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Biokinectic Parameter Investigation and Biological Treatment of Coffee Berry Effluents

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The coffee berry washing process is one of the most important sources of pollution in rural area, where pulp, husks and wastewater are produced. As a result, wastewater from this operation can have very high BOD sometimes exceeding 24,000 mg.L^{-1}. Wet and dry process are currently used in which the coffee berry is subjected to mechanical and biological operation in order to separate the been or seed from the exocarp, mesocarp, and endocarp (Gathuo et al. 1991). Much of this pulp is dumped in soils or water courses where its polyphenolic and recalcitrant substances cause serious damage to the terrestrial and aquatic ecosystems (Clifford and Martinez 1991). Wet processing of coffee is more sophisticated and leads to better quality coffee. This operation requires at least 80,000 L of water to produce one ton of clean coffee (Figure 1). Color pass through is a common problem found in some wastewater treatment process (Alwar et al.1990), and high molecular weight polyphenolic compounds usually remain as serious pollutants.

Activated sludge is a process for biodegrading organic contaminants in wastewater using a mixed population of microorganims at relatively high concentration in an aerobic environment (Ramalho 1983). For an easily biodegradable agro-industrial wastewater, COD removal better than 80 percent is achievable. For industrial wastewater, longer residence times are usually required because of organic contaminants refractory to biodegradation (Capps et al. 1995). Studies of biokinetic parameters of aerobical biological treatment yields the rate at which microorganisms degrade a specific waste, and therefore provides the basic information required for sizing biological aerobic reactors. Biokinetic parameters may be investigated using at least four batch scale reactors operating in parallel (Metcalf & Eddy 1991).

Natural polyelectrolytes, extracted from cactus natural of South America such as *Cereus peruvianus*, have been extensively used as auxiliary of flocculation and coagulation for pollutants removal before or after biological treatment of effluents (Nozaki et al. 1993). This paper is based on biokinetic parameters investigation and organic matter removal from wastewater of coffee berry washing process by biological treatment.

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MATERIALS AND METHODS

Wastewater of *coffee* berry *(C.arabica)* washing process was collected directly from COCAMAR (Cooperative of Agriculturist and Coffee Producer of Maringa) located in Fazenda Romaria, Nova Londrina District, Northwest of Parana State, Brazil. After collecting, the samples were kept at 4°C. Coffee berries were also collected (60 kg) and kept in the refrigerator until use. July, August, and September are the periods of coffee harvesting, and this is the end of winter season in the Northwest of Parana State with an average temperature of 21 ± 5°C. As shown in Table 2, polyphenolic compounds (tannin) and reducing sugars are the main components of dry coffee pulp (Clarke and Macrae 1989).

The inoculum was collected from SANEPAR (Sanitary Lagoon of Maringa, Parana State, Brazil) and was gradually acclimated to the wastewater. A bench scale biological reactor (Oliveira et a1.2000) was used during the acclimation period of 20 days with 5000 $mg.day^{-1}$ of commercial sucrose, 250 $mg.day^{-1}$ of nitrogen as NH_4Cl, 50 $mg.day^{-1}$ of phosphorus as K_2HPO_4. After the acclimation period, the artificial food was gradually substituted by influent of coffee berry washing process. Four parallel reactors of volumes of 1 liter, connected to four feeding flasks and also to four secondary clarifiers were used for biokinetic parameters investigation (Oliveira et al. 2000). Table 3 show the flow rate (Q_0), pH, temperature, SVI, S_o $X_{v,a,}$ q, etc., employed for biokinetic parameters determinations and used in sizing the aerobic biological reactor.

After biological treatment, the final effluent was investigated for suspended solids and fecal bacteria. The turbidity measurement was performed with a turbidimeter Micronal-B-250. The flocculation and coagulation studies were performed with aluminum sulfate ($50mg.L^{-1}$) and natural polyelectrolytes extracted from cactus *Cereus peruvianus* ($2.5mg.L^{-1}$). Six pyrex 2 liters beakers, a Jar-Test (Milan JT 101-1200 rpm), and the following conditions were used for flocculation and coagulation: 1 L of affluent volume, stirring time of 5 minutes, 100 rpm, and pH 7.4. After standing for 15 minutes, filtrations were performed using qualitative filter paper. The filtrate was then submitted to turbidity measurements and fecal coliform investigation.

