A PERIODICALLY LOADED AEROBIC BIODIGESTER

María del Pilar SÁNCHEZ SAAVEDRA AND Luis Fernando BÜCKLE RAMÍREZ

Departamento de Acuicultura, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Apdo. Postal 2732, Ave. Espinoza 843, Ensenada, Baja California 22800, México.

(Recibido marzo 1993, aceptado julio 1993)

Keywords: aerobic biodigestion, waste treatment, cow manure, chicken manure, Macrocystis pyrifera

ABSTRACT

Three equal biodigesters were built and operated separately with chicken and cow manure and with the brown macroalga *Macrocystis pyrifera* (L.) C. Agardh. The periodic loads which permitted the mechanical operation of the systems and a balanced nitrification process were 5% of the initial for chicken manure and 10% for cow manure and the macroalga. Once the load ratio was established, it was possible to recognize an initial period of adjustment of the bacteria populations, followed by a transition stage and finally a periodic stage, with constant nitrification rates. During the process the nitrogen balance was negative, with a net loss of 70.0, 82.0 and 11.9 percent of the total inorganic nitrogen in the biodigesters that operated with chicken and cow manure, and with the macroalga, respectively.

RESUMEN

Se construyeron tres sistemas iguales de biodigestión que funcionaron en forma periódica con excretas de gallina, de vaca y con la macroalga café *Macrocystis pyrifera* (L.) C. Agardh. Las cargas del 5% de la inicial (excretas de gallina) y 10% (excretas de vaca y macroalga) fueron las que permitieron el funcionamiento mecánico de los biodigestores y un proceso de nitrificación equilibrado. Una vez establecida la razón de carga, fue posible describir diferentes etapas: inicialmente un periodo de adaptación de las poblaciones bacterianas, una de transición y otra periódica con tasas constantes de nitrificación. El balance de nitrógeno resultó ser negativo, con una pérdida neta, durante el proceso de biodigestión del 70.0, 82.0 y del 11.9 porciento de nitrógeno inorgánico total en los biodigestores que funcionaron con excretas de gallina, de vaca y con macroalga respectivamente.

INTRODUCTION

Food production has been routed to the utilization of all type of natural resources (Porras 1981). The increasing agricultural and ranching activities result, in many cases, in local seasonal accumulations of organic residues exceeding the capacity of their traditional use, but which still have to be considered important natural resources.

Much research has been carried out during the '60s and '70s, on the technical treatment of residual waters originating from industrial, urban, agricultural, and cattle breeding activities (Ehrlich 1965, Prakasam and Loehr 1972, Gray and Biddlestone 1974, Vavilin *et al.* 1980, Hopwood and Barnes 1982), including among these anaerobic (Baquedaño *et al.* 1979), as well as aerobic systems (Adams *et al.* 1974, Bishop and Farmer 1978).

Through aerobic reactions the complex organic compounds of residual water are oxidized and transformed into stable inorganic and organic products. As a consequence, the effluent has a low BOD, few solids in suspension and its odour is lessened (Loehr 1965, Wong-Chong and Loehr 1978). This technique has the advantage of reducing the high concentrations of organic compounds, mainly nitrogen, phosphorus and organic carbon, in little space and in relatively simple systems, in comparison with anaerobic biodigesters or oxidation ponds.

The effluent of these processes still has a high content of inorganic nitrogen and phosphorus compounds, which can be used for other purposes, such as controlled eutrophication (Ryther et al. 1972, Goldman and Ryther 1975, 1976) or microalgae production (Kawasaki et al. 1982, Paniagua and Bückle 1984, Granados and Bückle 1984, Paniagua 1988, de la Noüe and Basseres 1989, Pouliot et al. 1986, 1989, Huntley 1989). In addition, these systems produce solid residues, that can be used as food supplement (Anwar et al. 1982, Paniagua and Granados 1987, Cordero and Voltolina 1990).

This work studies an alternative form of treatment, with a semicontinuous system of biodegradation, of the organic waste products originated from agricultural activities such as chicken and cow manures and of the brown macroalga *Macrocystis pyrifera* (L.) C. Agardh. Large quantities of this alga, due to the strong wave action, are continuosly stranded along the beaches of the Mexican Pacific NW and constitute a nuisance to the locally important tourist activities, for which reason they have to be continuosly removed and disposed according to local regulations.

MATERIAL AND METHODS

The biodigester used in this work (Fig. 1) is a modification of the model described by Paniagua (1988) for discrete loading.

