holding tank to store and clarify the liquid phase of the manure, (6) 12 outdoor basins to culture algae for the recovery of nutrients from the liquid waste, (7) product holding tank to store the biomass containing effluents from the culture basins, (8) centrifuge for concentrating the algal and bacterial biomass recovered from the swine waste, and (9) flush water holding tank to store fresh water or recycle waste water for flushing of the animal quarters.

Figure 7 shows a plan view of the actual facility indicating the positions of the components relative to each other and the routing of the manure through the system. The diagram shows that the areas of manure production, handling, pretreatment, and biomass recovery (top half of diagram) were spatially separated from the area of nutrient conversion into algae and/or bacteria (bottom half of diagram). The waste treatment and biomass recovery area consisted primarily of underground concrete tanks, a control panel, and a centrifuge for concentrating the algae. The area was partially covered to protect the control panel and the centrifuge from rain (Figure 8).

The outdoor basins are located approximately 15 m from the shed and were 2 to 3 m higher in elevation than the underground tanks. Figure 9 shows the basin area in the foreground and the shed with control panel, anaerobic digester, and centrifuge in the left background. The other buildings in the background are part of the OSU Swine Research Center.

Animal Quarters

Part of the finishing wing of the Swine Research Center was modified to house up to 90 pigs (Figures 7 and 8). A smooth concrete pad, 10 m long and 6.7 m wide, sloping towards a gutter 0.75 m wide and 0.10 m deep, was divided into six individual pens, each provided with automatic waterers and self-feeders.

Gutter Flushing System

The function of the flushing system was to provide hydraulic transport of the swine manure from the gutter to the sedimentation pit. According to Hazen (1973), a velocity of at least 60 cm/sec with a flow depth of 1 to 2 cm is necessary for hydraulic removal of manure from gutters to which animals have direct access. The duration of flushing must be long enough to assure uniform flow of the water along the entire length of the channel. The duration should equal or exceed the time it takes for the water to travel the length of the gutter at

its average velocity. With an effective gutter length of 9 m, the minimum flushing duration was calculated to be 15 sec.

In addition, it was required that the discharge rate be fast enough to establish the desired flow velocity and depth. Assuming a minimum flow velocity of 60 cm/sec and a flow depth of 2 cm, the discharge rate was calculated to be 560 liters/min. Given the 15 sec flush duration the flush volume, therefore, had to be 140 liters.

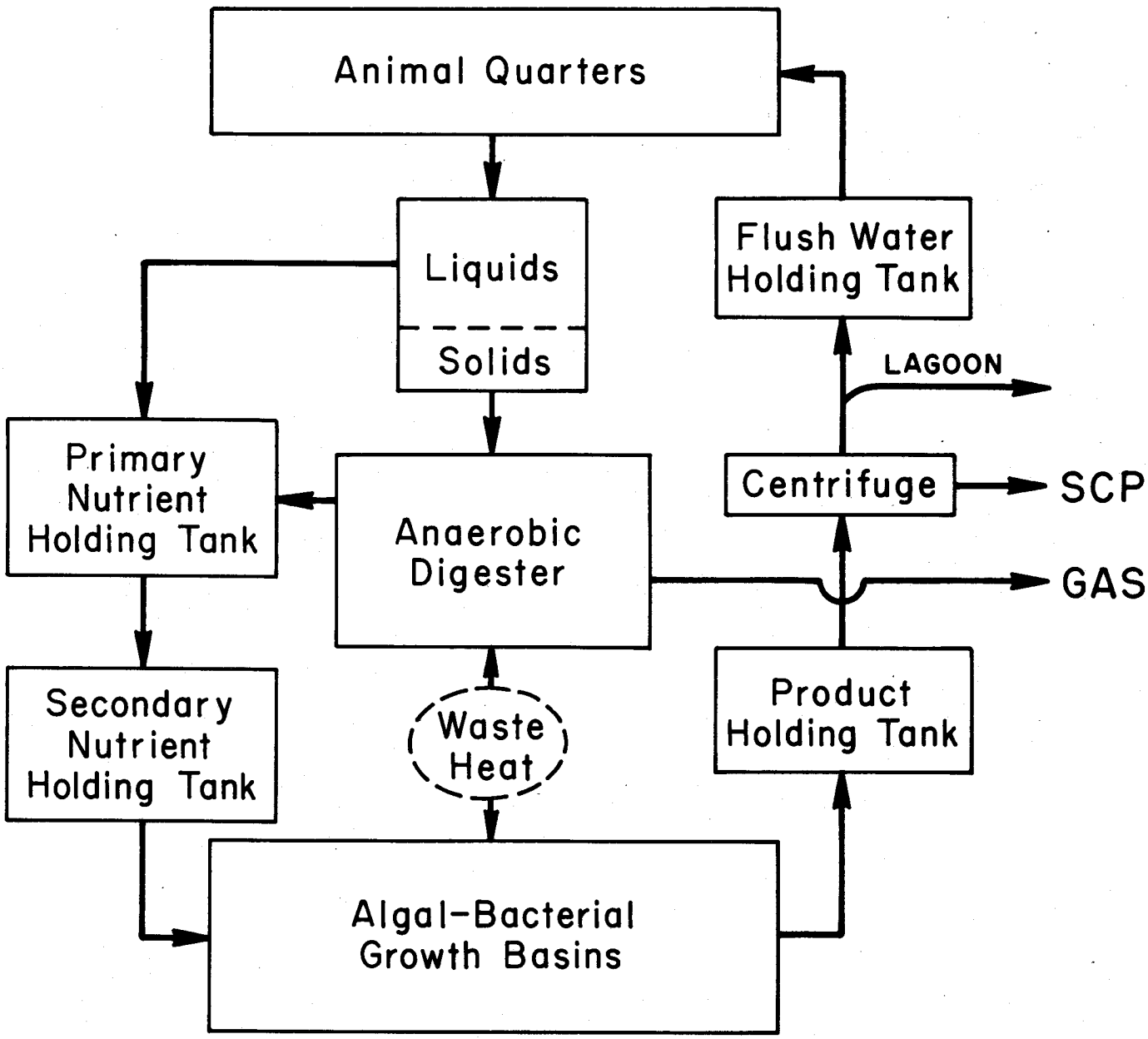

Figure 6. Flow diagram of the experimental system for the conversion of swine waste to single cell protein (SCP) and biogas.

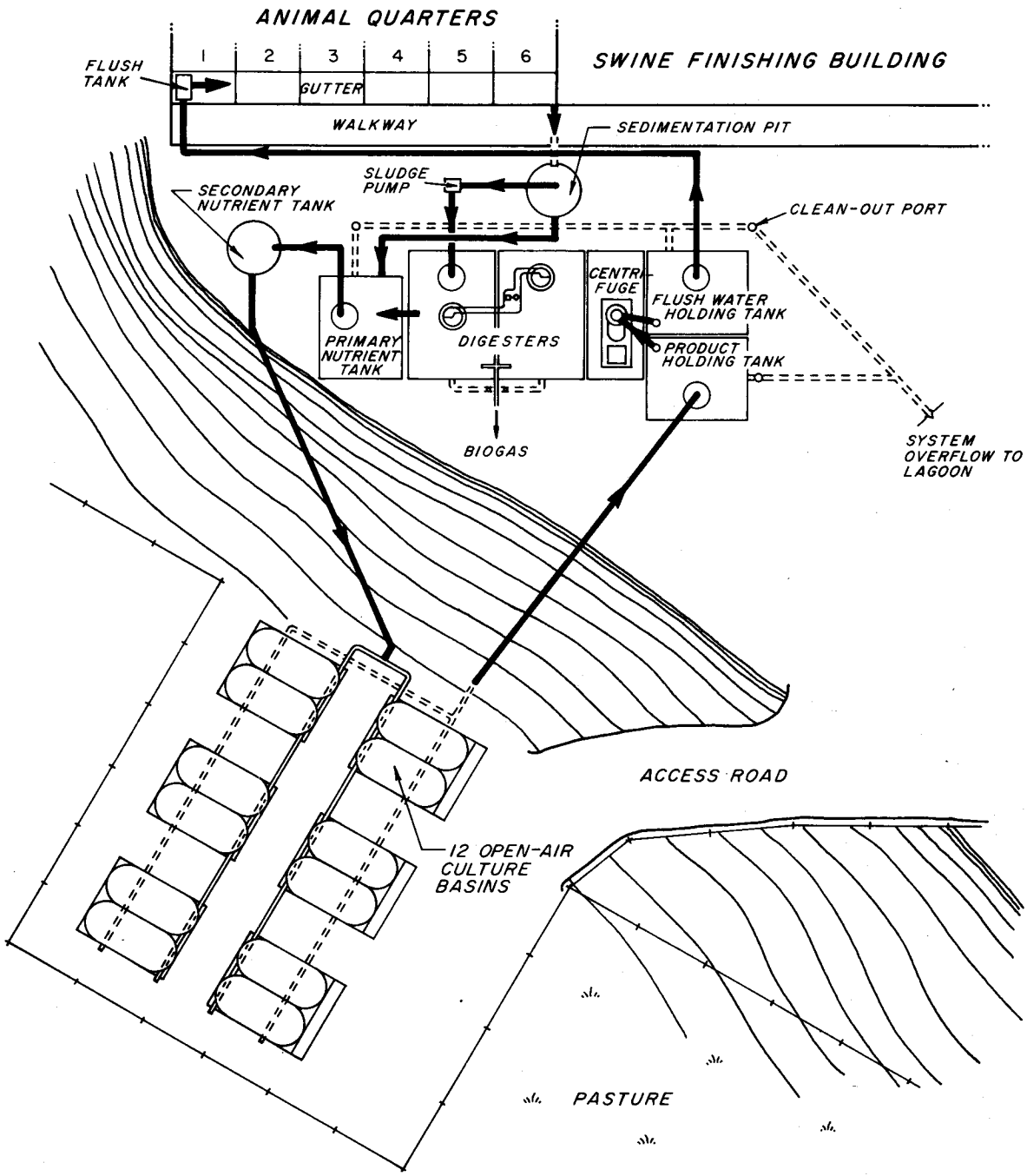

Figure 7. Plan view of experimental facility. Dark line with arrows indicates route of manure through system. Waste water may be recycled to flush animal quarters or may leave system to nearby lagoon.

Figure 8. Photograph of waste treatment and biomass recovery area. (1) Animal quarters, (2) sedimentation pit, (3) digester A, (4) digester B, (5) clean-out ports on digester inter-connect, (6) gas collection pipes, (7) support for digester heaters and warm water circulation pump, (8) control panel, (9) primary nutrient holding tank, (10) secondary nutrient holding tank added as a design modification but not actually used, (11) product (algae/bacteria) return pipe from basins, (12) product holding tank, (13) centrifuge, (14) flush water holding tank.

The flush tank itself was a 189 liter drum (Figure 10). Its design was based on a report by Person and Miner (1971). The theoretical relation between design parameters is:

$$F_o = T \frac{AB}{AB - AP} + Y,$$

where F_o (cm) is the height at which the tank discharges, T (cm) is the trap length, AB (cm^2) is the area of the bell, AP (cm^2) is the area of the discharge pipe under the bell, and Y is the snifter inlet height. The discharge height and the corresponding flush volume can be controlled by varying either the trap length (T), the area ratio (AB/AB-AP), and/or the snifter height (Y).

The use of a 10.2 cm diameter discharge pipe and a 19 liter bell (5 gallon plastic bucket) with a 25.4 cm diameter gave an area ratio of 1.2. Person and Miner (1971) reported that an area ratio of 1.2 and a trap length of T = 27.9 cm gave experimental discharge heights (F_o) within 1.3 cm of the theoretical values when the snifter height (Y) was varied. Given a trap length T = 27.9 cm and a discharge height F_o = 63.5 cm to correspond to the required flush volume of 140 liters, the snifter height was calculated to be Y = 30.5 cm.

The operation of the flush tank begins by filling the trap pipe with water through a valve or removable cap in the snifter tube. As the tank itself is filled, the water inside and outside the bell will rise at the same rate.

When the water level reaches the snifter tube, air is trapped between the water in the trap pipe and the water under the bell. As the tank continues to fill, the trapped air prevents the water under the bell from rising as fast as the water in the tank, thus establishing a difference in water levels.

This difference builds up pressure inside the discharge pipe and forces some water to leave the trap. An air bubble will leave with the water, reducing the air pressure inside the discharge pipe. In turn, the water under the bell continues to rise, again increasing the pressure inside the discharge pipe and forcing more water out of the trap.

Eventually, water under the bell will spill into the discharge pipe in a continuous flow and siphon action empties the tank. The siphon discharges until the water level in the tank reaches the opening of the bell. Air then enters and breaks the siphon. Sufficient water remains in the trap to start another flush cycle.

The siphon was installed just above the gutter in pen No. 1 at the end of the swine finishing building (Figure 7). In the operation of the experimental facility, the frequency of the flushing process was adjusted to maintain a desired concentration of nutrients in the liquid fed to the culture basins. For the majority of the experiments the gutter was flushed once every hour. The tank was filled on demand by a time clock-controlled pump, submersed in the holding tank for the flush water (Figure 7).

At the lower end of the gutter, the manure slurry dropped into a 75 cm wide x 75 cm long x 15 cm deep drop box and from it through a 15 cm diameter PVC pipe into the sedimentation pit (Figure 7).

Figure 9. Photograph of research facility. Foreground: six pairs of basins. Left background: barn with animal quarters. Under shed: control panel, anaerobic digester, and centrifuge.