Safe drinking water should not contain more than four fecal coliforms per 100 milliliters (Metcalf & Eddy 1991). Fecal coliforms were investigated by assaying water (final effluent) for the presence of *Escherichiu coli.* Effluent sample (100mL) was passed through membrane, which was aseptically transferred to a differential nutrient medium and incubated (24h). The following nutrient medium composition was used:meat extract ($1g.L^{-1}$), yeast extract ($2g.L^{-1}$), peptone($5g.L^{-1}$), sodium chloride ($5g.L^{-1}$), and final pH 7.4 at 37°C. After 24 hours, the number of coliform collonies were counted (Mckane and Kandel 1986).

Knowledge of MLVSS production and oxygen utilization were needed for design of aerobic biological reactors. Several biokinetic parameters related to oxygen utilization and biomass (VSS) production were investigated. Firstly, the sludge and

sample solution of each reactor were transferred to four BOD flasks of 250 mL. Using an air compressor the flasks were saturated with oxygen for 5 minutes. Then, the oxygen consumption were measured using the dissolved oxygen meter (Jenway-USA) in time intervals of 30 seconds. A plot of oxygen consumption ($mg.L^{-1}$) versus time (minutes) yields a straight line where the slope was the oxygen uptake rate (OUR).

Table 1. Definitions of symbols used

COD = chemical oxygen demand
VSS = volatile suspended solids in reactor = biomass produced
Affluent = effluent collected directly from coffee berry washing machine
Effluent = wastewater after biological treatment
MLVSS = mixed liquor volatile suspended solids = VSS in the reactor under strong aeration
S_o = soluble COD of combined feed (fresh feed + recycled sludge)
S_e = soluble COD of biological effluent = effluent COD
Q_o = flow rate of combined feed (fresh feed + recycled sludge)
$X_{v,a}$ = (subscripts: the first (v) = volume and the second (a) = aerator) = VSS inside the reactor
F/M = food to microorganism ratio (day^{-1}) = $(Q_O S_o)/(X_{v,a}.V)$
K = substrate removal rate = $L.mg.day^{-1}$; Y = mg of MLVSS produced per mg of COD removed = biomass production
K_d = endogenous respiration = mg of MLVSS oxydized per mg of MLVSS in reactor (day^{-1})
a = mg of oxygen utilized per mg of MLVSS in reactor
b = mg of oxygen utilized per day per mg of MLVSS in reactor for endogenous respiration. (day^{-1})
t_h = hydraulic residence time in reactor
q = specific substrate removal rate = $(S_o - S_e)/(X_{v,a}.t_h)$
OUR = oxygen uptake rate = mg of oxygen used = mg $O_2.L^{-1}.day^{-1}$
RO_2 = $OUR/X_{v,a}$ = aq + b = specific oxygen utilization rate
μ = specific growth rate = (mg MLVSS produced)/ (mg MLVSS) (day)
ΔX_v = net yield of MLVSS = the difference between MLVSS produced and MLVSS lost by endogenous respiration = $Y(So - Se)Qo - k_d.X_{v,a}.V$
SVI = sludge volume index = volume in milliliters occupied by 1 g of mixed liquor suspended solids (MLSS), dry weight, after settling for 30 minutes in a 1000 mL graduated cylinder.

