Performance of a Laboratory-scale Microalgae Pond for Secondary Treatment of Distillery Wastewaters

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Distillery waste disposal is one of the major problems being faced by all nations across the globe. To diminish its organic loading, the distillery waste is treated before its final disposal by biological processes. Microalgae pond gives a way to solve this situation. An evaluation of the performance of a laboratory-scale microalgae pond treating distillery waste previously treated in an anaerobic filter was carried out. The microalgae pond operated with an effluent recycling (*R*) of 10:1 with respect to the influent and at an hydraulic retention time (HRT) of 11 days based on the mixture (influent + recycling). The surface organic loading rates (SOLR*)* used were G = 418 kg COD ha^{-1} d^{-1} and G = 92 kg BOD_5 ha^{-1} d^{-1} according to the literature recommendations for microalgae ponds. Total chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS), total nitrogen, ammonia, total chlorophyll ($\gamma_{C,T}$) and chlorophyll *a* (γ_{ca}) concentrations were monitored. COD and BOD_5 removals of 83.2 % and 88.0 %, respectively were obtained. Removals of 60.6 %, 53.4 % and 78.8 % in the TS, TSS and VSS concentrations were achieved. The possibility to grow microalgae for biomass in this waste was also evaluated using the determinations of chlorophyll *a* (γ_{ca}) and VSS.

Key words:
Distillery waste, microalgae pond, secondary treatment, effluent recycling

Introduction

The disposal of distillery waste presents a serious challenge to the natural ecosystem and can cause considerable environmental problems. The manufacturing process of alcohol from sugar involves dilution of molasses with water followed by fermentation with cultured and developed yeast. The fermented solution contains 6–8 % alcohol and is distilled with low-pressure steam to obtain rectified spirit or neutral alcohol as the final product. The residue of the distillation process is a strong organic effluent. The production of distillery waste in a traditional alcohol factory is in the range of 9–14 litres per litre of ethanol obtained.[1] This waste is strongly acidic (pH 4–5) and has a high-organic content. Distillery waste holds the remaining soluble matter after the fermentation-distillation process of sugar cane molasses, as well as non-volatile fermentation by-products, being one of the most recalcitrant wastes.[1] Some researchers[2,3,4,5] have reviewed several methods for the treatment, utilization and disposal of distillery waste.

Some of the existing methods for the disposal of distillery waste are direct land application[5] and anaerobic digestion.[2,6–8] However, if distillery waste is discharged directly on land, the alkalinity of the soil is reduced so that crops may be destroyed,[9] a manganese deficiency in the soil occurs and seed germination can be inhibited.[10] Combined technology using anaerobic treatment followed by aerobic intensive treatment, tertiary treatment by stabilization pond and final disposal on land may be a good alternative for treatment and disposal in developing countries.[6–8] Secondary treatment by stabilization ponds or lagoons offers a simple and economical alternative of treating distillery waste in rural areas with the aim of using the final effluent as soil conditioner. Stabilization ponds have been widely studied by many authors and successfully applied.[10–15] They are one of the most effective and widely used methods for wastewater purification in developing countries, especially in hot climates because of the high values of natural radiation and temperatures

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usually achieved in these tropical countries. This is due to their efficiency in destroying pathogenic bacteria and other parasites, and to the low cost of construction, operation and maintenance. The natural processes of stabilizing organic waste by bacterial oxidation and oxygen production by algae are fundamental in the treatment of sewage and industrial wastewaters.[16–21] The oxygen required for aerobic bacteria respiration for the assimilation of organic materials is known as algae photosynthetic oxygen and implies no additional aeration.[16–19] In several recent studies, some investigators have also suggested that algae can remove colour from coloured wastes. In some cases, a colour reduction of 50–80 % was achieved by a mixed culture of microalgae.[22–25] Many algae cannot only grow photosynthetically, but also, by using organic substrates for biosynthesis.[25–28]

The wastewater treatment by microalgae cultures has another major advantage. It generates no additional pollution when the biomass is harvested and it allows efficient recycling of nutrients.[26,29] Nameche and Vasel[30] studied the hydraulics of stabilization ponds and concluded that almost all ponds with length/width ratios of below 4 or even below 8 corresponded reasonably well to completely mixed reactors, and first-order kinetics may be assumed with a maximum margin of error in estimating the performance of only 10 %.