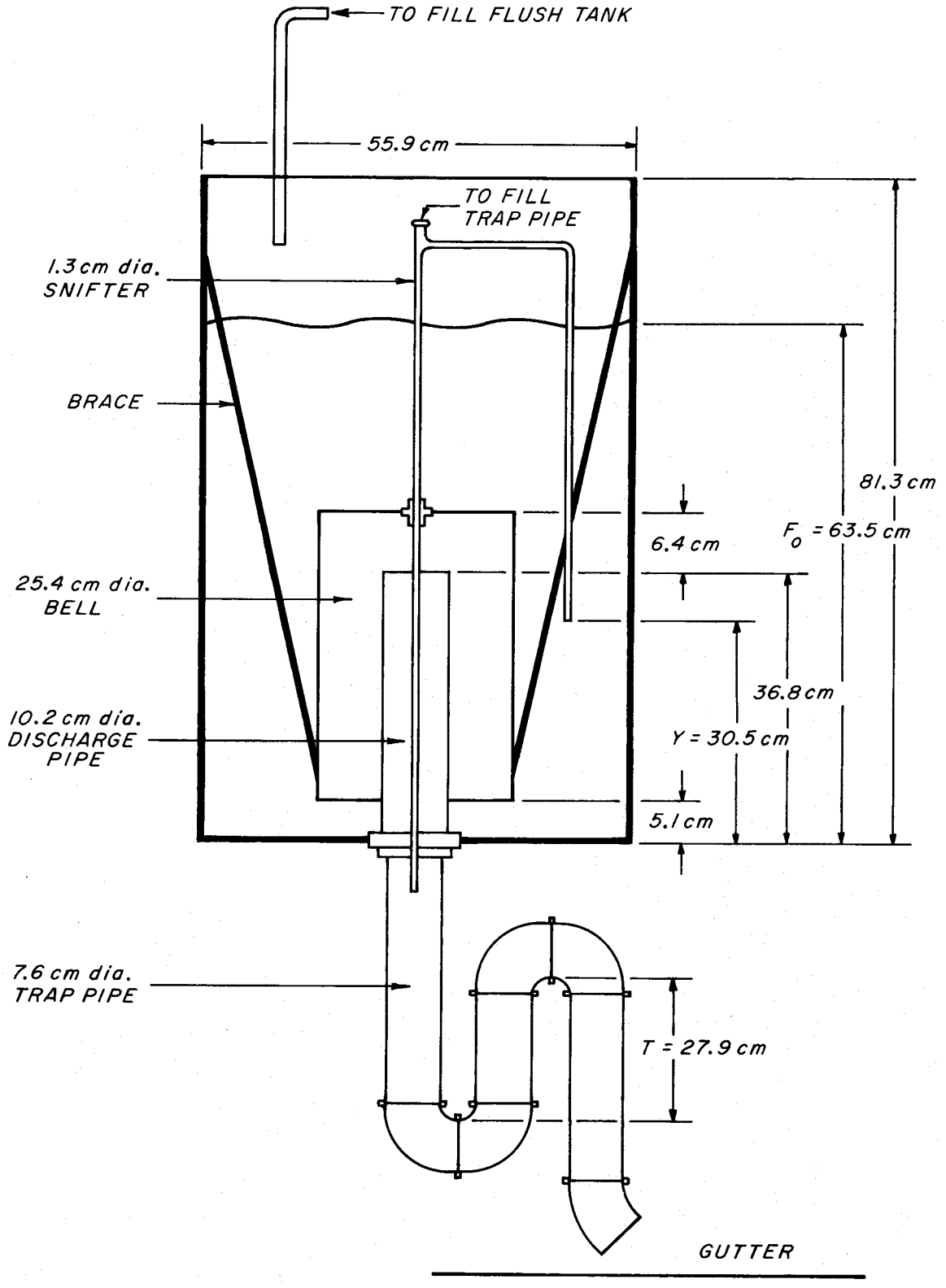

Figure 10. Flush tank with automatic dosing siphon used for hydraulic removal of swine manure from the gutter.

Sedimentation Pit

The sedimentation pit shown in Figure 11 was the first in a series of underground tanks outside the animal confinement area for storage of swine manure. The pit was constructed from a reinforced concrete culvert, 1.2 m in diameter and 1.8 m deep, set vertically in the ground with a 10 cm edge showing above ground level. The bottom was sealed with a 10 cm layer of concrete. It had a capacity of 1,800 liters which was equivalent to 12 to 13 hourly flush volumes.

Anaerobic Digester

The size of the digester was based on the amount of volatile solids (VS) produced by 50 pigs, which was at least 17 kg per day. Given a recommended loading rate of 2 kg VS/m^3 day (Miner and Smith, 1975) a minimum digester volume of 8.5 m^3 was required. The primary function of the digester was to solubilize plant nutrients for recovery by algae. The efficient generation of energy in the form of methane gas was a secondary objective.

Low cost and commercial availability indicated the use of a concrete septic tank without internal baffles. However, the largest tank available locally was smaller than the size needed for the experimental facility. It was therefore decided to install two tanks with a combined volume of 15 m^3. This was nearly twice as large as the required capacity of the digester and allowed for a conservative loading rate of 1.1 kg VS/day.

The two tanks were placed side-by-side with the top surfaces at ground level (Figure 8). The tanks were labelled "digester A" and "digester B". Figure 12 shows a profile of the digester units and the adjacent primary nutrient holding tank. Construction details of the digester tanks are given in the top, side, and end views presented in Figure 13.

Each tank was 3 m long, 2 m wide, and 1.7 m deep (outside dimensions) and had a wall thickness of 10 cm. A 10 cm diameter PVC pipe with clean-out ports connected them underground so that they could function as a single digestion unit (Figure 12). The location of this by-pass (Figures 12 and 13) created a bottom layer, 60 cm thick, of relatively undisturbed solids in digester B which was thought to be beneficial in promoting large numbers of bacteria for the digestion process.

Each digester had 60 cm diameter openings cast into the tank top. Digester A had two such openings, one to position the heat exchanger coil, the other to provide access to a mixing pump and to allow addition of fresh manure from the sedimentation pit (Figure 14). Digester B contained an opening for the heat exchanger coil only. Gas seals around the openings were established by sheet metal collars sealed to the concrete tops and extending 30 cm below the manure level in each tank. The collars were covered with several layers of epoxy paint to prevent corrosion.

Mixing. A 1/2 hp (9.5 Amp; 115 V) submersible sump pump was placed at the bottom of digester A to pump its contents to digester B through a 5 cm diameter PVC transfer pipe (Figure 14). This created a higher level in digester B thus forcing the manure slurry to return to digester A through the underground connecting pipe (Figures 12 and 13). At a pumping rate of 380 liters/min, the total digester volume was turned over once every 36 min. During

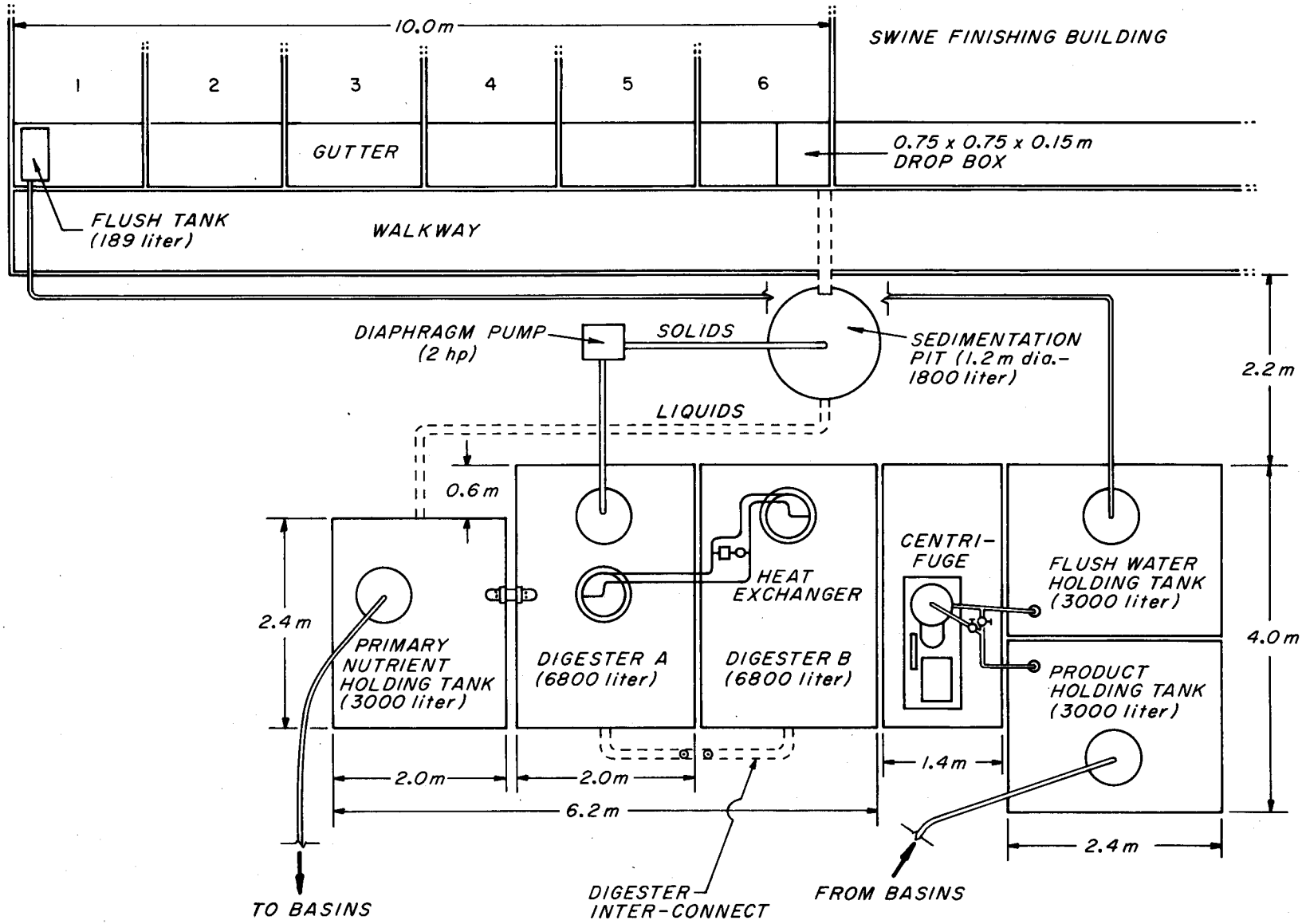

Figure 11. Plan view of area for collection, preparation, and treatment of swine waste.

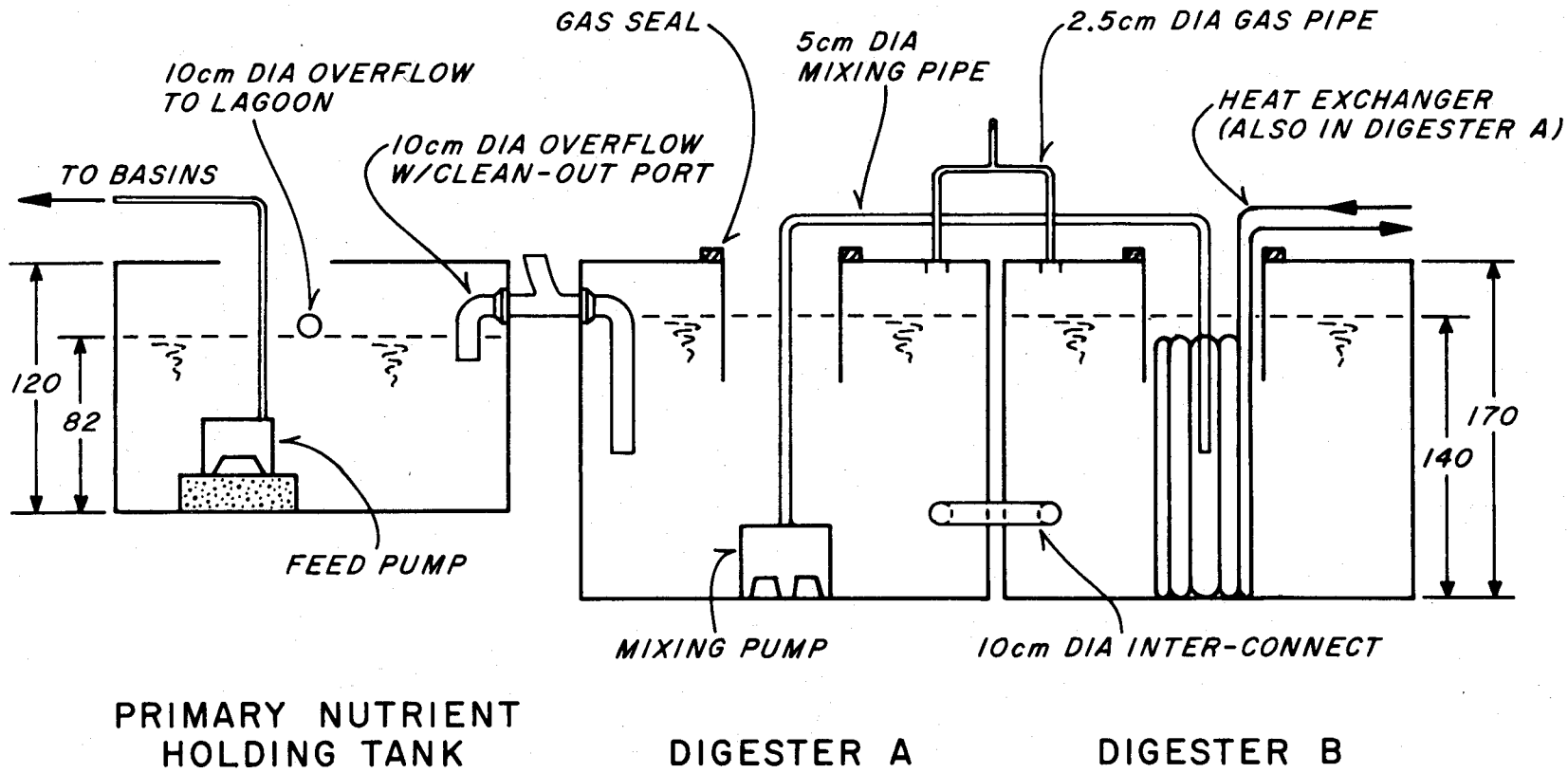

Figure 12. Profile of anerobic digester units and primary nutrient holding tank. Dimensions are outside dimensions, measured in cm. Wall thickness is 10 cm.