RESULTS AND DISCUSSION

Figure 1 shows the schematic diagram of coffee berry wet processing and the production of wastewater used in this investigation. As shown in Table 2, the most important recalcitrant organic compounds for biological degradation is tannin, that is about 8% of total organics, while the remaining organic compounds are easy for biological degradation. The effluent has a very high concentration of phosphate

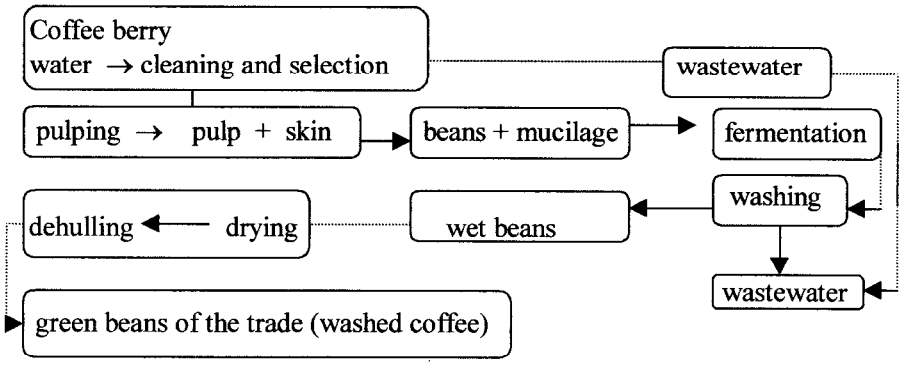

Figure 1. Schematic diagram of coffee-berries washing process and wastewater produced.

and this is a good indication in using activated sludge methods in wastewater treatment.
A plot of oxygen consumption ($mg.L^{-1}$) versus time (minutes) yields the values of oxygen uptake rate (OUR), and RO_2 were calculated as $RO_2 = (OUR)/(X_{v,a})$ as shown in Table 3.
Reactor 1: OUR = 1.0 $mg.L^{-1}.min^{-1}$, (y = 10.1242 - 0.9989x , r = 0.9987)
Reactor 2: OUR = 0.63 $mg.L^{-1}min^{-1}$, (y = 9.7835 -0.6275x), r = 0.9981)
Reactor 3: OUR = 0.45 $mg.L^{-1}.min^{-1}$, (y = 7.8195 - 0.4492x), r = 0.9998)
Reactor 4: OUR = 0.25 $mg.L^{-1}.min^{-1}$, (y = 7.9321 - 0.2553x), r = 0.9991)

The values of RO_2 were used for graphical determinations of K (substrate removal rate) as shown in Figure 2. The flow rate Q_0 were changed horn 0.08 to 0.16 $L.h^{-1}$, a variation of 50% from the lower to the higher flow rate. The influent S_0 were changed from 280 to 1215 $mg.L^{-1}$. As shown in Table 3, S_0, Q_0, pH, t_h,Xv,a, and reactors volumes were selected previously. SVI, Se, OUR, RO_2, q , μ, ΔXv, F/M, etc., were the parameters measured or calculated. From the data gathered in Table 3, graphical determinations of **a, b, Y, K,** and $\mathbf{K_d}$ were performed (Figures 2,3,4).

Biokinetic parameters are very important for the design of upper and lower operating limits capable of responding to variations in flow and pollutant concentrations, as pointed out previously (Ramalho 1983; Capps et al. 1995). The biodegradability criterion for wastewater is the ratio of COD to BOD. For domestic wastewater the ratio is about 1.6 and for industrial wastewater the ratio is usually larger than 2.5 (Capps et al. 1995).This paper reports the results always as COD removal instead of BOD.

When a substrate (SO) enters as affluent to a continuous biological reactor, the substrate follows two distinct ways: The first, called energy metabolism (main parameter = **a),** is the substrate oxidation to provide energy of maintenance, and the ends products are: CO_2, H_2O, N_2, P, etc. Graphical determination of **a** (slope) and **b** (intercept) of specific oxygen utilization rate (RO_2) versus specific substrate