Based on the literature reviewed, the subject of the current work was to evaluate the performance of a laboratory-scale pond used for the secondary treatment of distillery waste, which was previously treated in an anaerobic fixed bed reactor.

Materials and methods

Laboratory-scale pond used

Fig. 1 shows a flow diagram of the experimental set-up. This consisted of a mixing acrylic vessel of V = 1 L effective volume, while the laboratory-scale pond consisted of an acrylic vessel L = 40 cm in length, T = 25 cm in width and H = 30 cm in effective depth. The surface area and effective volume of the pond were A = 1000 cm^2 and 30 L, respectively. The pond was provided with two connections of d_i = 5 mm internal diameter for influent inlet and effluent outlet. The pond was placed and operated on the roof of the laboratory at bleakness conditions.

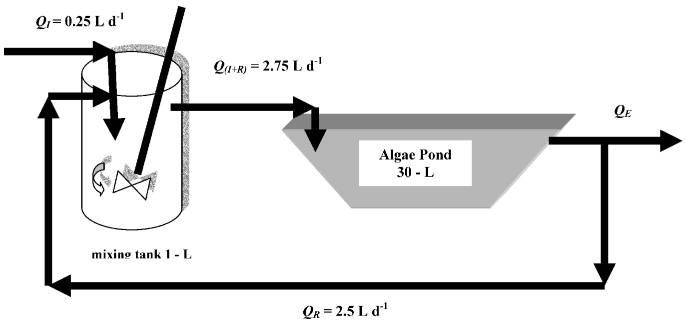


Fig. 1 – *Schematic diagram of the experimental set-up*

Inoculum

The pond was inoculated with *Chlorella vulgaris* SR/2 strain obtained from the Autotrophic Collection of the National Botanic Garden of Cienfuegos, Cuba. This strain is highly adaptable to load changes and is resistant to coloured growth media. The inoculum of the pond consisted of a mixture of a culture medium and microalgae with a concentration of microalgae of $C = 5 \cdot 10^6$ cells in 100 mL and a total suspended solids (TSS) concentration of γ = 10 g L^{-1}. The composition of the culture medium is shown in Table 1. A volume of V = 3 L of this inoculum was used for the pond, which represents 10 % of the pond total volume.

Table 1 – *Composition of the culture medium used for Chlorella vulgaris growth*

Reagents	Concentration, γ/mg L^{-1}
$(NH_4)NO_3$	400
$(NH_4)_2SO_4$	14
$MgSO_4 \cdot 7H_2O$	880
NaK_2PO_4	220
$FeSO_4 \cdot H_2O$	60
$CoCl_2 \cdot 6H_2O$	2
$CuSO_4 \cdot 5H_2O$	7
$CaCl_2$	38
H_3BO_3	49
$ZnSO_4 \cdot 7H_2O$	2
Na_2MoO_4	4
urea	86

Weather conditions during the experimental period

During the experimental period, temperatures ranged from 27 to 32 °C, while solar radiation was in the range of 6.5 to 7.5 kWh m^{-2} d^{-1}. Differences between evaporation and precipitation during the operational period were not significant and corrections due to this fact were not necessary.

Wastewater characteristics

The waste used as influent for feeding the laboratory-scale pond was the effluent derived from the anaerobic digestion of distillery waste. This pre-treatment process was carried out in an anaerobic fixed bed reactor (AFBR), which operated at steady-state conditions. The AFBR consisted of a glass column packed with ceramic raschig rings of 10 cm in diameter and 110 cm height. The bed porosity was 0.83, the specific surface area a = 1.18 $cm^2\,cm^{-3}$ and the effective volume was V = 5 L. The reactor was operated at a volumetric organic loading rate (VOLR) of Γ = 16.6 g COD $L^{-1}\,d^{-1}$, corresponding to an hydraulic retention time (HRT) of 5 days and a recycling ratio (R) equal to the unit. These operational conditions were considered to be optimum for the process after a long operation time. The characteristics and features of the raw distillery waste and the effluent of the AFBR, used as influent for the laboratory-scale pond are given in Table 2.