Each biodigester consists of a 200 L plastic cylindrical container of 28 cm diameter and 84 cm height (Fig. 1), containing a 15 L sample container with the walls profusely perforated with 2 mm holes (Fig. 1.1), located above the solids removal system (SRS) (Fig. 1.3).

The base of the main container has a 10 cm layer of gravel of 2 to 5 mm grading (Fig. 1.4), on top of which is installed the SRS system (Fig. 1.3). This consists of three acrylic plates (Fig. 2): the upper and intermediate ones with 2 cm holes and the lower one with 2 mm orifices. The last two plates are united by



Fig. 1. Periodic aerobic biodigester system. Arrows indicate flow direction 1. Sample container; 2. Supports of the sample container; 3. System of solids removal (SRS); 4. Base with gravel; 5. Perforated pipe; 6. SRS crank; 7. Air injection for the SRS; 8. Valve for solid collection; 9. Filter; 10. Recirculation system; 11. Heater; 12. Air injection for recirculation; 13. Thermometer; 14. Return of the biodigested liquid; 15. Collection of effluent; 16. Funnel (influent); 17. Foam eliminator; 18. Gas vent.

a series of 2 cm high supports (Fig. 2.4) and by an external wall. The upper plate has a crank (Figs 1.6 and 2.6), which allows to rotate the upper plate so that its orifices coincide with those of the intermediate one (Fig. 2a) to collect the accumulated solids (Fig. 2.7) or to close the communication between the two plates, leaving a closed lower chamber (Fig. 2b) (intermediate plus lower plates), which permits to eliminate the solids by air injection (Fig. 1.7), through a valve, connected to a filter (Fig.1.9). This consists of a PVC pipe of 6.25 cm diameter and 25 cm height filled with plastic cotton which retains the solid fraction and allows recirculation of the fluid into the system (Fig. 1.10). A heater in the recirculating system (Fig. 1.11) maintains the biodigestion process at 28 \pm 1°C.



Fig. 2. Solids removal system (SRS) a, open position to remove the solids; b. Closed system for solids evacuation; l. Lower plate; 2. Intermediate plate; 3. Upper plate; 4. Plates supports; 5. Rubber walls; 6. Valve for the SRS; 7. Orifices for the solids collection; 8. Stops for open and closed position; 9. Hose with drillings; 10. Air injection; 11. Valve for solids collection.

An airlift (Wheaton 1977) (Fig. 1.12) recirculates the fluid continuously into the sample container (Fig. 1.14). The foam generated in the process is liquefied in a container with a perforated bottom (Fig. 1.17) to recover the fluid. The gases generated in the process are vented through a pipe (Fig. 1.18).

The periodic sample addition was through a funnel (Fig. 1.16) connected to the internal sample container. The raw material was preserved in a commercial freezer at -25° C. The three bioreactors were first inoculated with 50 g of soil in 100 L of tap water (Paniagua 1984) and the liquid was recycled during a week to allow the establishment of an adequate bacterial community.

After this time each system was loaded with the amount of waste products which, according to Paniagua (1984), allows optimum performance of this bioreactor, when used with discrete loads of these raw materials: the first received 3.7 kg of chicken manure, the second 2.8 kg of cow manure and the third 4.4 kg of M. pyrifera (blades and stipes). All materials were previously homogenized with sufficient tap water to obtain a semisolid paste. Water recycling was then restarted and lasted, without addition of new material, until high nitrate and minimal quantities of ammonia were found in the effluent.

At this point, some preliminary trials were run with additions of different percentages (2, 5, 10 and 20%), in weight, of the initial load, to find the maximum amount of new material which could be treated periodically, and the periodicity with which it should be added. These trials, which considered the mechanical operation of each system, the nitrification variables and the loading factor (BOD/COD ratio), indicated that optimum performance was obtained adding at five days intervals 5% of the initial load, for chicken manure, and 10% for cow manure and for the macroalga (185, 280 and 440 g, respectively, Sánchez 1989).

The operation of each system was evaluated mea-



Fig. 3. Nitrogen concentrations in the digester loaded with chicken manure. Ammonia: •; nitrites: full circles; nitrates: open circles. Dotted lines: influent; continuous lines: effluent.