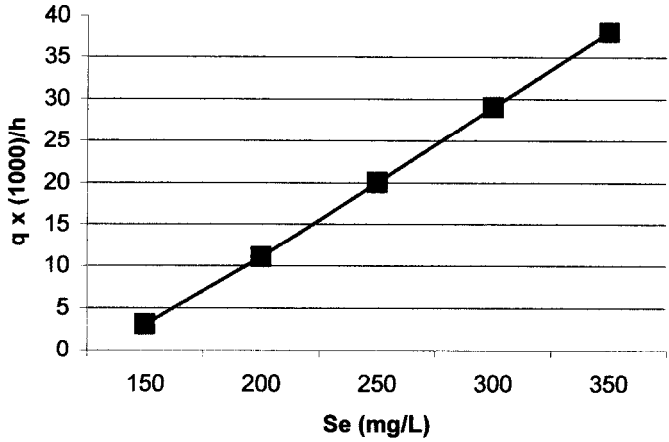

Figure 2. Graphical determination of biokinetic parameter k [q x 10^{3}(h^{-1}) versus Se ($mg.L^{-1}$); K = substrate removal rate = 4.4 x 10^{-3} $mg.day^{-1}.L^{-1}$; (r = 0.9939); (linerar regression = - 24.9544 + 0.1844x).

Table 2. Organic compounds composition (%) of pulp coffee (dry weight)*

Compounds	(%)
Tannin	1.80 – 8.50
Total pectins	6.50
Reducing sugars	1.40
Nonreducing sugars	2.0
Caffeine	1.30
Chlorogenic acid	2.60
Total caffeic acid	1.60
Coffee berry effluents characterization	
Average pH	5.50 (1)
COD ($mg.L^{-1}$)	6097 ± 223(2)
Dissolved oxygen ($mg.L^{-1}$)	5.80 ± 0.40(3)
NO_2^- ($mg.L^{-1}$)	0.455 ± 0.085(4)
PO_4^{3-} ($mg.L^{-1}$)	20.20 ± 1.55(5)

* from (Clarke and Macrae 1989)

(1) Average pH. (2) Average of five determinations for each sample ($p < 0.05$). (Standard Methods 1989). (3) Dissolved oxygen (O_2) apparatus (Jenway-USA). (4) α-naphthylamine (Sigma-USA) and sulfanilic acid (Sigma-USA) method, using a GBC-918-UV-VIS spectrophotometer with 10 mm cell (Standard Methods 1989). (5) Ammonium molybdate method (Standard Methods 1989)

Table 3. Experimental results for biokinetic parameters

Reactor number	1	2	3	4
S_0 (mg.L^{-1}) (affluent)(*)	1215 ± 45	1028 ± 40	654 ±30	280 ± 25
Se (mg.L^{-1}) (effluent)(*)	343 ± 24	274 ± 20	196 ± 16	159 ± 15
MLVSS = $X_{v,a}$ (mg.L^{1})	3749	3527	3390	3093
Q_0 (L.h^{-1})	0.16	0.13	0.09	0.08
t_h (h) = V.Q_0^{-1}	6.25	7.70	11.1	12.5
$X_{v,a}.t_h$ (mg.h.L^{-1})	23431	27158	37629	38663
$(S_0 - Se)/(X_{v,a}.t_h) = q$ (h^{-1})	0.0372	0.0278	0.0122	0.0031
ΔX_v (mg.day^{-1})	2616	1872	552	50
OUR (mg.L^{-1}.h^{-1})	60	37.8	27	15
RO_2 (h^{-1}) = (OUR)/($X_{v,a}$)	0.0160	0.0107	0.0080	0.0048
F/M (day^{-1}) = ($Q_0.S_0$)/($X_{v,a}.V$)	1.24	0.909	0.417	0.174
SVI	114	108	112	124
μ (h^{-1}) = ΔX_v (mg.h^{-1})/MLVSS	0.0291	0.0221	0.0068	0.0007

(*) Average of five determinations. Average temperature observed = 19 ±1^{0}C. The initial pH were adjusted to 7.3 ± 0.5 (reactors 1, 2, 3, and 4).