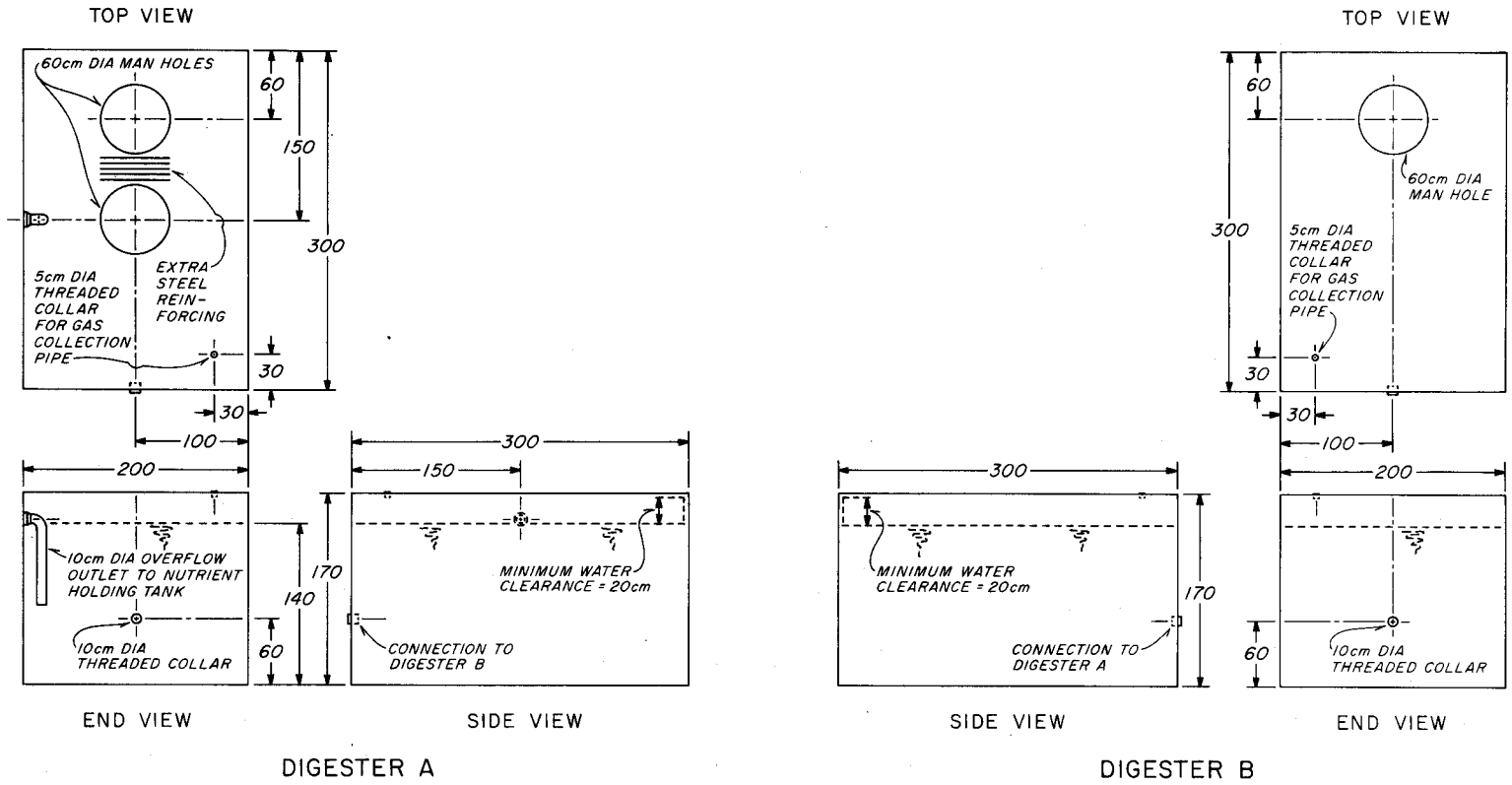

Figure 13. Top, end, and side views of anaerobic digester units. Two precast concrete tanks, each with a volume of 6,800 liters, were installed side-by-side and connected to function as a single digester. Measurements are in cm and dimensions are outside dimensions. Wall thickness is 10 cm (after Fukui, 1975).

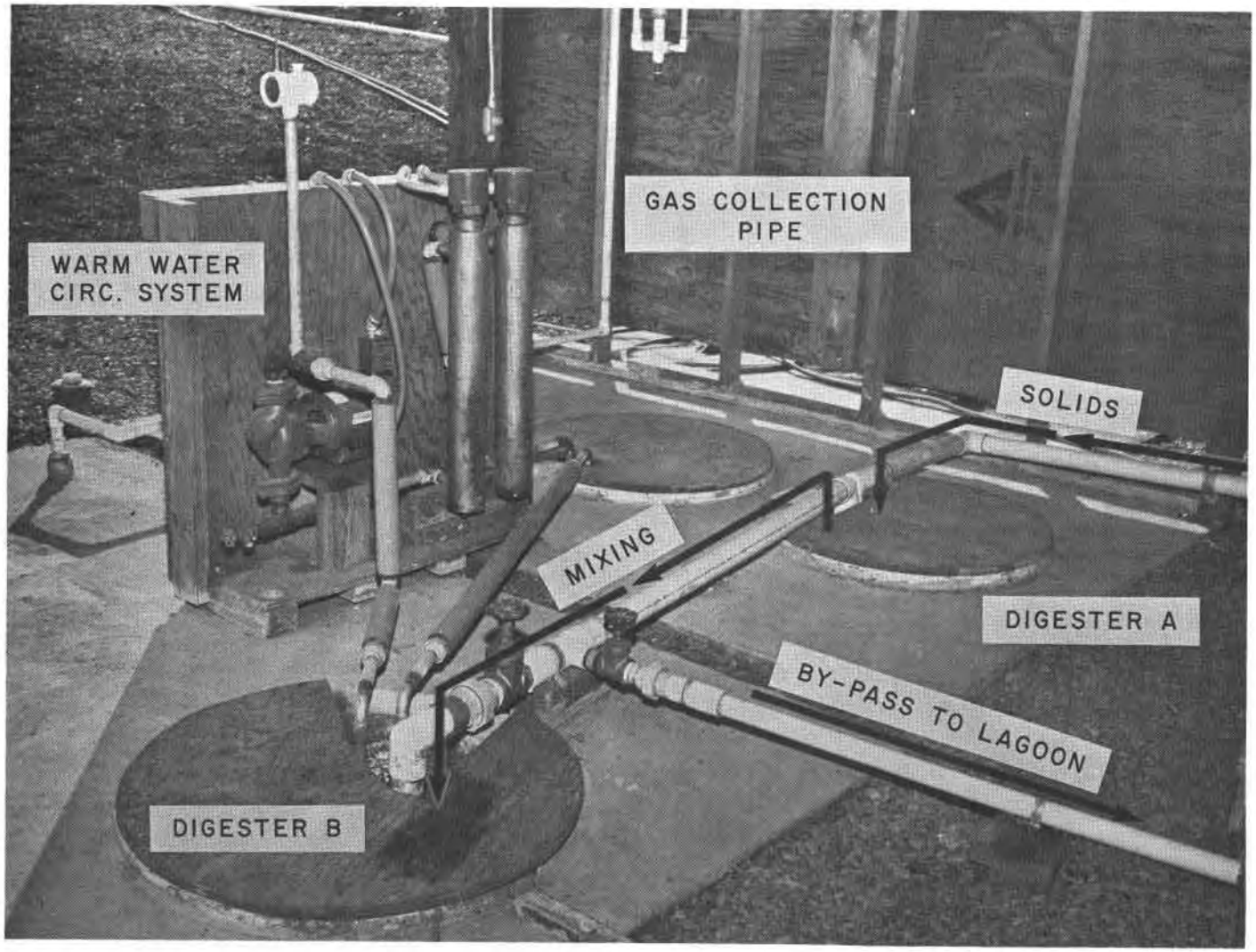

Figure 14. Photograph of digesters and warm water heating system. Manure solids from the sedimentation pit are discharged into digester A immediately above a mixing pump placed at the bottom of digester A. The pump transfers the manure slurry from digester A to digester B. It returns to digester A through an underground connecting pipe.

digester operation the pump was in continuous use. A by-pass in the transfer pipe from digester A to digester B allowed sampling of the digester contents as well as emptying into the overflow drain to the lagoon (Figure 14).

Fresh manure was added to digester A at a location immediately above the mixing pump. At the same time, an equal volume of digested manure overflowed through a 10 cm diameter effluent pipe which connected digester A with the nutrient holding tank (Figure 12).

Heating. In the absence of a nearby source of warm water, the waste heat was simulated by a warm water heating and circulating system. Water was heated to 37 C by two electric resistance heaters connected in series with a total installed capacity of 8,000 Watts (Figure 14). A 1/6 hp (2.5 Amp; 115V) circulation pump operated continuously to move the warm water through heat exchanger loops at the rate of 0.5 liters/sec. The temperature was controlled by a precision hydraulic thermostat with the sensing bulb strapped to the inlet of the resistance heaters. The bulb was insulated with 0.32 cm thick and 5 cm wide insulation tape. Operating time of the heaters was measured with an elapsed-time meter connected across the heater contactor.

The heat exchanger consisted of two coils, one for each digester, each constructed from nine loops of thin-walled stainless steel tubing (2.54 cm O.D. with a 0.05 cm wall thickness) arranged in a 50 cm diameter circle (Figure 15). One unit was placed upright in each digester tank (Figure 16), exposing approximately 2.3 m^2 of heat exchange surface to the contents of each digester.

Gas Collection and Measurement. Each tank was fitted with a 2.5 cm diameter PVC exhaust pipe to collect the biogas. These pipes were merged and joined to a precision dry-test gas meter to measure the production of gas (Figure 17). A trap on the inlet side of the meter allowed for the adjustment of back pressure on the digester and the scrubbing of CO_2 and H_2S from the biogas. The trap was build from a 30 cm long piece of acrylic tubing with a wall thickness of 0.6 cm and an inside diameter of 10 cm.

Nutrient Holding Tank

The effluents from the anaerobic digester and the liquid overflow from the sedimentation pit were collected in a 3,000 liter, precast concrete tank buried adjacent to digester A (Figure 12). Construction details of the tank are given in top, side, and end views in Figure 18. A submersible pump was anchored 30 cm above the bottom of the tank to avoid pumping the settled solids. The pump was capable of delivering clarified liquid waste to the open-air basins at a rate of 95 liters/min.

Culture Basins

This part of the nutrient recovery system was located approximately 15 m from the nutrient preparation area and 2 to 3 m higher in elevation (Figures 9 and 19). There were 12 identical basins each with heat exchangers and paddlewheels for mixing. To avoid toxicity and corrosion only fiberglass, acrylic, and stainless steel materials were used in the construction.