Fig. 4. Biochemical (BOD: •) and chemical (COD: Δ) oxygen demand (in mg/L) and BOD/COD ratio (*), for the influent (dotted line) and the effluent (continuous line) in the digester loaded with chicken manure.

suring in the effluent, at five days intervals, the indicators of the nitrification process (ammonia, nitrites and nitrates concentrations), the load factor (BOD/COD ratio), as well as temperature, flow rate of the recirculating liquid, pH, oxygen and hydrosoluble phosphates concentrations, which are related to this process. Nitrates, nitrites and phosphates were measured with the spectrophotometric methodology described in the Hach DR-EL/4 manual (Hach 1981) and ammonia concentrations with an ion autoanalyzer Orion 901; pH with a Litmax CP-20 potentiometer and dissolved oxygen concentrations and chemical and biochemical oxygen demand (COD-BOD) with the methodologies described in APHA (1980).

The mean values of each variable measured in the effluent during the different stages of the biodegradation process were compared with the non parametric analysis of variance test of Kruskal Wallis and, when necessary, with the non parametric version of the *a posteriori* Student-Neuman-Keuls test (Sokal and Rohlf 1969) elaborated at CICESE by Dr. A. Chagoya. The same variables were also measured in the inflow (mixture of raw material and water added periodically to each reactor).

The material accumulated in the SRS was removed during 15 min, with the same frequency as raw material addition, and weighed after removal of excess moisture by filtration. The constancy in time of the amount recovered was tested by linear regression analysis (Beta test, Sokal and Rohlf 1969) of the weight of the solids recovered (in g) vs time.

RESULTS AND DISCUSSION

The changes of the variables measured in the effluent of each system (Figs. 3 to 8), and a series of Kruskal-Wallis tests delimitated (Table I) three different stages in the biodigester operation: the initial (I) corresponds to the period when the biodigesters operated as static systems, with no addition of new digestible material. For the three reactors, this stage was characterized by a significant decrease of ammonia, a marked increase in nitrates and a slight increase in nitrites concentrations (Figs. 3, 5 and 7).



Fig. 5. Nitrogen concentrations in the digester loaded with cow manure. Ammonia: *; nitrites: full circles; nitrates: open circles. Dotted lines: influent; continuous lines: effluent.

		Kruskal-Wallis		,	А	posterio	ori		
Var	riable	(H) value	Pro	obability	comparison via S N K				
Hen ma	nure				I	Т	Р		
O_2	(mg/L)	1.150	0.5804	NS					
Т	(°C)	6.184	0.0374	*					
pН		2.101	0.3688	NS					
DBO	(mg/L)	10.422	0.0011	* *					
DQO	(mg/L)	12.788	0.0000	* * *					
NH_4 +	(mg/L)	11.133	0.0081	* *					
NO_2^-	(mg/L)	13.124	0.0002	* * *					
NO ₃ -	(mg/L)	10.134	0.0049	* *					
PO_4^{-3}	(mg/L)	9.138	0.0045	* *					
Cow ma	nure								
O_2	(mg/L)	0.750	0.7086	NS					
Т	(°C)	2.322	0.3332	NS					
pН		0.794	0.6946	NS					
DBO	(mg/L)	7.881	0.0094	* *					
DQO	(mg/L)	9.168	0.0029	* *					
NH4 ⁺	(mg/L)	10.003	0.0089	* *					
NO_2^-	(mg/L)	6.032	0.0308	*					
NO ₃ -	(mg/L)	11.366	0.0002	* * *					
PO_4^{-3}	(mg/L)	5.123	0.0691	NS					
Macroal	ga								
O ₂	(mg/L)	3.002	0.2343	NS					
Т	(°C)	2.260	0.3417	NS					
pН		1.158	0.5443	NS	-				
DBO	(mg/L)	9.102	0.0038	* *					
DQO	(mg/L)	6.677	0.0259	*					
NH4 +	(mg/L)	12.888	0.0000	* * *					
NO2-	(mg/L)	5.459	0.0278	*					
NO ₃ -	(mg/L)	9.774	0.0020	**					

TABLE I. KRUSKAL-WALLIS TEST FOR THE VARIABLES MEASURED IN THE INFLUENT DURING THE INITIAL (I) TRANSITION (T) AND PERIODIC (P) STAGE FOR THE BIODIGESTERS OPERATED WITH CHICKEN AND COW MANURE, AND WITH THE MACROALGA *M. pyrifera*

* = P < 0.05 Significative

** = P < 0.01 highly significative

*** = P<0.001 Very Highly significative

NS = Not significative

In the three reactors BOD increased while COD decreased (Figs. 4, 6 and 8) which, according to Stones (1981) may be taken as indicative of increased availability of degradable matter, possibly as a result of the action of organic refractary-degrading microorganisms (Oswald 1988).