Table 2 – *Characteristics of the raw distillery waste and the effluent of the anaerobic fixed bed reactor (AFBR), used as influent in the secondary pond*

Parameters	Raw distillery waste*	AFBR-effluent*
COD, γ/mg L^{-1}	76960 ± 8465	16685 ± 1210
BOD_5, γ/mg L^{-1}	38600 ± 5790	3651 ± 307
TS, γ/mg L^{-1}	70615 ± 9866	26119 ± 3233
TVS, γ/mg L^{-1}	46778 ± 5613	13754 ± 1702
TSS, γ/mg L^{-1}	7690 ± 846	11074 ± 638
VSS, γ/mg L^{-1}	5433 ± 815	6190 ± 841
pH	5.3 ± 0.5	6.6 ± 0.2
Alkalinity, γ_{CaCO_3}/mg L^{-1}	7500 ± 1130	9436 ± 136

*Average and standard deviation values of 30 samples

Chemical analysis

The analyses of the waste used as influent and the effluents of the pond included: total chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total solids (TS), total volatile solids (TVS), total suspended solids (TSS), volatile suspended solids (VSS), organic nitrogen (γ_{ON}), ammonia nitrogen (γ_{AN}), total phosphorous ($\gamma_{P,T}$), orthophosphate (γ_{OP}), alkalinity, total chlorophyll ($\gamma_{C,T}$), chlorophyll a (γ_{ca}) and pH. All analyses were performed according to Standard Methods for the Examination of Waters and Wastewaters.[31]

Experimental procedure

The experiments were carried out in continuous mode. A flow diagram of the process studied is shown in Fig. 1. The effluent of the AFBR was continuously pumped at a flow-rate (Q_I) of 0.25 L d^{-1} to the mixing tank where it is mixed with the re-circulated effluent of the pond (Q_R) at a flow of 2.5 L d^{-1}, the recirculation ratio (R) being equal to 10. The mixture of AFBR effluent and recycling was also continuously pumped to the pond at a flow-rate ($Q_{(I+R)}$) of 2.75 L d^{-1}. Therefore, the pond operated at an HRT of 11 days. A multichannel peristaltic pump was used during the operational time. The experiment was carried out during a period from April to June of 2005. Therefore, the experiment lasted three months. The non-steady state or transient period was assumed to be three times the value of the HRT. After the day 34^{th}, steady-state conditions were assumed and sampling was initiated.

Additionally, these conditions were corroborated by the low standard deviations of the different parameters evaluated in the effluent of the pond. Samples were taken in the AFBR effluent, the mixed liquor fed to the pond and in the final pond effluent. According to the geometry of the pond, it could be assumed to have completely mixed reactor behaviour, as it was reported in the literature.[30]

Results and discussion

Table 3 shows the variation ranges of COD and BOD_5 for the effluent of the AFBR, which corresponds to the influent of the process (I), the mixture of influent and recycling, which corresponds to the pond influent (I + R), and the pond effluent (E) during the experimental period, once steady-state conditions were achieved. Influent COD and BOD_5 had a very similar pattern of variation, the average ratio BOD_5/COD being equal to 0.22, with coefficients of variation of 7.0 % and 8.4 % for COD and BOD_5, respectively. During the experimental period, the average volumetric organic loading rate (VOLR) added to the pond was Γ = 0.14 kg COD $m^{-3}\,d^{-1}$, with a variation in a range of Γ = 0.13–0.15 kg COD $m^{-3}\,d^{-1}$. These values corresponded to Γ = 0.03 kg BOD_5 $m^{-3}\,d^{-1}$ with a variation in a range of Γ = 0.027–0.033 kg BOD_5 $m^{-3}\,d^{-1}$. These values were equivalent to surface organic loading rates (*SOLR)* of G = 418 kg COD $ha^{-1}\,d^{-1}$ and G = 92 kg BOD_5 $ha^{-1}\,d^{-1}$ respectively. The average concentration of COD and BOD_5 in the mixing tank was considerably lower than in the influent, decreasing the variation coefficients from 7.0 % to 5.0 % for COD and from 8.4 % to 4.3 % for BOD_5.