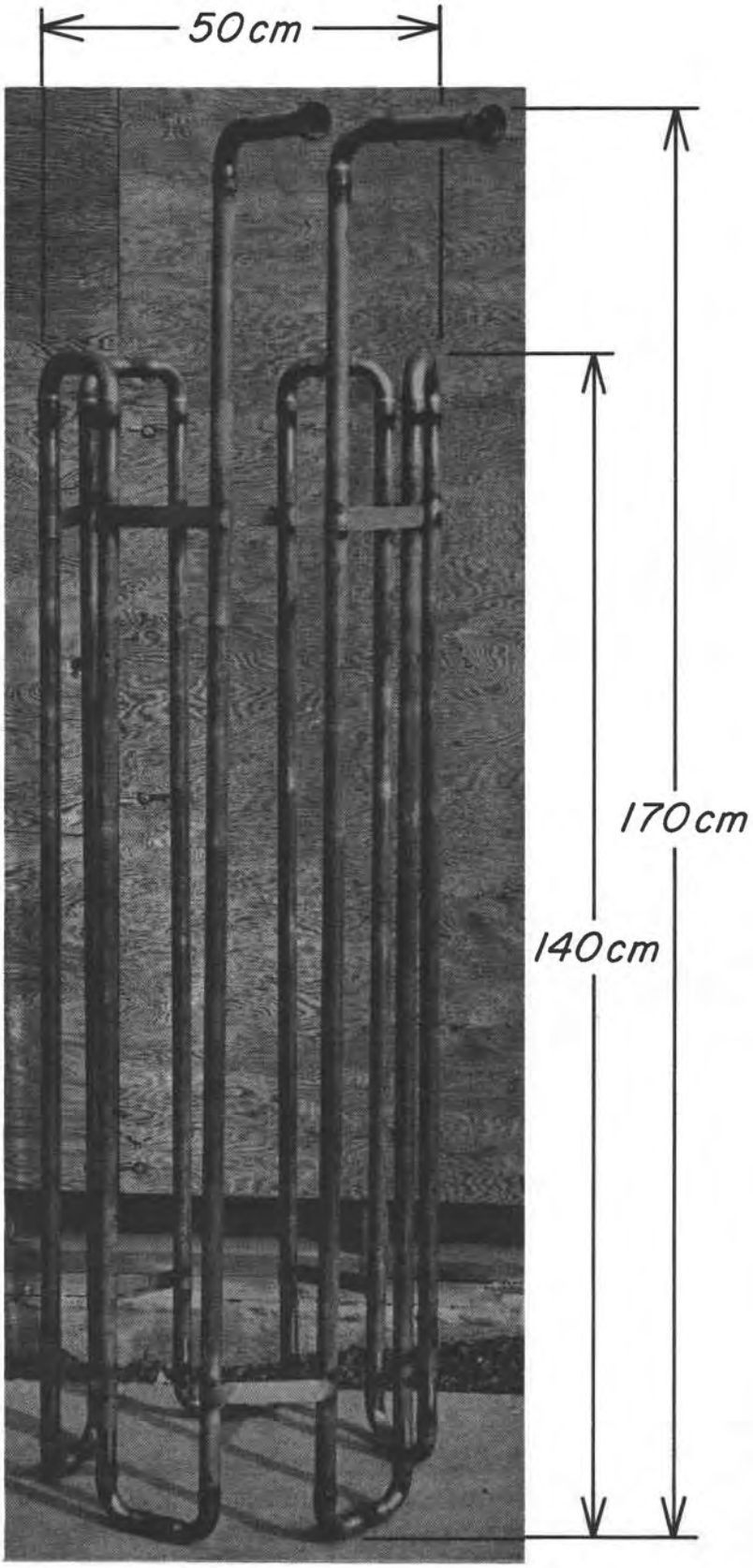

Figure 15. Stainless steel heat exchanger coil used for heating digester A.

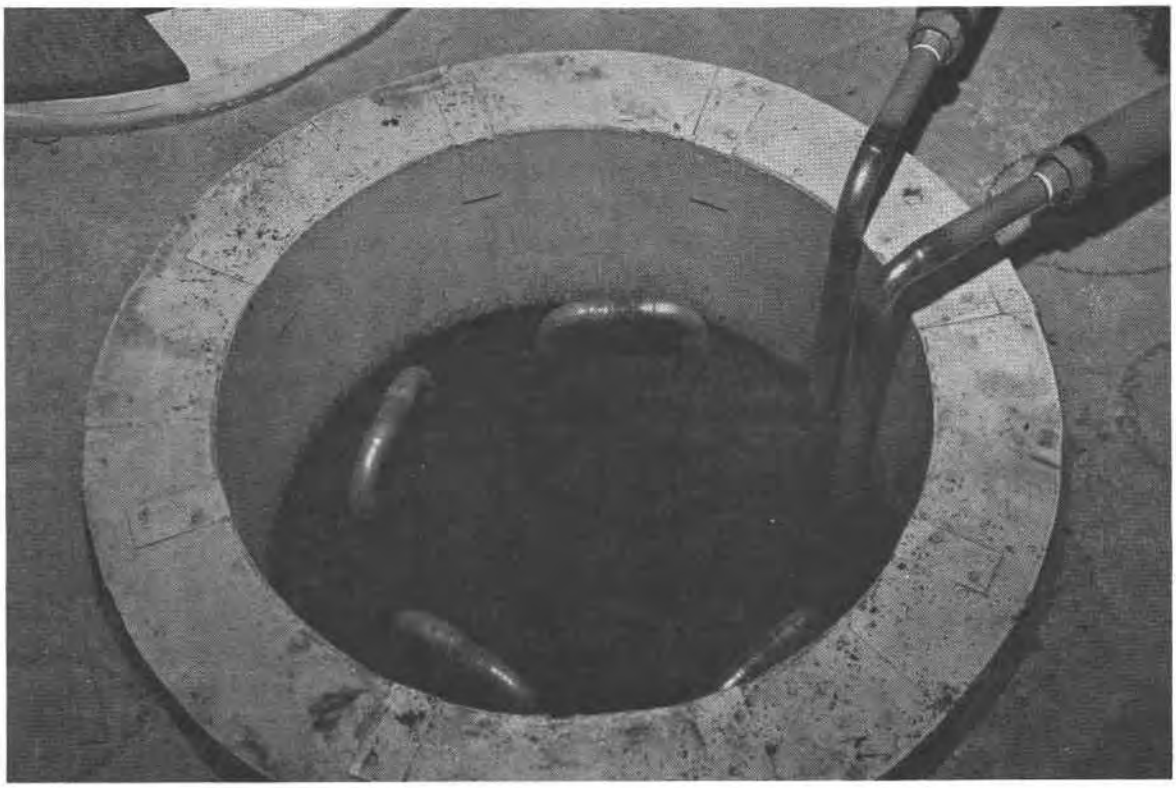

Figure 16. Digester A with exposed manhole showing tops of submerged heat exchanger loops.

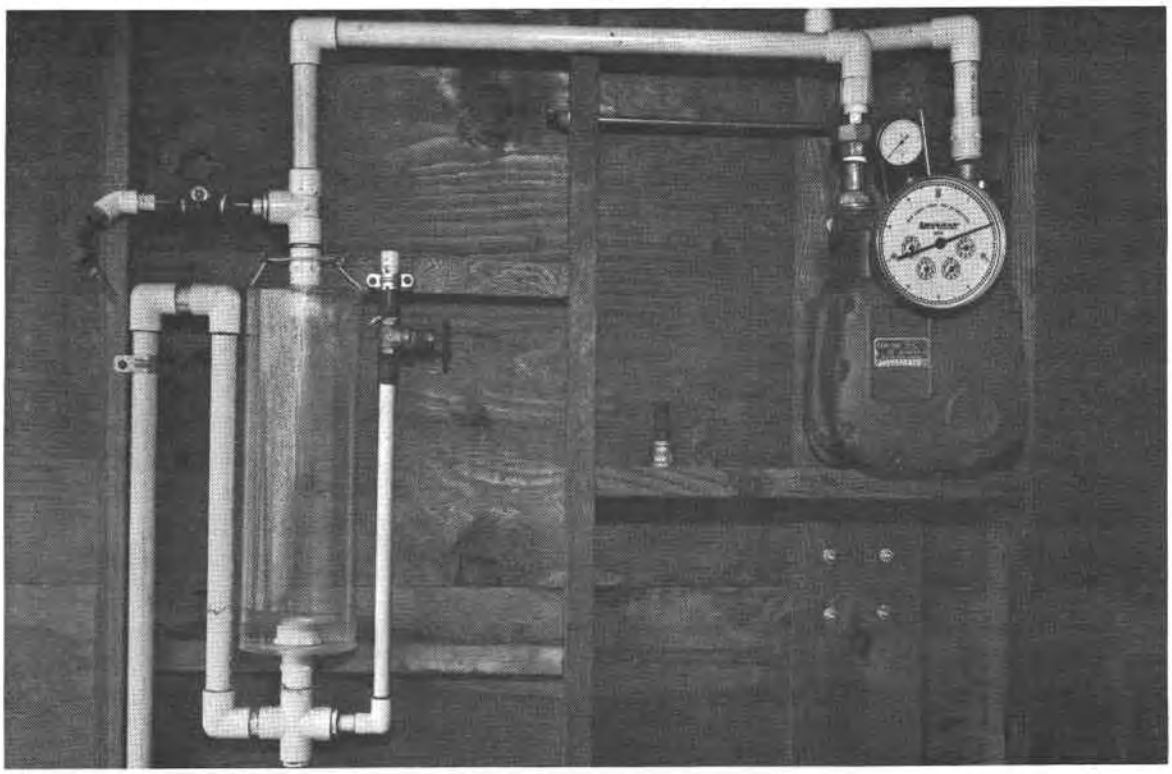

Figure 17. Dry-test gas meter used for measuring gas production from the anaerobic digestion of swine manure.

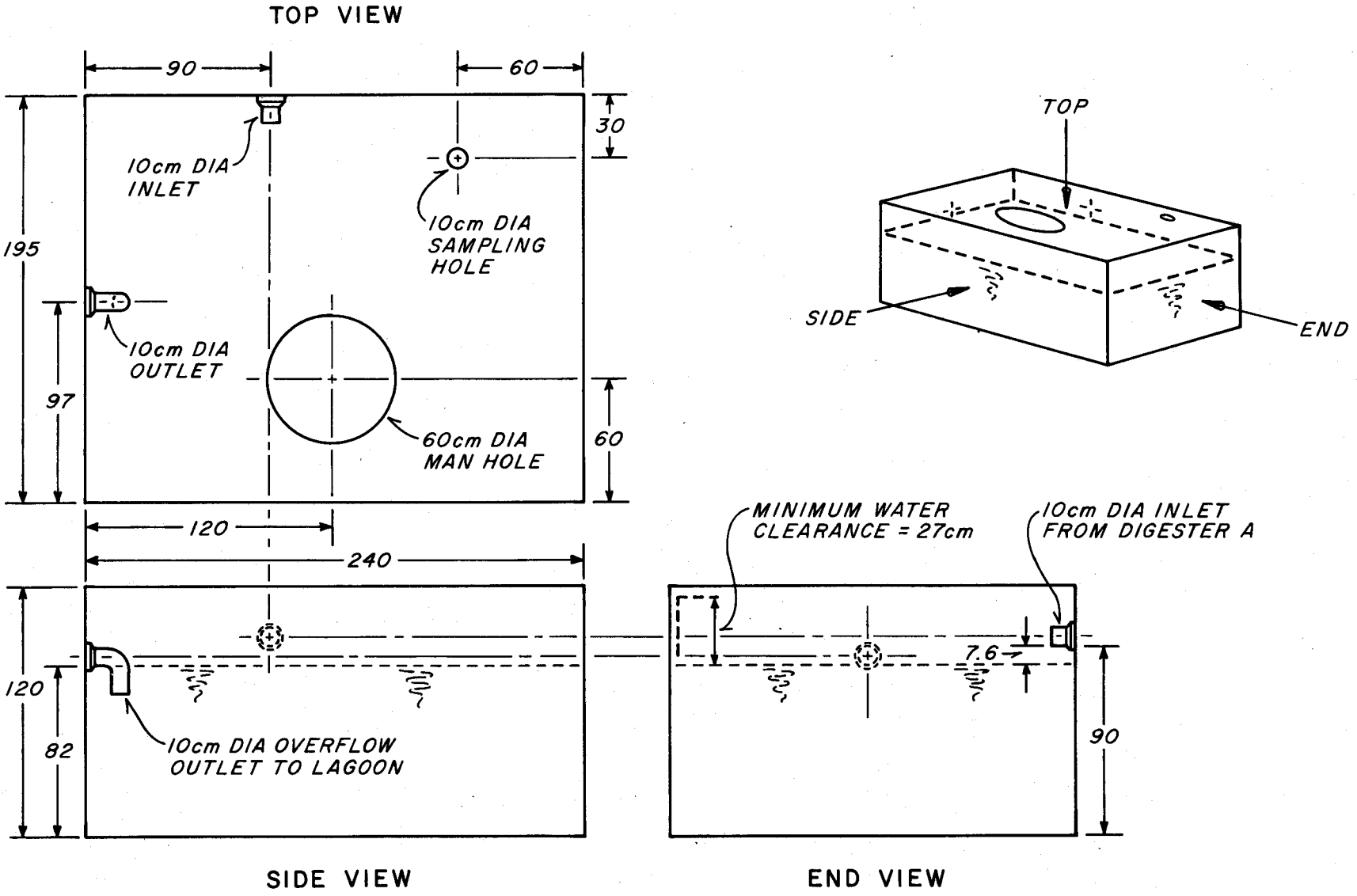

Figure 18. Top, side, and end views of nutrient holding tank. Dimensions are outside measurements in cm. Wall thickness is 10 cm.

