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Chlorella vulgaris Growth on Pretreated Cane Sugar Mill Waste

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The growth of unicellular microorganisms is generally described by a first order kinetic model. First order kinetic equations can also be applied for mixed populations of microorganisms. If endogenous respiration and death coefficients are not taken into account, growth can be expressed by:

$$dX/dt = \mu X \quad (1)$$

where X is the microorganism concentration, μ is the microorganism specific growth rate and t is the time.

Monod (1950), proposed the Michaelis-Menten equation for describing the ratio between microorganism growth and limitant concentration of substrate.

For microalgae culture there are different parameters that could affect a satisfactory growth (light, nutrient and organic matter concentrations, temperature and pH).

Microalgal cultures offer interesting alternatives for wastewater treatment as secondary or tertiary biotreatments to remove inorganic nutrients, such as nitrogen and phosphorus, while producing potentially valuable biomass. Effluents from secondary domestic wastewater treatment plants and from anaerobically-digested animal wastes are the most common types of wastes used for the growth of microalgae. These effluents are well suited for the growth of algae because they contain high amounts of inorganic nutrients and relatively low amounts of organic compounds. In addition, recent publications indicate that microalgae and cyanobacteria can also be grown, given appropriate dilution, in other types of effluents, such as *Spirulina* on starch wastewater (Tanticharoen et al. 1993) and *Anacystis* on dairy wastewater (Thangaraj and Kulandaivelu 1994). The aim of the present work was to obtain the *Chlorella vulgaris* specific and maximum growth rates, growing on raw and pretreated cane sugar mill wastes.

MATERIALS AND METHODS

A strain of eucaryotic unicellular green algae *Chlorella vulgaris* SR/2, supplied by the Autotrophic Collection of the Botanical Garden of Cienfuegos (Cuban

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Academy of Science), was used. The cells were kept on flasks with Bold's Basal Medium (BBM) (Endo 1977).

The cane sugar mill waste used in the experiments was collected from an industrial factory located in Havana city. The samples were taken during the sugar cane harvesting seasons of 1989-90, 1990-91 and 1991-92. All the samples taken showed a statistical correlation agreement with regard to chemical oxygen demand (COD). The samples were settled at 29 °C for 2 hours and supernatant stored at - 40 °C before to be used. Afterwards the supernatant was treated by three different biological processes: activated sludge (AS), anaerobic filter (AF) and facultative lagoon (FL).

The analytical determinations were chemical oxygen demand (COD), chlorophyll and volatile suspended solids (VSS) for the determination of growth rates (American Public Health Association 1985). Algae/bacteria ratio was calculated knowing that 2% of the cellular dry weight of *Chlorella* is chlorophyll a, this algae concentration is directly proportional to concentration of chlorophyll a (Ca):

$$[algae] = Ca/0.02$$

The bacterial concentration was calculated assuming that:

$$[bacteria] = VSS - [algae]$$

The algae/bacteria ratio was over 0.01 during all the experiments.

Specific maximum growth rates (μ_{max}) and saturation coefficients (K_s were calculated using the Lineweaver-Burk method (Aiba and Humphrey 1965).

Batch experiments were carried out in order to obtain the biokinetic parameters for *Chlorella vulgaris* growth. These experiments were performed in cylinders of 2-litre effective volume.

The reactors used for the continuous cultivation consisted of plastic pools (ponds) of 40 cm lenght, 30 cm width and 30 cm depth, each one with an overflow weir. The plastic pools were inoculated with 3 litres of microalgae solution. After the continuous pumping began, the system was allowed to reach the steady-state, which was considered after 7 days. The feed was pumped to the pond by means of a peristaltic pump. The temperature and pH ranged between 30 and 35° C and 6.8 and 10, respectively. Hydraulic retention times (HRT) of 4, 6, 8 and 10 days were assayed.