T a b l e 3 – *COD and BOD_5 values variation during the experimental period*

Statistic parameter	S_I COD γ/mg L^{-1}	$S_{(I+R)}$ COD γ/mg L^{-1}	S_E COD γ/mg L^{-1}	S_I BOD_5 γ/mg L^{-1}	$S_{(I+R)}$ BOD_5 γ/mg L^{-1}	S_E BOD_5 γ/mg L^{-1}
r	14960–19170	1610–1980	250–340	3030–4020	80–400	39–52
$\bar{x}$	16685	1776	299	3651	366	45
S.D.	1210	105	28	307	16	4
v.c./%	7.0	5.0	9.0	8.4	4.3	7.9
N	15	15	15	15	15	15

I: effluent of the anaerobic fixed bed reactor, which corresponds to the influent of the process. (I + R): mixture of the influent and recycling, which corresponds to the influent of the pond. E: effluent of the pond; r: range of values variation; $\bar{x}$: average value; S.D.: standard deviation; v.c.: variation coefficient (%). N: number of determinations carried out after the steady-state conditions had been reached.

Finally, the average values of COD and BOD_5 in the pond effluent decreased considerably with respect to the values of the influent and the mixture of influent and recycling. Average values and variation coefficients were 299 mg L^{-1} and 9.0 % and 45 mg L^{-1} and 7.9 % for COD and BOD_5, respectively. Therefore, the average efficiency of COD and BOD_5 removals with respect to the influent was determined to be η = 98.2 % with a variation coefficient of 2.5 % and η = 98.8 % with a variation coefficient of 1 %, respectively. Taking into account the mixture (I + R), the average removals were found to be 83.2 % and 88.0 % with variation coefficients of 3.0 % and 2.0 % for COD and BOD_5, respectively. The low values of the variation coefficients obtained show that the pond operated adequately and at very stable conditions. Due to the organic matter oxidation during the process, the average BOD_5/COD ratio decreased from 0.22 to a final value of 0.15 for the pond effluent.

These COD and BOD_5 removal efficiency values were higher than those obtained in laboratory and full-scale microalgae ponds for tertiary treatment of piggery wastes, operating at HRT of 3.8 days (57 % and 69 % for COD and BOD_5, respectively)[32] and than those reported in laboratory-scale stabilization ponds for tertiary treatment of distillery waste previously treated by a combined anaerobic filter-aerobic trickling system (68 % and 75 % for COD and BOD_5), which operated at similar HRT (τ = 10 d) and influent substrate concentrations (γ = 1670 mg COD L^{-1} and γ = 341 mg BOD_5 L^{-1}).[33]

Table 4 shows the range of variation of the solids concentration (TS, TSS and VSS) during the experimental time after achieving the steady-state conditions. The concentrations of TS, TSS and VSS in the effluent of AFBR, which corresponds to the influent of the process (I), had variations of 12.4 %, 5.8 % and 13.6 % respectively, while in the mixture (I + R), the variation coefficients of TS, TSS and VSS decreased to 8.2 %, 7.6 % and 11.5 %, respectively favoring the pond performance. As can be seen, the values of the solids in the mixture (I + R) and in the effluent (E) were considerably lower than those observed in the influent (I). Therefore, the average removals of TS, TSS and VSS were very high with values of 94.9 %, 92.6 % and 97.6 % respectively considering the values of the influent. Taking into account the values of the mixture (I + R), the average removal values were equal to 60.6 %, 53.4 % and 78.8 % for TS, TSS and VSS, respectively. TS and VSS removal efficiency values were