New raw material was added at the time of maximum nitrates and minimum ammonia concentrations, and every fifth day thereafter, until the end of the experiment. The first addition marked the beginning of the transition stage (T), during which the nitrification variables had a "Gaussian" behaviour (Figs. 3, 5 and 7). COD had a similar trend, while BOD rose continuously (Fig. 4, 6 and 8).

This stage was followed by the periodic stage (P), during which most of the variables measured in the effluent did not change significantly (Figs. 3 to 8). The only exceptions were phosphates for the chicken biodigester, nitrites for the cow and pH for the macroalgae (Tabla II).

Temperature, oxygen and pH of the three effluents (Table II) remained at levels which allow a good nitrification process (Wild *et al.* 1971, Bishop and Farmer 1978), and showed no significant changes ($\alpha = 0.05$). On the three bioreactors oxygen level in the three stages was 5.8 mg/L.



Fig. 6. Biochemical (BOD: •) and chemical (COD: Δ) oxygen demand (in mg/L) and BOD/COD (•) ratio, for the influent (dotted line) and the effluent (continuous line) in the digester loaded with cow manure.

		DILO		1101101							
Influent											
		O_2	^{o}C	pН		DBO	DQO	NH_4	NO_2	NO_3	PO_4
Chicken manure	$\overline{\mathbf{X}}$	7.42	22.90	7.80		292.67	550.46	85.80	0.037	4.60	1.45
	SD	0.34	0.91	0.18		58.42	202.59	4.37	0.200	1.15	0.26
Cow manure	$\overline{\mathbf{X}}$	7.57	22.70	7.70		443.92	785.62	38.83	0.006	4.70	0.33
	SD	0.43	0.74	0.17		123.76	234.55	6.45	0.002	2.26	0.02
Macroalga	$\overline{\mathbf{X}}$	8.14	22.70	8.00		318.27	274.76	20.36	0.002	0.57	ND
	SD	0.36	0.84	0.17		3.78	10.36	1.86	0.0005	0.50	ND
Effluent											
		Flow	O_2	^{o}C	pН	DBO	DQO	NH_4	NO_2	NO_3	PO_4
Chicken manure	$\overline{\mathbf{X}}$	2.50	7.90	29.10	8.10	1618.50	733.39	12.70	0.340	43.20	5.27
	SD	0.14	0.18	0.51	0.06	568.03	485.51	1.95	0.200	5.11	2.03
Cow manure	$\overline{\mathbf{X}}$	0.52	7.69	27.40	8.00	1212.90	506.51	5.49	0.075	5.78	2.46
	SD	0.03	0.25	1.32	0.07	138.58	58.76	1.83	0.060	2.48	0.71
Macroalga	$\overline{\mathbf{X}}$	2.76	7.97	27.57	8.00	2662.30	865.27	12.25	0.0003	20.60	ND
	SD	0.11	0.50	1.03	0.09	230.04	92.74	1.17	0.001	4.22	ND

TABLE II. MEANS (\overline{X}) AND STANDARD DEVIATIONS (SD) OF THE VARIABLES MEASURED IN THE INFLUENT AND EFFLUENT DURING THE PERIODIC STAGE, FOR THE THREE PRODUCTS USED IN THE DIGESTERS. CHEMICAL VARIABLES IN mg/L. FLOW IN L/MIN IN THE CASE OF THE MACROALGA, PHOSPHATE CONCENTRATIONS WERE BELOW THE DETECTION LIMITS OF THE METHOD (ND)



Fig. 7. Nitrogen concentrations in the digester loaded with macroalgae. Ammonia: *; nitrites: full circles; nitrates: open circles. Dotted lines: influent; continuous lines: effluent.

TABLE III. SOLIDS RECOVERY PER DAY (WET WEIGHT, GRAMS) IN THE SRS, FOR THE THREE BIOREACTORS

Days	Chicken manure	Cow manure	Macroalga
0			
5			
10		45.10	
15		30.23	
20		48.11	39.80
25	60.80	29.14	27.31
30	46.26	19.27	23.00
35	43.00	18.36	15.90
40	38.00	15.27	17.39
45	29.00	17.33	13.00
50	26.10	20.15	12.69
55	29.60	26.19	15.03
60	25.40	23.36	12.80
65	23.18	25.27	17.26
70	26.37	26.31	15.29
75	27.15	29.30	17.11
80	25.23	21.40	10.23
85	24.60		15.37

Accrued solids were removed immediately before every addition of new material in each digester. Their quantities decreased during the transition stage (Table III) and did not change significantly thereafter (Table IV), which confirms the stabilization of the oxidation processes during the periodic stage.