Table 4. Final treatment using aluminum sulfate and natural polyelectrolytes

Reactor number	1	2	3	4
Se (mg.L^{-1}) (BR)	343	274	196	159
Se (mg.L^{-1}) (final)	32	24	18	16
Turbidity (IUT) (final)	3.2	3.0	3.0	2.9
Fecal coliforms (NMP/100 mL) (BR)	LD	LD	LD	LD
Total coliforms (NMP/100 mL) (BR)	> 16	> 16	> 16	> 16
Total coliforms (NMP/100 mL) (final)	LD	LD	LD	LD
NO_3^- (mg.L^{-1}) (BR)	LD	LD	LD	LD
PO_4^{3-} (mg.L^{-1}) (final)	LD	LD	LD	LD

BR = effluent of biological reactor. Final = after final treatment of flocculation and coagulation. LD = lower than detection limit of the method employed (Standard Methods 1989). IUT = international unity of turbidity. Use of aluminum sulfate (50 mg.L^{-1}) and natural polyelectrolytes (2.5 mg.L^{-1}) with a Jar-test for flocculation and coagulation studies.

removal rate (q) appears in Figure 3. The second way and the most important, is the cell metabolism synthesis phase (parameter Y) with production of new cells (VSS). When substrate becomes exhausted, the end products of the biomass produced (VSS) are nonbiodegradable products, known as endogenous respiration phase (parameters **b** and $\mathbf{K_d}$). Graphical determinations of Y (slope) and $\mathbf{K_d}$ (intercept) are shown in Figure 4 as plot of specific growth rate (μ) versus specific substrate removal rate (q).

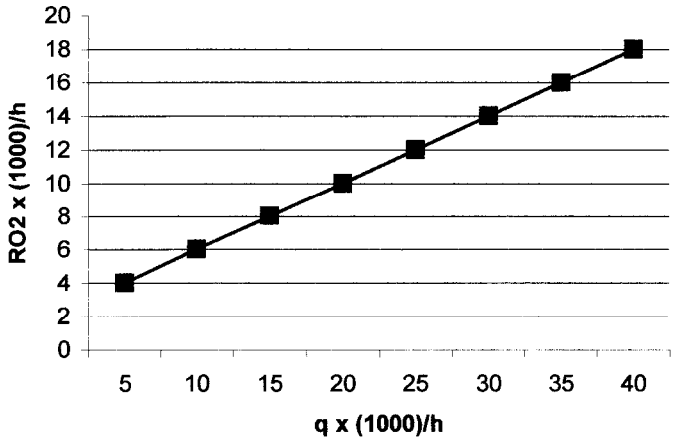

Figure 3. Graphical determination of parameters **a** and **b.** [specific oxygen utilization rate = $RO_2 \times 10^3 (h^{-1})$ versus specific substrate removal rate = q x 10^3 (h^{-1})]; **a** = slope = 0.38 mg O_2/mgCOD; **b** = intercept = 0.0912 d^{-1}; (r = 0.9742); (linear regression = 3.8058 + 0.3818x).

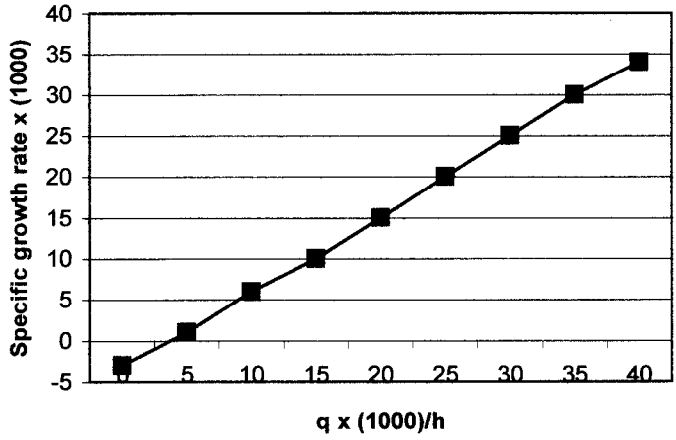

Figure 4. Graphical determination of Y and $\mathbf{k_d}$. [specific growth rate = μ x 10^3 (h^{-1}) versus specific substrate removal rate = q x $10^3 (h^{-1})$]; Y = slope = 0.86 mg MLVSS/mg COD; $\mathbf{k_d}$ = intercept = 0.063 d^{-1}; (r = 0.9974); (linear regression = -2.6576 + 0.8597x).