T a b l e 4 – *Variation range of the different solid concentrations (TS, TSS, VSS) during the experimental period*

Statistic parameter	TS_I γ/mg L^{-1}	$TS_{(I+R)}$ γ/mg L^{-1}	TS_E γ/mg L^{-1}	TSS_I γ/mg L^{-1}	$TSS_{(I+R)}$ γ/mg L^{-1}	TSS_E γ/mg L^{-1}	VSS_I γ/mg L^{-1}	$VSS_{(I+R)}$ γ/mg L^{-1}	VSS_E γ/mg L^{-1}
r	21300–30750	3300–4200	1350–1600	9800–12100	720–890	120–200	5100–7300	550–790	140–190
$\bar{x}$	26119	3709	1463	11074	809	150	6190	697	172
S.D.	3233	305	77	638	62	10	841	80	7
v.c./%	12.4	8.2	5.3	5.8	7.6	6.6	13.6	11.5	4.0
N	15	15	15	15	15	15	15	15	15

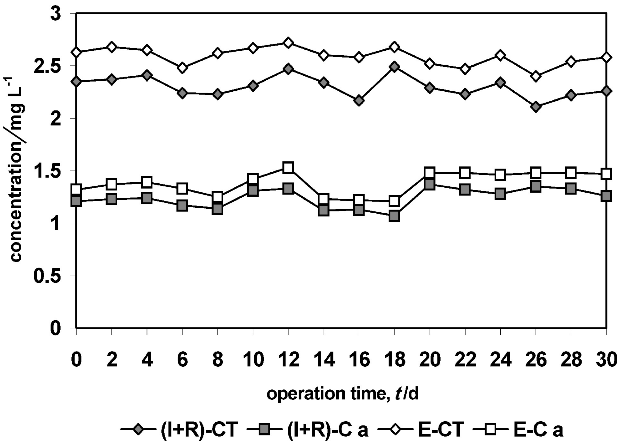
higher than those obtained for TSS, probably, because soluble organic compounds are better assimilated and degraded by microorganisms.

On the other hand, the concentration of γ_{ON} for the influent was considerably higher (545–1011) mg L^{-1}, when compared to ammonia nitrogen γ_{AN} = 40–62 mg L^{-1}. The concentration of γ_{ON} decreased significantly in the mixture (I + R) and finally in the pond effluent. The average γ_{ON} removal efficiency was η = 90.7 % when compared the values of the pond effluent (E) and influent (I) and 46.9 % when compared the values of (I + R) and the pond effluent (E).

The average removal efficiency of ammonia was η = 84.0 % when compared the values of influent and pond effluent and η = 33.3 % when compared the pond effluent (E) and the mixture (I + R). Ammonia removal could be attributed mainly to the assimilation of this compound by the microalgae present in the pond for their metabolism.

The fraction (%) of O.P. in the T.P., decreased through the process, being 76.1 % in the influent and decreasing to 67.0 % in the effluent, which shows that the degradation of O.P. is carried out at higher rate than that corresponding to the organic phosphorus or polyphosphates uptake. The average removal efficiency of T.P. was η = 85.5 % considering the influent (I) concentration (41–96) mg L^{-1} and effluent (E) values (7–10) mg L^{-1} and 7.6 % if the (I + R) concentration (10–13) mg L^{-1} and effluent (E) values are considered. In the case of O.P., the corresponding values of removal efficiencies were η = 87.3 % and η = 19.8 % using the influent and the mixture (I + R), respectively. This fact also corroborates that O.P. was assimilated at a higher rate than the other forms of phosphorus and that microalgae play an important role in the process. In the same way, previous studies showed that there was little phosphorus removal in tertiary lagoons where there was little algal growth.[9] The phosphorus removal efficiencies obtained in our study were also slightly higher than those reported in the above-mentioned studies[20] using the same microalgae (*Chlorella vulgaris*).