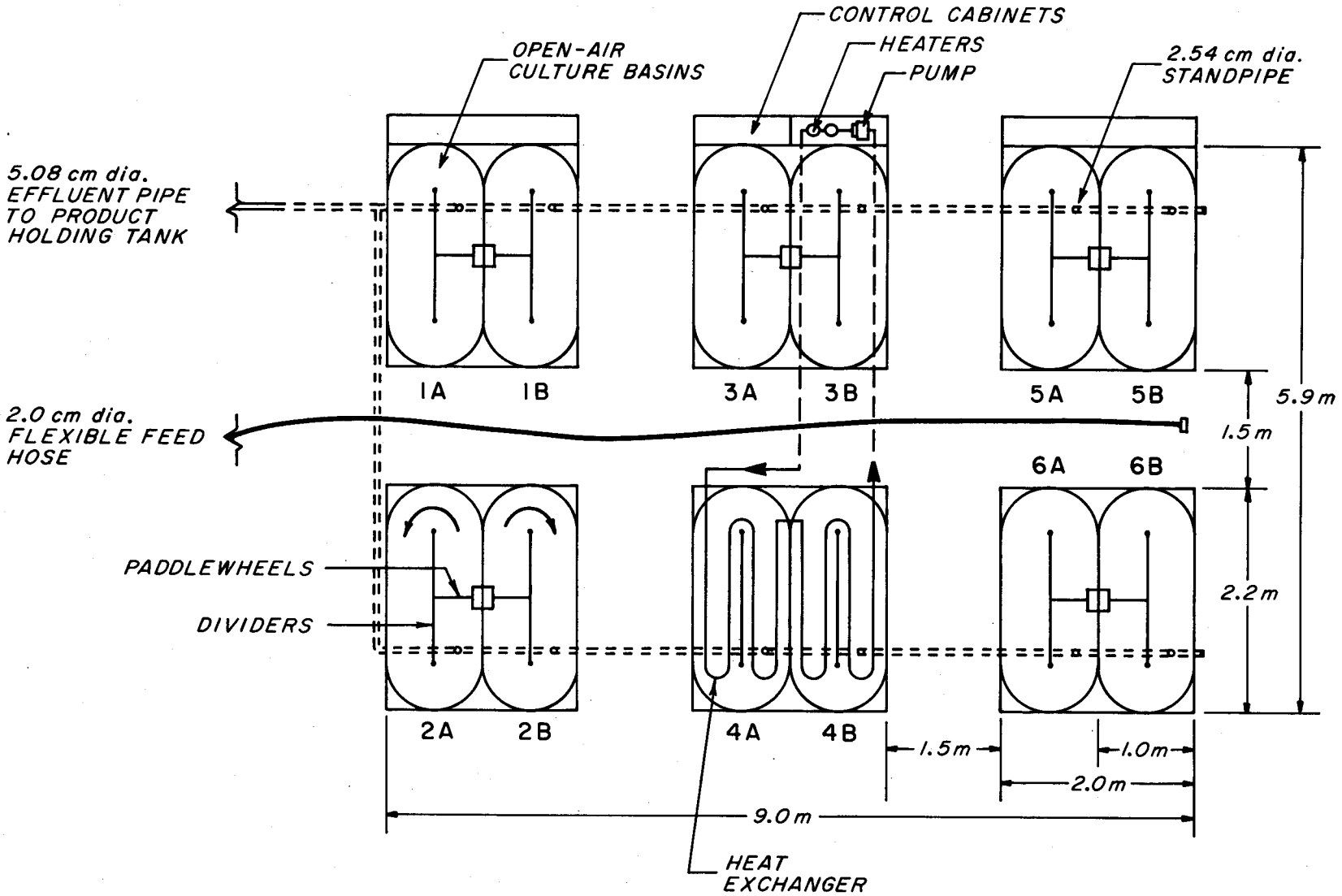

Figure 19. Plan view of basins. There are 12 identical basins paired into 6 sets of two basins. Each set can be operated independently from any other set. Each set contains a stainless steel heat exchanger, shown only for set 4, and two paddlewheels, one for each basin, driven by a variable speed motor. Each basin contains a standpipe for collecting effluent and a central divider around which the basin fluid is circulated as shown in set 2.

Each basin was molded from polyester reinforced with fiberglass in the form of a rectangle with semicircular ends. Each basin was 1 m wide, 2.2 m long, 0.3 m deep, and had a surface area of 2.01 m^2. Each 1 cm in depth corresponded to 20 liters in volume. The exterior surfaces were thermally insulated with 8 cm of polyurethane foam so that only heat losses from the water surface had to be considered.

The basins were grouped in pairs on six wooden platforms (Figures 9 and 19) of post and joist construction. Each platform was 2.2 x 2.4 m. They were from 0.5 to 1.5 m above the sloping soil surface. There were two rows of three pairs each, with a 1.5 m service space between the rows (Figure 19). All platforms were at the same elevation and low enough to allow easy maintenance and servicing of the basins. Each pair of basins was operated as a single physical unit with respect to temperature control and mixing rates, independently from any of the other parts. The necessary control equipment, pumps, and heaters were housed in wooden cabinets attached to basin sets 1, 3, and 5 (Figure 19). Culture temperature, depth, retention time, and mixing speeds could all be varied independently for each pair of basins.

Heating. Temperatures of the basin contents were controlled by a stainless steel heat exchanger (Figure 20) made of four tubes, 2.54 cm O.D. with 0.05 cm wall thickness, which were 20 cm apart and connected to function as a continuous pipe. They were placed 2.5 cm from the bottom of the basin and exposed 0.7 m^2 of heat exchange surface to the fluid in each basin. The two basins in each set were served by heat exchangers connected in series. Galvanized iron pipes, 2.5 cm O.D. connected the heat exchangers to pump and heaters. All exposed pipes were insulated with 1.3 cm of urethane insulating material backed by 0.1 cm PVC sheeting.

The water in each heat exchanger was heated by two electrical resistance heaters hooked up in series with a total capacity of 8,000 Watts (Figure 21). A 1/6 hp pump moved the water continuously through the pipes at a rate of 0.5 liters/sec. The temperature was controlled by a precision hydraulic thermostat whose sensing bulb was strapped to the inlet pipe of the heaters with insulating tape. It was possible to heat the water to a maximum temperature of 40 C.

The heaters were protected by a high temperature cut-off switch which could shut down the resistance heaters but allow the circulation pump to continue operating if overheating occurred. However, when the circulation pump was stopped, the power to the resistance heaters was also shut off. The circulation pump itself was protected by an external fuse. Heating time was obtained with an elapsed-time meter connected across the heater contactor.

Mixing. The fluid in each basin was mixed continuously with a paddlewheel (Figure 20). The mixing device consisted of two acrylic paddlewheels, one in each basin, attached to the ends of a 2.5 cm diameter steel shaft driven by a variable speed motor through a sprocket and chain drive mounted between each basin pair. Each paddlewheel was 65 cm in diameter and had six paddles made of acrylic sheets. These were 0.6 cm thick and cemented to an acrylic hub. The paddles reached within 2.5 cm of the basin floor and were notched for clearance around the pipes of the heat exchanger. Mixing speeds were adjustable from 2 to 20 rpm, corresponding to 6.8 to 68 cm/sec in linear velocity of the paddlewheel tips.

Nutrient Supply. The Nutrient substrate for the growth of photosynthetic and non-photosynthetic microorganisms was pumped to the basins from the nutrient holding tank through a flexible hose (Figure 7). Once each day, all basins were harvested by draining a

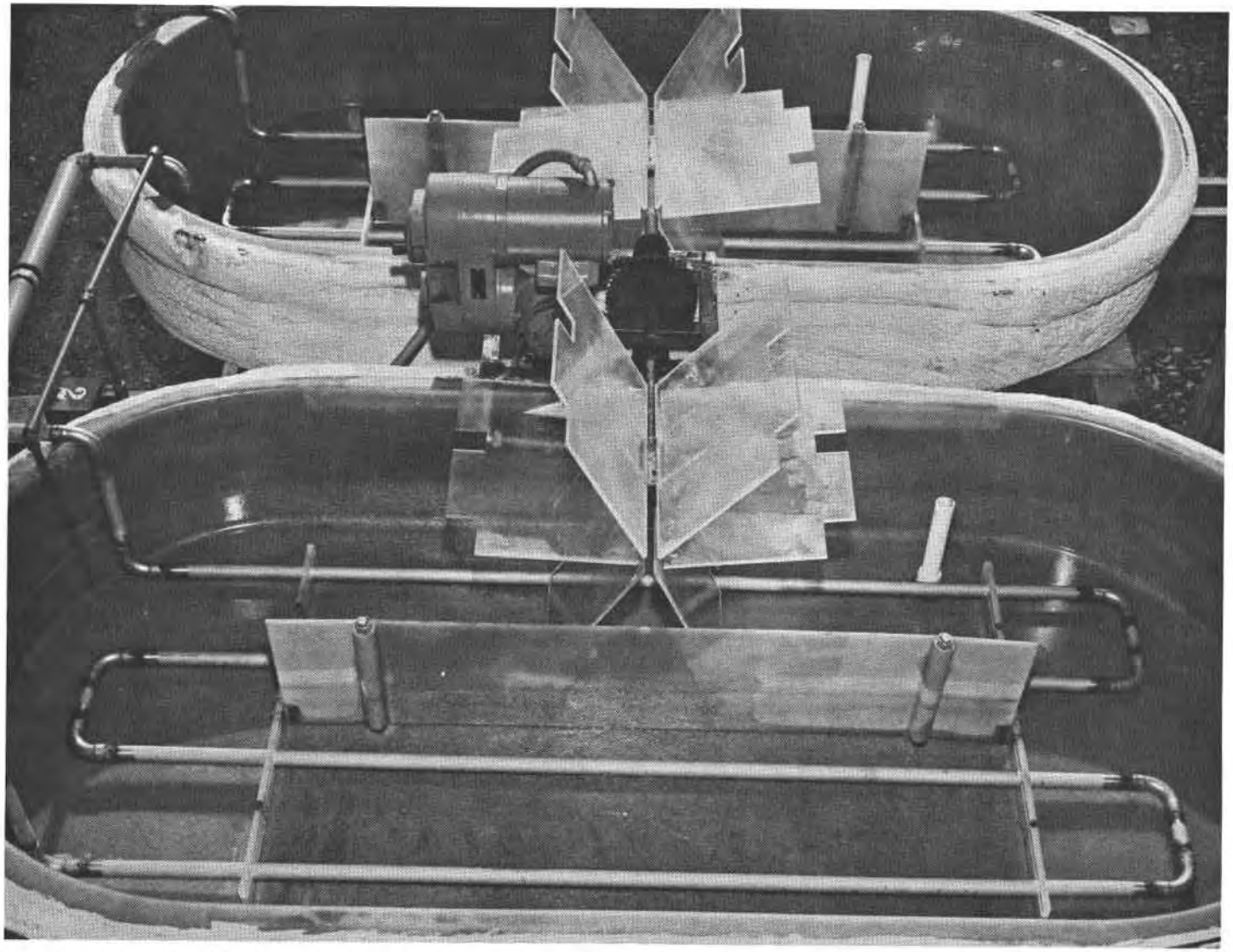

Figure 20. Details of a pair of basins showing heat exchangers made of stainless steel tubing, paddles made of acrylic plastic, variable speed motor, and overflow pipes.

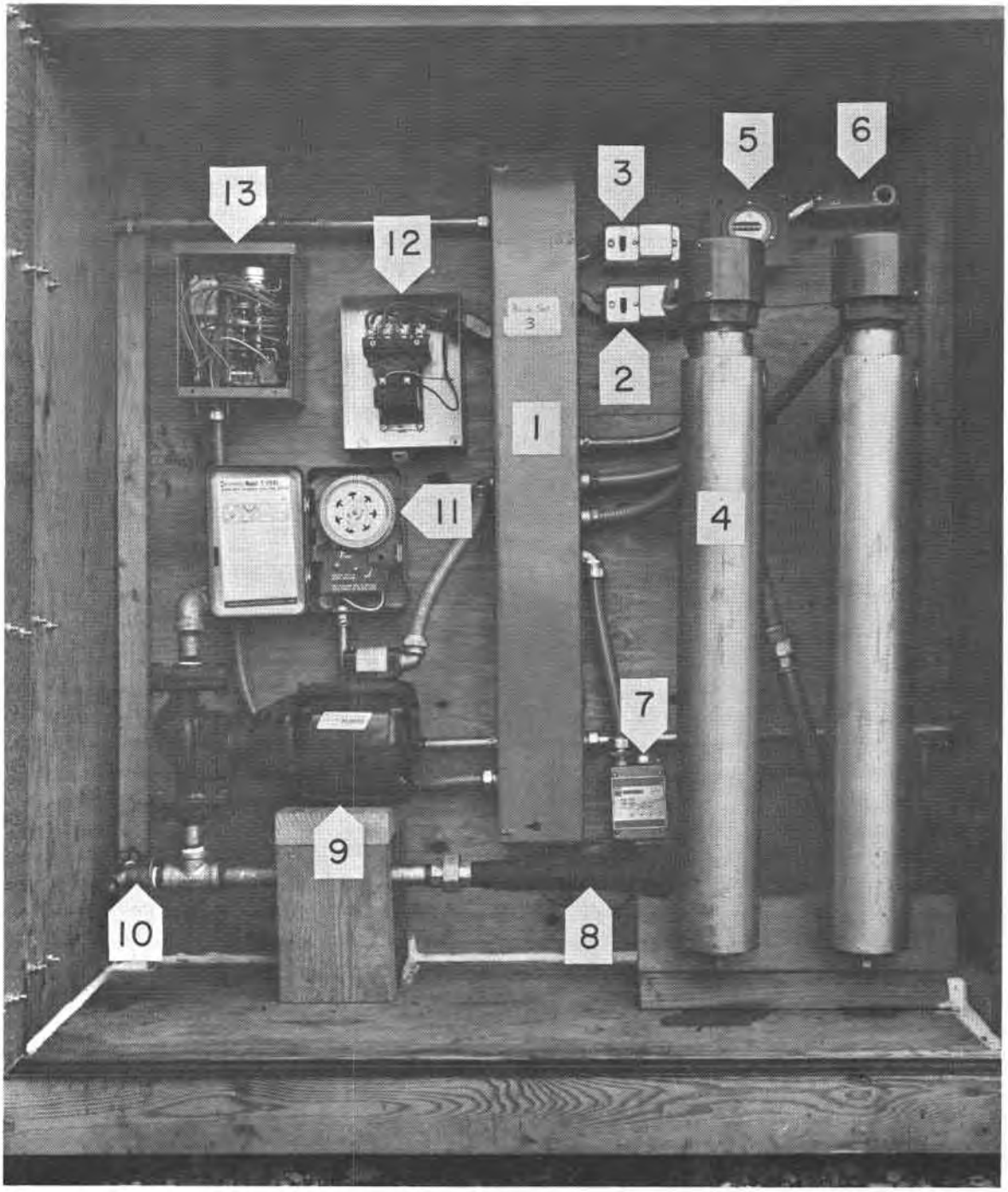

Figure 21. Control cabinet with equipment for one pair of basins. (1) Raceway, (2) on-off switch with fuse for warm water circulating pump, (3) on-off switch with fuse for paddlewheel motor, (4) resistance heaters, (5) elapsed-time meter for heater operation, (6) high temperature cut-off switch for heaters, (7) thermostat, (8) sensor bulb strapped to heater inlet pipe with insulation tape, (9) warm water circulating pump, (10) fill and drain valve, (11) 7-day time clock installed for automated waste addition but not actually used, (12) heater contactor, and (13) sequence timer installed for automated waste addition but not actually used.

specified volume. The operator would then replenish the drained volume in each basin with fresh liquid waste from the nutrient holding tank.