For the activated sludge (AS) treatment a completely mixed reactor was used. It consisted of a 100 mm internal diameter Pyrex cylinder of 2.5 litre capacity. The air used was saturared. The pH was maintained between 7.0-7.5. The HRT of the AS system was 24 hours and the average food/microorganisms (F/M) ratio was 0.2 d^{-1}. The average sludge age was 5 days.

The effluent of the facultative lagoon (FL) was taken directly from a secondary lagoon of an industrial sugar mill.

The anaerobic filter (AF) consisted of a cylinder of 9.5 cm internal diameter with 6-litre working volume. The cylinder was filled with 4 cm diameter stones. It has an average porosity of 0.51. The HRT used in the anaerobic filter ranged from 1 to 5 days.

RESULTS AND DISCUSSION

Figure 1 shows the preliminary experiments performed with raw cane sugar mill waste. There was a sensible decrease of chlorophyll (Ct) concentration and algae/bateria ratio when the operation time increased. These two facts indicate that raw cane sugar mill waste is not a satisfactory culture medium for *Chlorella vulgaris* (Benítez 1979).

Tables 1, 2 and 3 show the total chlorophyll, volatile suspended solids and COD concentrations for batch experiments carried out during a 119 hours operation time using effluents from AS, AF, and FL, respectively. These values were plotted against experimental time to find out the logarithmic phase. The different growth rates (μ) were obtained by applying the Monod (1950) model for each experimental run with different initial substrate concentration. This model was selected according to the literature recommendations (Benneman et al. 1986). Later, μ_{max} and K_s were determined.

Table 1. Activated sludge (AS) effluent batch cultures

Infl. COD (g/l)	1.55		2.30		3.36		4.02	
T (hours)	Ct	VSS	Ct	VSS	Ct	VSS	Ct	VSS
0	2.1	0.35	1.7	0.20	1.0	0.17	1.5	0.18
17	4.9	0.61	3.7	0.43	2.8	0.38	2.9	0.40
34	5.9	0.84	3.8	0.67	2.9	0.36	3.0	0.45
51	8.4	0.92	5.1	0.68	4.2	0.42	4.5	0.47
68	9.5	1.25	6.4	0.97	5.1	0.64	5.9	0.81
85	10.9	1.29	8.8	0.97	7.0	0.67	8.5	0.83
102	11.3	1.30	8.9	0.97	7.0	0.66	8.8	0.83
119	11.5	1.30	8.9	0.97	7.0	0.66	8.8	0.83
Effl. COD (g/l)	0.78		1.09		1.57		2.01	
μ (d^{-1})	0.27		0.38		0.41		0.48	
μ_{max} (d^{-1}) = 0.47 (±0.03)					K_s (g/l) = 0.80 (±0.04)			

Ct = total chlorophyll (mg/l). VSS = volatile suspended solids (g/l).
All the experimental values are averages of five determinations and showed deviations of less than 3 % from their corresponding means, which are reported.

The highest value of μ_{max}, 0.47 (± 0.03 standard deviation throughout the paper) day^{-1}, was obtained when the effluent from AS system was used as culture medium of *Chlorella* (Table 1). The saturation constant K_s was 0.80 (± 0.04) g/l. These values are normal for this species of microalgae (Borowitzka and Borowitzka 1988).

The lowest value for μ_{max}, 0.35 (± 0.02) d^{-1} and its corresponding K_s value, 0.15 (± 0.01) g/l, were found when the effluent from the AF was used.

The results of cultivation in the effluent from a FL showed values of μ_{max}, 0.40 (± 0.01) d^{-1} and K_s, 1.40 (± 0.07) g/l.

K_s values obtained were within the values reported in the literature for other substrates. For the effluent from a facultative lagoon, high concentrations of substrate (more than 1.4 g COD/l) were necessary to reach the half value of maximum growth rate. This fact represents a problem when this type of effluent is used as a culture medium to approach μ_{max}.