Fig. 2 shows the variation of total chlorophyll ($\gamma_{C,T}$) and chlorophyll *a* (γ_{ca}) concentrations representatives of the microalgae concentration during the operation time. The concentration of these parameters was zero in the influent (I) but was higher in the effluent (E) than in the mixture (I + R), showing that microalgae may grow under these conditions. The average concentration of $\gamma_{C,T}$ in the effluent was 2.59 mg L^{-1}, while in the mixture (I + R) the $\gamma_{C,T}$ concentration was 2.30 mg L^{-1}, which represents an increase of 12.6 %. In the case of γ_{ca}, the concentration increased from 1.24 mg L^{-1} to 1.38 mg L^{-1}, which represents an increase of 11.3 %. Taking into account the concentrations of



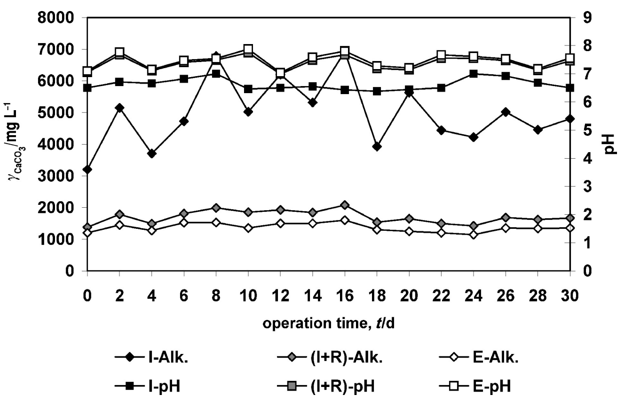
F i g . 2 – *Variation of the concentrations of $\gamma_{C,T}$ and γ_{ca} for the mixture of influent and recycling (I + R) and pond effluent (E) during the operation time*

$\gamma_{C,T}$ and γ_{ca} in the pond effluent and that the HRT was τ = 120 days, taking into account the flow-rate of the influent (Q_I = 0.25 L d^{-1}), the average values of the rate of $\gamma_{C,T}$ and γ_{ca} generation may be estimated to be 0.02 mg L^{-1} d^{-1} and 0.01 mg L^{-1} d^{-1}, respectively. Considering that the pond behaved as a complete mixed reactor, the concentration of $\gamma_{C,T}$ and γ_{Ca} in the pond being equal to the concentration in the effluent, the specific growth rate (μ) may be determined by the following equation:[34]

$$\mu = (1/\gamma_X)\cdot d\gamma_X/dt \qquad (1)$$

where the term dγ_X/dt is the rate of $\gamma_{C,T}$ or γ_{Ca} increase and γ_X may be considered as the $\gamma_{C,T}$ or γ_{Ca} concentration or concentration of total chlorophyll or chlorophyll *a* in the pond, respectively. Thus, the average values of μ were determined to be 0.01 d^{-1} for both $\gamma_{C,T}$ and γ_{ca}.

Fig. 3 shows the variation of the pH and alkalinity during the operation time. A great variation coefficient of the influent alkalinity was observed with a value of 21.6 %, while in the case of pH the



F i g . 3 – *Variation of the alkalinity γ_{CaCO_3}/mg L^{-1} and pH for the influent (I), mixture of influent and recycling (I + R) and pond effluent (E) during the operation time* variation coefficient was 3.4 %. The alkalinity in the mixture (I + R) decreased and pH increased with respect to the values observed in the influent. In the case of the final pond effluent (E), again, the alkalinity decreased and pH increased with respect to the influent and mixture (I + R) values. In addition, it was observed that the color of the influent and effluent samples remained practically invariable throughout the experiment.