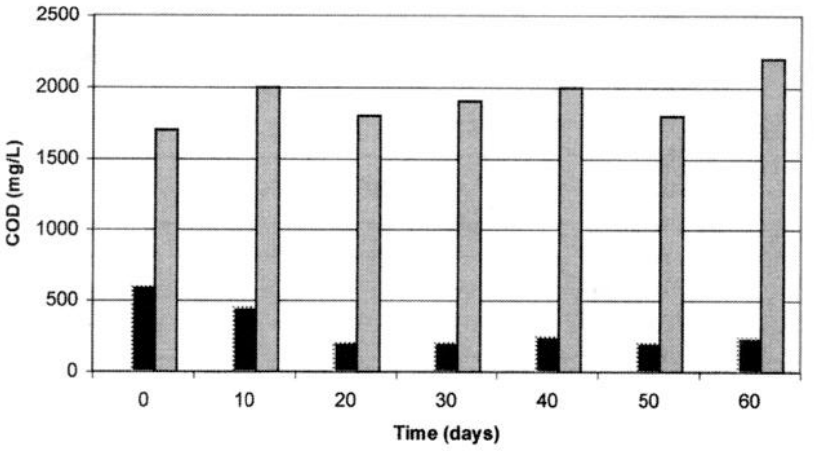

Figure 5. Biological COD removal efficiency as function of time. [COD affluent and removed ($mg.L^{-1}$) versus time of biological treatment (days)]; gray bar = affluent COD an average of (2000 $mg.L^{-1}$) ; black bar = effluent COD; flow rate (Qo) = 0.64 $L.h^{-1}$; t_h = hydraulic residence time = 1.3 day.

Table 5. Biokinetic parameters comparison with other effluents

Effluents	K ($L.mg^{-1}.day^{-1}$)	Y (mg)	K_d (day^{-1})	a (mg)	b (mg)
This work(*)	0.0044	0.86	0.063	0.38	0.091
Cassava meal industry(1)	0.0034	0.64	0.039	0.43	0.120
Dairy breed(2)	0.0029	0.84	0.160	0.18	0.020
Domestid sewage(3)	0.0170	0.73	0.075	0.52	0.160

(*) Sized biological reactor: volume = 20 liters; Q_0 = 0.64 $L.h^{-1}$. Residence time (t_h) 1.3 day = 31h. Affluent COD = 2000 $mg.L^{-1}$. Starting pH = 7.4 ± 0.5. Room temperature = 21 ± 3^0C. Introduction rate of oxygen inside the reactor = 6.0 $mg.L^{-1}$
(1,2) Oliveira et al. 2000. (3) Metcalf & Eddy 1991.

Preliminary studies demonstrated that activated sludge works efficiently with COD in the range of 500 to 2000 $mg.L^{-1}$, and the original wastewater was diluted with tap water to an average COD concentration of 2000 $mg.L^{-1}$ before using in biological reactor. The sized biological reactor was a PVC reactor of 20 liters, with strong aeration using an air compressor. The sludge was recycled back continuously using a four channel peristaltic pump. After the biological treatment, the efficiency of COD removal was 87-88% (Figure 5) with hydraulic residence time of 1.3 day. The small sludge volume index (SVI) also indicated a good settleability for the biological process (Table 3). The final treatment with aluminum sulfate and natural polyelectrolytes reduced the COD concentration to

around 32 $mg.L^{-1}$, with the overal COD removal efficiency of 97% and a complete bacteria removal by a mechanisms of adhesion, flocculation, and coagulation (Table 4).

Comparing the biokinetic parameters found for effluents of coffee berry washing process with effluents of domestic sewage, dairy breed, and cassava meal industry, as shown in Table 5, the substrate removal rate (K) was lower than the domestic sewage, indicating higher biodegradation for domestic sewage. The higher VSS consumption for endogenous respiration (K_d) was observed for dairy breed effluents. The biomass (VSS) production (Y) was slightly higher than the other effluents.

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