Harvesting of Biomass. Each basin was fitted with a 2.5 cm diameter standpipe which was inserted into a tank adapter of corresponding size (Figure 20). The adapter, in turn, was connected by flexible tubing to a 5 cm diameter PVC effluent collection pipe (Figure 19) which drained into a 3,000 liter, precast concrete tank buried next to the flush water holding tank (Figures 7 and 8). The length of the standpipe determined the depth of the culture volume in each basin. Both product holding tank and flush water holding tank were identical in construction and size to the nutrient holding tank (Figure 18).

Once each day, all standpipes were pulled manually and a predetermined volume was drained or harvested from each basin. The drained volume was a function of retention time and culture depth according to the formula:

$$\text{Harvested volume (l/day)} = K \frac{\text{Culture depth (cm)}}{\text{Retention (days)}}$$

where K is the basin volume per cm of culture depth (K = 20 liters/cm). Knowing the specific volume per cm of culture depth, the operator allowed the liquid level in each basin to fall a specified distance, replaced the standpipe, and replenished the harvested volume with an equivalent volume of fresh swine waste. For example, a basin with a culture depth of 20 cm contained 400 liters of culture fluid (20 cm x 20 liters/cm). If the retention time was 2 days, then 200 liters of culture fluid had to be drained or harvested each day and replaced with 200 liters of fresh nutrient solution. The operator therefore removed the standpipe once each day and allowed the level of culture fluid to fall by 10 cm (200 liters divided by 20 liters/cm) along a measuring tape affixed to the wall of the basin divider at a location near the standpipe. When the fluid level had dropped 10 cm, the operator replaced the standpipe and replenished the culture volume remaining in the basin with 200 liters of fresh liquid swine waste by pumping the waste water from the nutrient holding tank into the basin (Figure 12) until the overflow level of the standpipe was reached.

Table 10 summarizes the relationship between retention time, culture depth, culture volume, and the daily harvest volume and its equivalent culture depth.

The harvested biomass was concentrated to the consistency of a paste with a dry weight content of 15 to 20 percent (w/w) by a Sharples/Fletcher Mark III* continuous flow centrifuge. The centrifuge had a 2 hp (10 Amp; 230 V) electrically driven hydraulic drive and an adjustable basket speed up to 3250 rpm which created a maximum of 2,100 x G (Figure 22).

Mode of Operation

Selection of Photosynthetic Organism

In order to have as much control as possible over the quality and uniformity of the SCP end product, it was desirable to grow a single species of a non-toxic alga as the predominant organism in the liquid portion of the swine waste.

*Sharples-Stokes Division, Pennwalt Corporation, 1415 Rollins Rd., Burlingame, CA 94010.

Table 10. Relationship between retention time, culture depth, culture volume, and daily harvest volume and its equivalent culture depth.

Retention time	Culture depth	Culture volume	Daily harvest	
			Volume	Equivalent culture depth
days	cm	l	l/day	cm/day
2	7.5	150	75	3.7
	10	200	100	5.0
	15	300	150	7.5
	20	400	200	10.0
3	7.5	150	50	2.5
	10	200	67	3.3
	15	300	100	5.0
	20	400	133	6.6
4	7.5	150	37	1.8
	10	200	50	2.5
	15	300	75	3.7
	20	400	100	5.0
6	7.5	150	25	1.2
	10	200	33	1.6
	15	300	50	2.5
	20	400	67	3.3
8	7.5	150	19	0.9
	10	200	25	1.2
	15	300	37	1.8
	20	400	50	2.5

In screening experiments with several species of *Chlorella, Scenedesmus, Spirulina,* and *Botyrococcus,* the thermophilic green alga *Chlorella vulgaris* 211/8K was found to be best adapted for growth in dilute swine manure (Boersma et al., 1975). The organism was strongly competitive in the nonsterile substrate, possessed a biochemical composition required of a prospective feed supplement, and as a member of the Chlorophycophyta, was not known to produce substances toxic to man or animal.

Preparation of Swine Waste

The dilution of the liquid waste was conveniently monitored by its ammonium nitrogen content (Table 11). Using the steam distillation method of Bremner (1966) the ammonium nitrogen could be determined within 10-20 minutes after sampling the waste. By manipulating the flush rate and the size and number of pigs in the confinement area, the concentration of the liquid waste could be altered to suit experimental purposes. Table 11 shows some combinations of flush rate and weight and number of pigs with which ammonium nitrogen concentrations of 100 to 850 mg/l were achieved. Partial clarification of the waste was accomplished by gravity settling in the sedimentation pit and the nutrient holding tank. Although the restricted light penetration into suspensions of swine manure was recognized and of concern

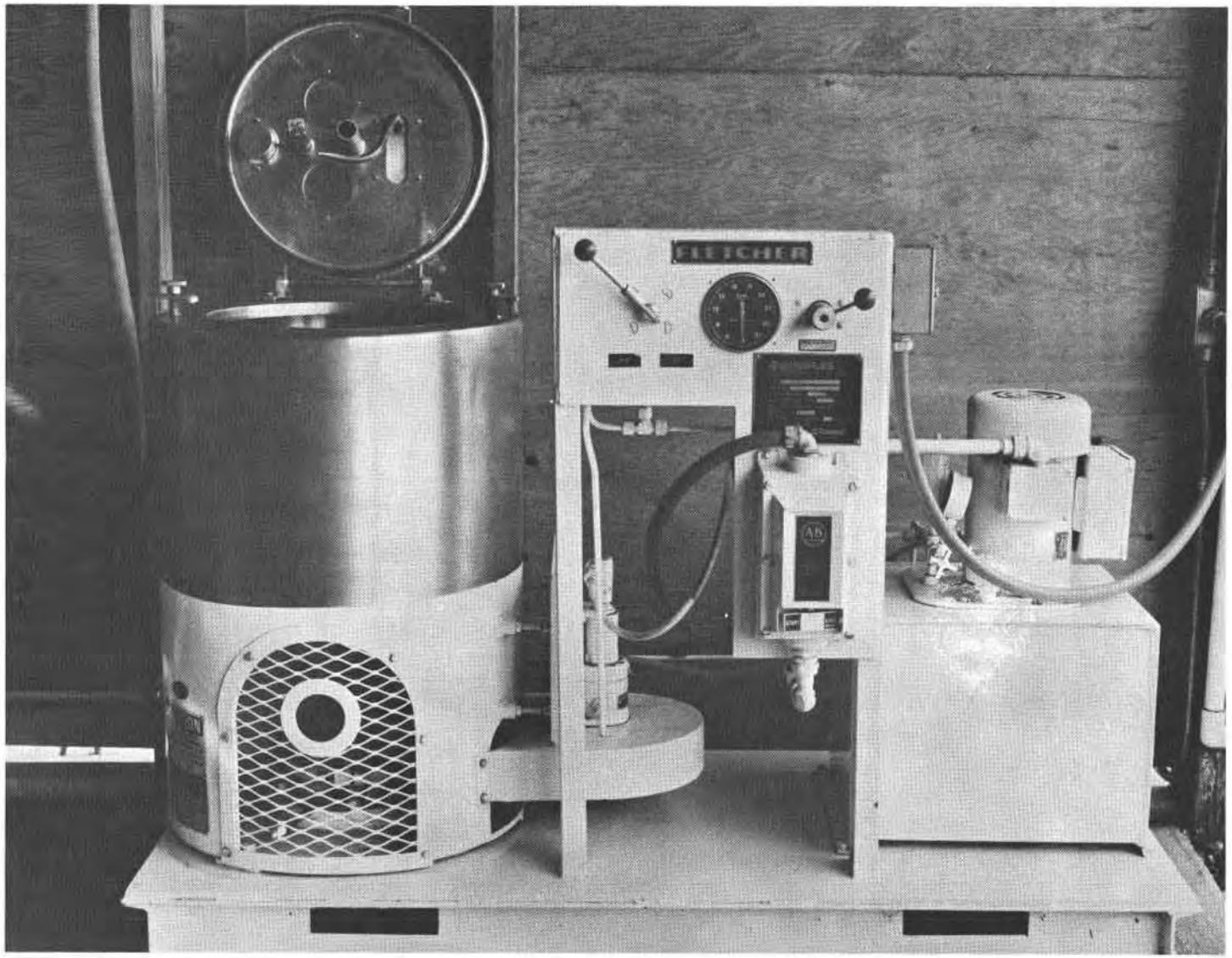

Figure 22. Continuous-flow centrifuge for concentrating algal-bacterial biomass.

(Table 12), Barlow et al. (1975) had shown that further clarification by flocculation with ferric chloride or filtration through activated carbon reduced the growth rate of both *Chlorella vulgaris* 211/8K and *Scenedesmus obliquus*. The superiority of the untreated liquid waste was attributed to its content of organic matter and the availability of carbon dioxide for photosynthesis from the respiratory activity of bacteria.

Table 11. Ammonium nitrogen concentration in the liquid portion of swine waste as a function of the gutter flushing rate, weight of pigs, and number of pigs in the confinement area.

Gutter flushing rate	Weight per pig	Number of pigs	NH_4-N
liters/hr	kg		mg/l
140	36	20	100-150
140	54	20	200-250
140	54	50	350-400
140	73	50	450-550
70	91	50	750-850

Table 12. Percent light transmission as a function of wavelength through 1 cm of swine manure. Samples were passed through cheesecloth to remove large particles and diluted to contain the indicated amounts of ammonium-nitrogen. One sample was clarified by adding $FeCl_3$, which precipitated solids.

Substrate tested	NH_4-N	Wave length – nm			
		400	500	600	700
	mg/l	.....% transmission			
Fresh manure	250.0	2.0	6.0	7.5	19.0
	125.0	9.0	20.9	30.0	39.0
	62.5	35.0	50.0	61.0	68.5
$FeCl_3$ treated	250.0	77.5	86.5	91.5	94.0

Table 13 shows the composition of the swine ration during the period of experimentation. The animals had continuous access to feed and water, and each consumed from 2.5 to 3.5 kg of feed per day. About 75 to 80 percent of the ration was digestible (NRC, 1971). The daily production of fresh manure was estimated to be 3 to 4 liters per pig with the solids content ranging from 15 to 20 percent (Meek et al., 1975).

Table 13. Composition of feed during period of experimentation. Grower pigs ranged in weight from 27 to 54 kg each, finishing pigs from 54 to 100 kg.

Feed ingredients	Feed rations	
	Grower pigs	Finishing pigs
	..... g/kg diet	
Barley	591	558
Wheat millrun	136	181
Soybean oil meal	82	23
Meat meal	45	45
Cottonseed meal	–	23
Dehydrated alfalfa meal	23	–
Sun cured alfalfa	–	45
Dried whey	23	23
Trace minerals salt	4.5	4.5
Ground limestone	4.5	4.5
Chemotherapeutic agent*	0.91	–

*Provides 110 mg chlortetracycline, 110 mg sulfamethazine, and 55 mg penicillin per kg diet.

Preparation of Algal Inoculum

Chlorella vulgaris 211/8K was obtained from Dr. N.I. Bishop, Dept. of Botany and Plant Pathology, Oregon State University. The culture was maintained in ASMG medium (McLachlan, 1963) at 25 C, a light intensity of 2150 lumen/m^2, and a 16-hour photoperiod. Mass cultures were grown from 100 ml stock cultures by adding the culture to increasing volumes of ASMG medium (100 ml, 1, 2, 10, and 20 liters) at 3 to 5 day intervals. The 10 and 20 liter cultures were aerated. ASMG medium was prepared as a concentrated stock solution so that 10 ml of stock added to one liter of distilled water would yield a properly diluted culture medium. The pH was then adjusted to 7.0 and the solution autoclaved.

Inoculation of Culture Basins

At the beginning of each experiment basins 1A, 1B, 2A, and 2B (Figure 19) were each filled with 110 liters of fresh liquid manure pumped up from the nutrient holding tank and the temperature was set at 25 C. Each basin was then seeded with a 10 liter culture of *Chlorella vulgaris* 211/8K to bring the final volume to 120 liters. This volume corresponded to a culture depth of 6 cm. The final cell density was approximately 10^6 algal cells/ml. The temperature was then increased to the desired experimental temperature within the next two hours. Mixing was continuous at 15 cm/sec as measured at the outer edge of the paddles. No swine waste was fed or biomass removed during the following two days so that the algae could establish themselves and increase their cell numbers by approximately one order of magnitude.