Table 2. Anaerobic filter (AF) effluent batch cultures

Infl. COD (g/l)	1.60		1.75		1.90		2.10	
T (hours)	Ct	VSS	Ct	VSS	Ct	VSS	Ct	VSS
0	1.3	0.18	1.1	0.20	1.1	0.19	1.4	0.25
17	3.5	0.22	3.9	0.26	3.5	0.26	3.9	0.30
34	5.1	0.36	4.1	0.40	5.7	0.46	6.5	0.41
51	6.5	0.45	7.0	0.50	6.5	0.50	8.7	0.52
68	8.9	0.53	8.9	0.60	7.9	0.63	9.1	0.65
85	9.2	0.54	9.2	0.66	8.2	0.64	10.9	0.66
102	11.0	0.56	10.0	0.67	9.1	0.66	11.9	0.69
119	11.3	0.61	10.3	0.68	9.3	0.68	12.1	0.70
Effl. COD (g/l)	0.78		0.90		0.97		1.02	
μ (d^{-1})	0.32		0.31		0.33		0.33	

μ_{max} (d^{-1}) = 0.35 (±0.02) K_s (g/l) = 0.15 (±0.01)

Ct = total chlorophyll (mg/l).   VSS = volatile suspended solids (g/l).
All the experimental values are averages of five determinations and showed deviations of less than 4% from their corresponding means, which are reported.

Figure 2 shows the results obtained for the continuous culture experiments. In all cases the effluent of FL would be worst because the growth process was very slow. Even so it could be used for high concentrated wastes as culture