High concentrations of nitrogen compounds were detected in the biodigester loaded with chicken manure (inflow and outflow), probably because chicken feed on high protein food and because they excrete uric acid and insoluble urates (Murray *et al.* 1975). Cow manure contains considerable fiber quantities, and a lower nitrogen concentration (Porras 1981). Due to its composition (North 1986), *M. pyrifera* has the lowest concentration of nitrogen.

The trends of the nitrification variables during operation of the three systems (Figs. 3, 5 and 7) indicate a fast growth rate of the nitrifying bacteria inoculated initially and a fast oxidation of ammonia to



Fig. 8. Biochemical (BOD: •) and chemical (COD: Δ) oxygen demand (in mg/L) and BOD/COD (•) ratio, for the influent (dotted line) and the effluent (continuous line) in the biodigester loaded with macroalga.

TABLE IV. MEANS $(x10^{23})$, ANI	D DIFFERENCES (DIF) OF	ATOMS OF INORGA	NIC NITROGEN IN THE	INFLUENT (I)
AND THE EFFLUENT (E),	, DURING THE PERIODI	C STAGE, FOR THE	THREE BIODIGESTION	SYSTEMS

	F Relation	<i>d.f.</i>	Decision
Chicken manure	0.4988	6	accepted
Cow manure	1.2380	7	accepted
Macroalga	2.2780	7	accepted

TABLE V. BETA TESTS WITH THE SOLIDS RECOVERED PER DAY (WET WEIGHT, GRAMS) IN THE SRS, FOR THE THREE DIGESTERS DURING THE PERIODIC STAGE

	<i>N-NH</i> ₄ ⁺			$N-NO_2^-$		N-NO3 ⁻			Total			
	Ι	Ε	DIF	Ι	Ε	DIF	Ι	E	DIF	I	E	DIF
Chicken manure	28.5900	4.2710	-24.3190	0.0049	0.0445	0.0396	0.5226	4.1992	3.6766	29.1220	8.5147	-20.6073
Cow manure	12.9440	1.8333	-11.1107	0.0049	0.0098	0.0089	0.4442	0.5611	0.1169	13.3890	2.4046	-10.9844
Macroalga	6.7987	3.9857	-2.8130	0.0003	0.0004	0.0001	0.0552	1.8864	1.8312	6.6960	5.8724	-0.8236

Decision level $\alpha < 0.05$, I = Influent, E = Effluent, DIF = Difference

TABLE VI. MEAN (\overline{X}) , STANDARD DEVIATION (SD) AND MEDIAN DIFFERENCE TEST, OF THE INORGANIC NITROGEN CONTENT OF THE INFLUENT AND THE EFFLUENT DURING THE PERIODIC STAGE, IN THE THREE BIODIGESTION SYSTEMS

	Influent (atoms-g $N/1$)				Decision		
	n	\overline{X}	SD	n	\overline{X}	SD	$(\alpha = 0.05)$
Chicken manure	8	4.8344	0.2330	8	1.4137	0.1350	T = 35.884 reject (P = 0.0000)
Cow manure	9	2.2230	0.3920	9	0.3992	0.0975	T = 13.543 reject (P = 0.0000)
Macroalga	7	1.1117	0.0888	9	0.9750	0.0397	T = 3.791 reject (P = 0.0068)

nitrates, reflected in the low nitrite concentrations (Table II). This process in basically similar to that of a biological "filtration" system (Wheaton 1977), with higher ammonia concentration in the influent and higher nitrites and nitrates in the effluent (Table V).

The total number of nitrogen atoms calculated for the periodic stage (Table VI) is significantly greater in the influents than in the effluents, with a net loss of 70, 82 and 11.9% of the total inorganic nitrogen in the biodigesters loaded with chicken and cow manure, and with the macroalga, respectively.

This negative nitrogen balance may be considered as due to high bacterial protein synthesis with consequent increase in their biomass, which is necessary for the oxidation of the organic matter. The fluid and the resulting solid residues of the process are serendipitous resources, which may be used for other purposes.

ACKNOWLEDGEMENTS

We thank Guillermo Díaz de Cossío Batani for his continuous help during this work and Domenico Voltolina for his valuable assistance in elaborating and translating it. We also thank Karla López and María Elena Corona who typed the manuscript.

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