The cultures in the two pairs of basins served as inoculum for the other basins. The cultures in basin set 1 were used to inoculate sets 3 and 5, the cultures in basin set 2 served to inoculate sets 4 and 6 (Figure 19). The inoculum consisted of 40 liters of starter culture

transferred to 100 liters of fresh liquid waste maintained at the desired experimental temperature. This left approximately 40 liters of algal culture in each of the four starter basins. Fresh waste was added to the starter basins to raise their volumes to 140 liters.

In this manner, all cultures were started under similar conditions. Again, no waste was added or biomass removed for the next two days in order to increase the density of algal cells. All basins were then adjusted to their respective experimental depths by pumping in more fresh waste. Depending on the final culture depth and prevailing weather conditions another two or three days without harvesting were required to raise the algal cell densities from 1×10^7/ml to 5×10^7/ml. Routine harvesting was then initiated.

Experiments

The initial set of experiments tested (1) the photosynthetic conversion of nutrients into algal biomass, (2) the heterotrophic conversion into bacterial biomass, and (3) the conversion of solids into methane-rich fuel gas by anaerobic digestion. To provide the necessary waste of known composition and concentration for these experiments, the facility was operated in a non-recirculating mode using only fresh water for flushing. Table 14 summarizes the experimental variables tested and the parameters measured.

As a consequence of operational difficulties encountered soon after start-up of the facility in June, 1975, the digester and basins were operated independently of each other during these experiments. The digester was found to have gas leaks so that the daily production of biogas could not be measured. Furthermore, the overflow of the digested manure into the nutrient holding tank from digester A (Figures 12 and 13) ceased as a layer of solids 20 to 30 cm thick began to accumulate at the surface of the manure slurry. This layer suppressed the liquid level in digester A below the overflow level to the nutrient holding tank and plugged the opening of the overflow pipe with solids.

In view of the limited time available for experimentation, priority was given to the algal growth experiments while the deficiencies of the digester were corrected. Only the untreated liquid phase of the manure from the sedimentation pit (Figure 6) was used as the substrate for algal growth.

The leaks in the digesters were sealed by applying a roofing mastic to the inside surfaces. The difficulty with the overflow to the nutrient holding tank was solved by lengthening the overflow pipe towards the bottom of digester A (Figure 12).

After the digester became operational again, it was noted that its effluent required further clarification before it could be used as a substrate for the growth of algae. It was therefore necessary to add another tank which is labeled as "secondary nutrient holding tank" in Figures 7 and 8. During the construction of the tank, provisions were also made for the automated addition of waste to the basins (Figure 21). While these design modifications were undertaken, the outdoor basins and the digester were operated separately by closing the overflow from digester A to the nutrient holding tank (Figure 12). In this mode of operation, the effluents from the digester and the basins were not recycled but diverted into the lagoon after samples were taken for analysis and the algal/bacterial biomass was harvested.

Table 14. Summary of experimental variables and measurements.

EXPERIMENTAL VARIABLES

A. ALGAL GROWTH
   1. Swine waste concentration: 100, 200, and 400 mg/l NH_4-N
   2. Retention time : 2, 3, 4, 6, and 8 days
   3. Temperature : ambient, 30 C, and 35 C
   4. Depth : 7.5, 10, 15, and 20 cm

B. BACTERIAL GROWTH
   1. Swine waste concentration: 100, 200, 400, 600, and 800 mg/l NH_4-N
   2. Retention time : 1.5, 2, 3, and 4 days
   3. Temperature : 15, 20, 25, 30, and 35 C

C. ANAEROBIC DIGESTION
   1. Swine waste concentration: solids from 50 pigs diluted 1:2

MEASUREMENTS

A. BIOMASS
   1. Total dry matter, g/l
   2. Cell and particle counts (algal cultures only)
      a. hemocytometer, algal cells/ml
      b. Coulter counter, particles/ml
   3. Mean cell volume (algal cultures only), μm^3
   4. Protein content
      a. total Kjeldahl nitrogen, mg/l
      b. ammonium nitrogen, mg/l
      c. amino acid spectrum, g/100 g

B. WATER QUALITY (POND INFLUENTS AND EFFLUENTS)
   1. Total Kjeldahl nitrogen, mg/l
   2. Ammonium nitrogen, mg/l
   3. Nitrate nitrogen, mg/l
   4. COD, mg/l
   5. BOD, mg/l
   6. pH
   7. Dissolved oxygen, mg/l

C. ENERGY DISSIPATION
   1. Power consumption (ponds and digester), kWh/day
   2. Evaporation losses (ponds), l/m^2 day

D. ANAEROBIC DIGESTION
   1. Liquid phase (digester influent and effluent)
      a. total solids (TS), mg/l
      b. volatile solids (VS), mg/l
      c. COD, mg/l
      d. pH
   2. Gas phase
      a. production, m^3/day
      b. composition, CH_4/CO_2 (v/v)

E. CLIMATE
   1. Temperature, C
   2. Solar radiation, $kcal/m^2$ day

The initial experiments during the summer and early fall of 1975 defined the operational and cultural conditions such as retention time, culture depth, temperature, solar radiation, and ammonium nitrogen concentration which did or did not support the growth of *Chlorella vulgaris* 211/8K in outdoor basins when the liquid phase of untreated swine manure is used as the growth medium. Experiments in late fall and early winter of 1975 determined that algal growth was severely reduced when the photoperiod decreased to less than 11 h per day and the rate of solar radiation decreased to less than 2,000 kcal/m^2 day. A detailed analysis of the growth of *Chlorella* in diluted swine waste in response to the operational and cultural variables, therefore, had to be postponed until the spring of 1976. The time available for experimentation then prevented the implementation of the experiments during which the system was to have been operated in a closed-loop mode, recycling the waste water. The purpose was to test the biological stability and productivity of the bioconversion units under conditions of recycling nutrients, salts, and possibly toxic metabolic end products. An accumulation of these compounds, in particular salts, was expected with the passage of time.

Algal Growth

Dry matter yields of algal biomass were measured as a function of swine waste concentration, retention time, culture depth, and temperature under prevailing climatic conditions. The concentration of ammonium nitrogen in the liquid waste was adjusted by varying the combinations of flush rate and size and number of pigs as shown in Table 11. The solids which accumulated in the sedimentation pit were pumped out periodically and diverted to the lagoon. The basins were mixed continuously at 15 cm/sec and harvested once each day between 16:00 and 17:00 hours. The harvested biomass was concentrated by centrifugation. The water passing the centrifuge was discharged into the lagoon. The pH and the dissolved carbon dioxide levels in the culture medium were not controlled.

Bacterial Growth

A study of the potential for bacterial protein production from the swine manure was conducted for several reasons. The growing urgency in the U.S. for waste recovery and recycling, of which the production of non-photosynthetic SCP from animal wastes for refeeding is just one example, has been recognized and was recommended to the National Science Foundation, Research Applications Directorate, as a priority area for research and funding (Scrimshaw et al., 1975). Bacteria have some advantages over algae. In general, they are more digestible by monogastric animals, have much shorter generation times and higher protein and sulfur amino acid content. On the other hand, bacteria have a high nucleic acid content which may be undesirable from a nutritional point of view and they are subject to phage infection and lysis.

Open-air ponds used for the photosynthetic reclamation of animal manures inevitably produce a mixture of algae and bacteria. The extent to which either group of organisms contributes to the total biomass depends on such factors as light intensity, nutrient concentration, organic and inorganic carbon concentration, culture depth, retention time, and temperature. If a predominantly algal product is to be obtained for use as a protein supplement in livestock rations, it is necessary to know which combination of factors will give the algae a competitive advantage over the bacteria and which will favor bacterial growth.

The bacterial growth experiments were conducted in the same manner as the algal experiments. The bacterial biomass was derived from the mixed populations existing in the liquid portion of the manure. The cultures were adjusted to a depth of 15 cm and stirring was continuous at 15 cm/sec. Harvesting and replacing the harvested volume with fresh medium took place once each day between 08:00 and 09:00 hours. Biomass production was measured as a function of swine waste concentration, temperature, and retention time (Table 14).

Anaerobic Digestion

The manure from 50 animals with a combined weight of 4,000 kg was collected in the sedimentation pit using an hourly flush rate of 140 l. The solids were mixed with equal volumes of liquid and pumped into the digester. In this manner the digester was filled to capacity within 5 weeks. The manure slurry had a final solids content of about 7 percent. During the time interval of filling the digester no heating or mixing took place.

Once the digester was filled past the water seal, loading of the digester was halted and the mixing pump and heating system were activated (Figures 12 and 14). Mixing was now continuous. It took approximately 4 days for the digester temperature to stabilize at 37 ± 1 C. Gas production was measurable 7 days after the heating system was activated and reached a constant rate one week later.

At this point, regular feeding of the digester was continued. The solid manure was loaded into the digester at the rate of 635 liters every other day for a hydraulic retention time of 47 days. The total solids content of the digester influent was 6 percent. The 635 liters represented 38 percent of the daily flush volume used to remove the manure from the animal quarters. The excess liquid was discharged into the nutrient holding tank and overflowed from there to the lagoon together with the effluent from the digester.

Digester influent and effluent were examined for total solids (TS), volatile solids (VS), chemical oxygen demand (COD), and pH. The composition of the gas was analyzed by gas chromatography.

Data Collection

On-site data were collected early in the morning and late in the afternoon. For the open-air basins a record was made of overnight and day-time evaporation losses, temperatures, pH, dissolved oxygen, and the power consumed for heating and stirring. At harvest time, pond influent and effluents were sampled for determinations of nitrogen, biomass dry weight, algal counts, particle counts, COD, and BOD.

For the anaerobic digester a daily record was made of the biogas production and the power consumed for heating and mixing. The CO_2 and CH_4 composition of the gas was analyzed at least twice each week. At the time of feeding the digester, influent and effluent were sampled for determinations of pH, total solids, volatile solids, and chemical oxygen demand.

Measurements of Growth

Concentration of Total Dry Matter

Determinations of total dry matter concentration were made by passing measured portions of culture suspension through tared Millipore(R) filters. This technique was modified from that used by the Environmental Protection Agency in its eutrophication research program (EPA, 1971). Type RA filters with a pore size of 1.2 μm were leached with 500 ml of distilled water, dried in an oven at 30 C for 45 minutes and then for 24 hours in a desiccator under vacuum. The filters were weighed and after passing 3 to 10 ml samples through them, the drying and weighing procedure was repeated. All determinations of dry matter concentration were made in triplicate.

Microscopic examination of filtrates from the 1.2 μm filters showed that in mixed cultures of *Chlorella* and bacteria all algal cells are retained on the filters while some bacteria pass through. The effect of the loss of bacteria through the filter on the accuracy of the measurement of concentration of dry matter was tested by statistical methods (Goldstein, 1965). Also tested was the precision of the sampling technique.

Nine 100 ml samples were taken within a time interval of several minutes from a mixed culture of bacteria growing in dilute swine waste at a temperature of 30 C. Determinations of dry matter were made in triplicate for each sample, using 5 ml aliquots of culture for each measurement. The concentrations of dry matter are shown in Table 15 and results of the statistical treatment in Table 16.

The calculations show that the variance of the mean for the population of 27 measurements of dry matter was 0.026 g^2/l^2 and the standard deviation was 0.162 g/l. The variance of the mean for the 9 samples was 0.027 g^2/l^2 and the standard deviation was 0.165 g/l. The sampling procedure and the Millipore filter technique, therefore, had errors of about 6 percent each.

Table 15. Dry matter determinations of a mixed culture of bacteria growing in liquid swine waste at 30 C. Nine 100 ml samples were taken from the culture at the same time and three 5 ml aliquots from each sample were filtered through tared Millipore filters of type RA with 1.2 μm pore size.

Sample #	Dry matter concentration		
	..... g/l		
1	2.72	2.70	2.80
2	2.75	2.77	2.62
3	2.82	2.87	2.85
4	2.70	2.75	2.87
5	2.87	2.75	2.85
6	2.72	2.77	2.72
7	2.70	2.70	2.70
8	2.75	2.72	2.85
9	2.47	2.45	2.50

Table 16. Statistical analysis of data shown in Table 15.

Parameter	Value
No. of samples	9
No. of determinations per sample	3
Mean (g/l)	2.62
Range (g/l)	0.42
Variance (g^2/l^2)	
a. population	0.026
b. sample	0.027
Standard deviation (g/l)	
a. population	0.162
b. sample	0.165
Coefficient of variation (%)	
a. population	6.20
b. sample	6.30
99% confidence interval estimate of mean using S^2 (g/l)	2.53 to 2.71

Using the same filtration technique, Seward (1976) obtained a standard deviation of 0.1 g/l for measurements of dry matter concentrations in mixed cultures of *Chlorella* and bacteria. His alga was grown in dilute swine waste under laboratory conditions at different culture depths, temperatures, retention times, and radiation intensities.

Chlorophyll Fluorescence

Chlorophyll, like many other organic molecules, possesses the ability to fluoresce by absorbing light energy at one wavelength and emitting the energy at a longer wavelength. It has been attempted to correlate units of fluorescence with cells of algae per ml of sample. A linear relationship of cell numbers of fluorescence has been shown for the green alga *Selenastrum capricornutum* during its logarithmic phase of growth, demonstrating the potential usefulness of fluorescence to evaluate productivity (EPA, 1971).