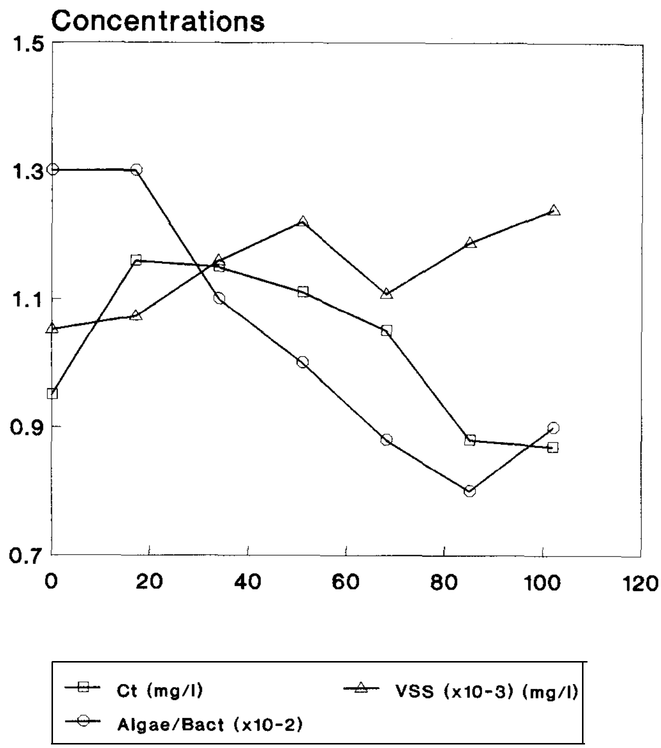

Figure l. Sugar cane raw waste batch cultures

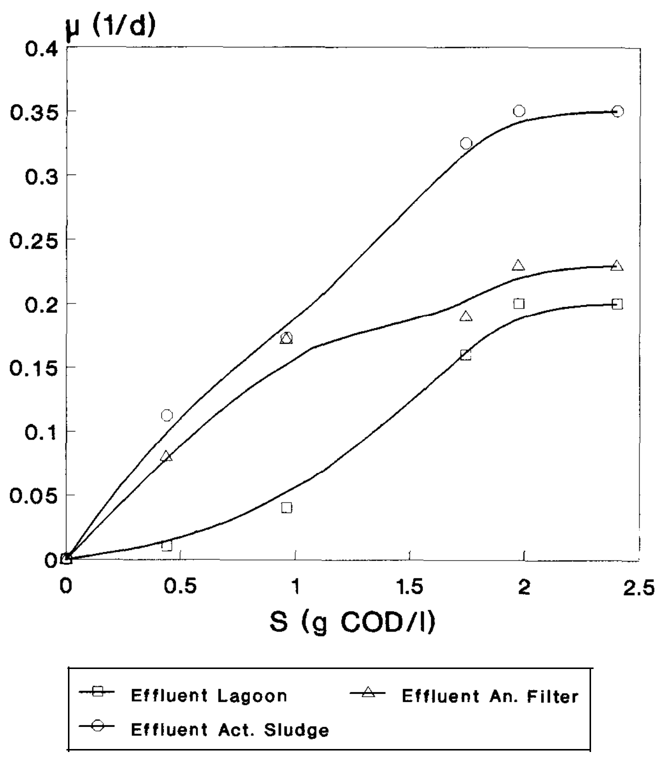

Figure 2. Continuous cultures growth rate variations.

medium for microalgae. The comparison of the use of AS and AF effluents indicates that for low concentrations of substrate it is slightly better to use the latter as culture medium of *Chlorella vulgaris,* and for high concentrations (over 1000 mg COD/1), the former. This analysis was done taking into account only the biokinetic parameters (without an specific regard to economical and operational conditions).

Table 3. Facultative lagoon (FL) effluent batch cultures

Infl. COD (g/l)	2.80		3.00		3.20		3.40	
T (hours)	Ct	VSS	Ct	VSS	Ct	VSS	Ct	VSS
0	1.2	0.22	1.3	0.22	1.0	0.22	1.1	0.26
17	2.3	0.39	2.9	0.40	3.0	0.39	2.3	0.39
34	3.1	0.40	4.6	0.45	4.0	0.42	4.4	0.42
51	4.4	0.46	6.0	0.48	5.2	0.47	6.6	0.52
68	5.2	0.53	6.7	0.52	6.5	0.60	7.4	0.59
85	7.8	0.54	8.0	0.59	8.0	0.62	8.0	0.63
102	8.3	0.56	9.0	0.62	9.0	0.64	8.9	0.65
119	8.5	0.61	10.5	0.62	10.2	0.65	9.1	0.66
Effl. COD (g/l)	1.39		1.50		1.59		1.68	
μ (d^{-1})	0.26		0.27		0.28		0.27	
μ_{max} (d^{-1}) = 0.40 ($\pm$0.01)					**K_s (g/l) = 1.40 ($\pm$0.07)**			

Ct = Total chlorophyll (mg/l). VSS = Volatile suspended solids (g/l).
All the experimental values are averages of five determinations and showed deviations of less than 3 % from their corresponding means, which are reported.

It is interesting to note that the shape of the curve μ vs S for the effluent of facultative lagoon agree with the model proposed by Just (1972) for microorganisms growing on the same substrate via a two step process. In this case the growth rate becomes:

$$\mu = \mu_{max} S^2 / K_1 + S)(K_2 + S) \qquad (2)$$

where K_1 and K_2 are the saturation constants for the first and second limiting substrates respectively.

The results presented indicate that the batch biokinetic parameters found for the different effluents used as culture media of microalgae *Chlorella vulgaris* were similar to those reported in the literature when domestic sewage and synthetic media were used as substrates (Just 1972; Oh-Hamma and Miyachi 1988; Shelef and Soeder 1980). The values obtained in continuous cultivations were somewhat lower but they remain within the range of the above-mentioned previous reports using domestic sewage and synthetic media.

The effluent from AS treatment appears to have the best characteristics for *Chlorella* growth, but also satisfactory results were obtained using the effluent of the AF, which is a less expensive process than the AS system. The effluent from the FL is of relatively low quality for *Chlorella* culture, but it has an easy and quick applicability because of the lagoon treatment systems already existing in cuban sugar cane mills.

The cultivation of algae in secondary effluents of cane sugar mill waste treatment combines the environmental protection effect of a waste final treatment with the economical effect of the use of a cheap utilizable biomass for livestock farming.

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