In acetone, commonly used to extract chlorophyll from plants, maximum rate of absorption or excitation takes place at a wavelength of 430 nm and maximum rate of emission occurs between 650 and 675 nm (Yentsch, 1963). Fluorescence of *in vivo* chlorophyll is only 10 percent as efficient per unit weight of chlorophyll than fluorescence of extracted and dissolved chlorophyll (Lorenzen, 1966).

The extraction of chlorophyll with acetone takes at least 24 h and may not be complete as many plant cells resist extraction of the pigment. In contrast, *in vivo* fluorescence with a Turner Fluorometer can be determined within minutes. Both methods have the disadvantage that the chlorophyll *a* content of algal cells is not always constant but may vary according to the nutritional environment, light intensity, and the physiological condition of the cells (Bain, 1969). For example, nitrogen deficient cells usually have a lower chlorophyll *a* content than cells which are not deficient in nitrogen although the number of cells and the cell mas produced per ml of sample may be in close agreement. Thus, chlorophyll values recorded either as

relative fluorescence units or as mg of chlorophyll *a* per m^3 of culture volume may not always correlate to the number of cells of algae.

Samples from the basins were blended for 15 seconds in a Sorvall blender after which aliquots were poured into a cuvette for *in vivo* measurement of relative fluorescence in a Turner Fluorometer. When the scale deflection showed over 90 relative units, the sensitivity of the instrument was lowered, when the reading showed less than 15 units, the sensitivity was increased, and when the reading failed to stay in the range from 15 to 90 units, the samples were diluted accordingly. Relative fluorescence units were then recorded based on a common sensitivity factor.

Hemocytometer Counts

Direct determination of the number of algal cells by means of a hemocytometer was deemed necessary because the Millipore technique measures only the total biomass concentration and makes no distinction between the algae and the other organisms which contribute to the total dry matter such as bacteria, zooplankton, fibers, and detritus.

It was realized that the direct cell count has limitations as a method for the determination of algal growth. The principal disadvantage is that counts alone can not be used to calculate the dry matter because the size of the cells varies with age, availability of light, and the nutritional environment of the algae. Nevertheless, direct counting of cells offers a relative measure for comparing the growth of algae under different environmental and nutritional conditions.

The counting technique itself is subject to a minimum variation of 10 percent for *Chlorella* (Oswald and Gaonkar, 1969). At least 100 cells must be counted in the 5 central squares of the hemocytometer in order to reach a minimum variance of 10 percent in the counts. When only 10 cells are counted, the coefficient of variance increases to 25 percent.

Coulter Counts

The use of an electronic particle counter was deemed desirable because it allows the calculation of the algal biomass as the product of particle counts or hemocytometer counts, mean cell volume, and specific gravity of the algal cells, so that an estimate of the contribution of the algae to the total dry matter can be made. Furthermore, by comparing particle counts and direct algal counts, it may be possible to replace visual counting with the speed and ease of electronic counting.

A model ZBI Coulter counter with a mean cell volume (MCV) computer became available in July, 1976. The sensitivity of the instrument was linear for particles between 2 and 7 μm in diameter using a 100 μm diameter orifice. The instrument was calibrated with ragweed pollen which had a mean cell diameter of 19.5 $\pm$ 1.0 μm. The multiplier settings of the counter were adjusted to "1 over 1/4" and "1 over 1/2" to count particles 2 to 7 μm in diameter which included the cells of *Chlorella.*

The samples from the outdoor basins were prepared for counting by 15 seconds of high speed blending in a Sorvall blender to break up bacterial floc and detritus and to reduce as

much as possible the number of particles other than algae in the 2 to 7 μm diameter range. Sample aliquots were added to the electrolyte, a 1 percent (w/v) solution of NaCl, to obtain counts in the range from 10,000 to 40,000 particles/count with a standard error of 3 percent.

Dry Matter of Algae

The Millipore filter technique used to obtain total dry matter concentrations of the cultures was adequate for the determinations of biomass yield. However, in order to obtain the contribution made by the algal cells to the total dry matter, it was necessary to determine the dry matter concentrations of the algae. The hemocytometer counts did not provide this information nor did the particle counts with the Coulter counter.

The dry matter of the algae can be estimated from the number of algal cells, their mean cell volume (MCV), specific gravity, and moisture content by using the following formula:

$$\text{Dry matter (g/l)} = \text{No. of cells/l} \times \text{MCV } (\mu m^3) \times \text{sp. gr. } (g/cm^3)$$

$$\times 10^{-12} \ (cm^3/\mu m^3) \times (\text{l-moisture content})$$

where the number of cells per liter of culture can be obtained from hemocytometer and/or Coulter counts, the mean cell volume from a Coulter counter with MCV computer, the specific gravity of the algal cells can be assumed to be 1.0 to 1.1 g/cm^3, and the moisture content can be calculated from dry matter determinations and packed cell volumes (PCV) of axenic cultures of the algae. Oswald and Gaonkar (1969) indicated that the packed cell volume usually contains about 14 percent dry matter of algae.

Moisture Content of Algae

Knowledge of the moisture content of *Chlorella vulgaris* 211/8K was essential in estimating the contribution made by the algae to the total dry matter as a function of changes in solar radiation intensities, culture depth, retention time, and temperature. Knowing the concentrations of algal dry matter permitted the calculations of algal yields and photosynthetic efficiencies.

The moisture content and other physical characteristics of *Chlorella vulgaris* 211/8K were ascertained in the following manner. The growth of axenic cultures was followed under laboratory conditions in ASMG medium (McLachlan, 1963) at a temperature of 25 C, 2,150 lumen/m^2, and a photoperiod of 16 h to construct the growth curve shown in Figure 23.

Six identical culture flasks with 1 liter of ASMG medium each were inoculated to contain approximately 10^6 cells/ml and growth of the algae was allowed to proceed according to Figure 23. Three of the six cultures were removed from the growth chamber during the exponential phase of growth (Figure 23, point A) and the remaining cultures during the stationary phase of growth (Figure 23, point B). A 10 ml sample was obtained from each culture for hemocytometer counts, Coulter counts, and measurement of the mean cell volume with the Coulter counter. Determinations of dry matter concentrations were made in triplicate by using the Millipore filter technique with 5 ml of sample for each determination. The remaining 975 ml in each flask were centrifuged at 500 x g for 10 min to determine the packed cell volume (PCV) in tubes with graduated stems.

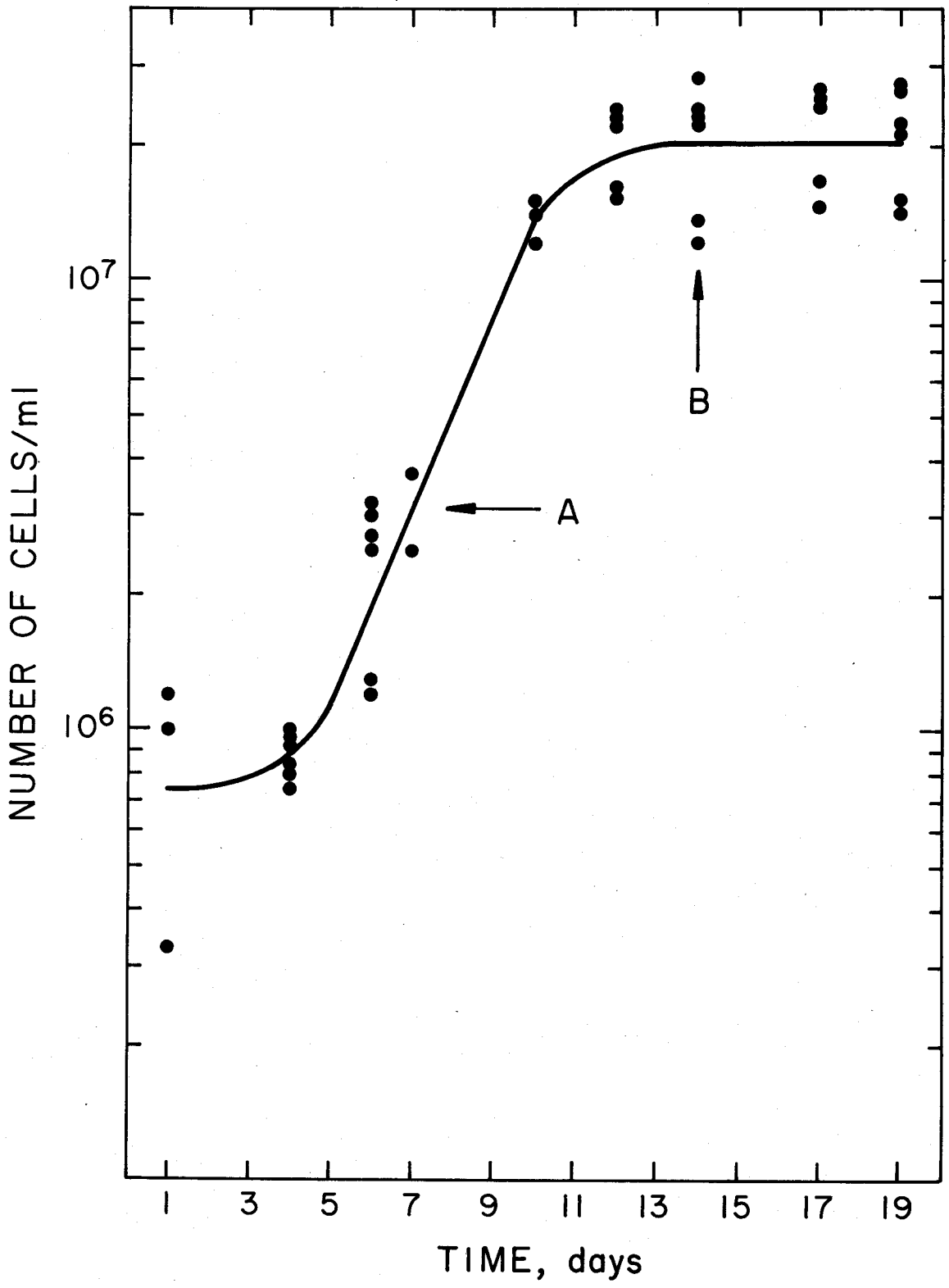

Figure 23. Growth of axenic cultures of *Chlorella vulgaris* 211/8K in ASMG medium (McLachlan, 1963) at 25 C, 2,150 lumen/m^2, and a 16 h photoperiod.

The results of these measurements are shown in Table 17. The number of cells and particles, the packed cell volume, and the dry matter per liter of culture increased about 10-fold as growth proceeded from the exponential phase to the stationary phase. The mean cell volume increased from 17 μm^3 to 29 μm^3 which corresponds to an increase in cell diameter from 3.2 μm to 3.8 μm.

Table 17. Physical properties of axenic cultures of *Chlorella vulgaris* 211/8K during the exponential and stationary phase of growth. The cultures were grown in ASMG medium (McLachlan, 1963) at 25 C, 2,150 lumen/m^2, and a 16 h photoperiod.

Physical properties	Exponential phase of growth	Stationary phase of growth
Hemocytometer counts, cells/l x 10^{-9}	2.32 ± 0.57	20.37 ± 3.03
Coulter counts, particles/l x 10^{-9}	1.57 ± 0.34	20.67 ± 3.91
Mean cell diameter, μm	3.20 ± 0.03	3.79 ± 0.08
Mean cell volume (MCV), μm^3	17.23 ± 0.48	28.87 ± 1.96
Packed cell volume (PCV), $\mu l/l$	46.70 ± 11.50	493.30 ± 31.90
Dry matter, mg/l	11.03 ± 1.96	118.00 ± 12.37
Dry matter/PCV, mg/μl[1]	0.26 ± 0.03	0.24 ± 0.02
Moisture content, % w/w		
a. based on MCV[1]	71.10 ± 2.30	79.50 ± 0.30
b. based on PCV	73.70 ± 3.20	76.10 ± 2.00

[1] Calculations assume a specific gravity of 1.0 g/cm^3 for the algal cells.

Calculations of the moisture content, based on dry matter determinations and the mean cell volume of wet cells, showed that the algae contained 71 percent moisture in the exponential phase of growth and 79 percent in the stationary phase. Calculations based on dry matter determinations and the packed cell volume showed a moisture content of 74 percent and 76 percent, respectively.

In calculating the contribution made by the algal dry matter to the total biomass, an average moisture content of 75 percent was used.

Other Measurements

Amino acid profiles of lyophylized *Chlorella,* bacteria, and mixed culture samples were determined with a modified Backman Spinco Model 120 B analyzer. Acid hydrolysis was performed in evacuated and sealed glass tubes with constant boiling HCl at 110 C for 18 hours (Speckman et al., 1958). Tryptophan was analyzed by alkaline hydrolysis according to Hugli and Moore (1972).

Nitrogen, phosphate, and elemental analyses were performed by the Soil Testing Laboratory at Oregon State University (Kauffman and Gardner, 1976).

Chemical and biological oxygen demands, total, and volatile solids were determined according to the American Public Health Association (1971).

Evaporation losses in the open-air basins were replaced manually by fresh make-up water using a flexible water hose. An in-line water meter measured the replacement volume with an accuracy of ± 1.5 liters equivalent to the volume in 0.75 mm of culture depth.