The experimental results obtained demonstrated the feasibility of microalgae pond for secondary treatment of distillery wastewaters previously digested in an anaerobic fixed bed reactor. Based on the experimental results obtained for a distillery with a daily production of 500 m^3 of wastewater and using the data of Table 2, the organic loading of the anaerobic effluent would be q = 8350 kg COD d^{-1}. Therefore, if the pond operates at an organic loading rate of Γ = 0.14 kg COD m^{-3} d^{-1}, the total volume of pond required would be 59 650 m^3. In the case of a microalgae pond with 1.5 m depth, the surface area required for the secondary treatment would be of 3.9 ha. Given the characteristics of the final effluent, this pond may be used as a reservoir for the wastewater with a better quality for irrigation purpose.

Conclusions

The results obtained demonstrated the suitability of microalgae pond for secondary treatment of distillery wastewaters previously digested in an anaerobic fixed bed reactor. COD and BOD_5 removal efficiencies of η = 83.2 % and η = 88.0 %, respectively were obtained when operated at surface organic loading rates of G = 418 kg COD ha^{-1} d^{-1} (92 kg BOD_5 ha^{-1} $d^{-1)}$ and an HRT of τ = 11 days based on the mixture (influent + recycling). TSS and VSS removal efficiencies of η = 53.4 % and η = 78.8 % were also achieved operating at the above-mentioned conditions.

It was observed that the pond behaved as a complete mixed reactor. The average value of the microorganism specific growth rate obtained was μ = 0.01 d^{-1}. Effluent obtained from the pond at the mentioned operating conditions was acceptable for final disposal or irrigation.

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List of symbols

η – efficiency, %
μ – microorganisms specific growth rate, d^{-1}
Γ – volumetric organic loading rate, kg COD m^{-3} pond d^{-1}
G – surface organic loading rate, kg COD or BOD_5 ha^{-1} d^{-1}
γ – mass concentration, mg L^{-1}
γ_{AN} – ammonia nitrogen concentration, mg L^{-1}
γ_{ca} – chlorophyll *a* concentration, mg L^{-1}
$\gamma_{C,T}$ – total chlorophyll concentration, mg L^{-1}
γ_{ON} – organic nitrogen concentration, mg L^{-1}
γ_{OP} – orthophosphate concentration, mg L^{-1}
γ_{S} – organic loading, mg COD or BOD_5 L^{-1}
γ_{TP} – total phosphorous concentration, mg L^{-1}
γ_{TS} – total solids concentration, mg L^{-1}
γ_{TSS} – total suspended solids concentration, mg L^{-1}
γ_{TVS} – total volatile solids concentration, mg L^{-1}
Q – volumetric flow rate, L d^{-1}
q – mass flow rate, kg d^{-1}
v.c. – variation coefficient, %
$\bar{x}$ – average value

Abbreviations

AFBR – anaerobic fixed bed reactor
BOD_5 – biochemical oxygen demand
COD – chemical oxygen demand
E – effluent of the pond
HRT – hydraulic retention time
I – effluent of the AFBR, which corresponds to the influent of the process
I + R – mixture of the influent and recycling, which corresponds to the influent of the pond
R – effluent recycling ratio
S.D. – standard deviation
SOLR – surface organic loading rate
VOLR – volumetric organic loading rate
VSS – volatile suspended solids

References

1. *Borja, R., Sánchez, E., Martín, A., Jiménez, A. M.,* Bioproc. Eng. **16** (1996) 17.
2. *Fitzgibbon, F. J., Nigam, P., Singh, D., Marchant, R.,* J. Basic Microbiol. **35** (5) (1995) 293.
3. *Navarro, A. R., Sepúlveda, M. C., Rubio, M. C.,* Waste Manag. **20** (7) (2000) 581.
4. *Jiménez, A. M., Borja, R., Martín, A.,* Biochem. Eng. J. **18** (2) (2004) 121.
5. *Maiorella, B. L., Blanch, H. W., Wilkie, G. R.,* Process Biochem. **18** (4) (1983) 5.
6. *Sánchez, E., Travieso, L.,* Biotechnol. Letters **10** (6) (1990) 520.
7. *Baneerje, S., Biswas, G. K.,* Chem. Eng. J. **102** (2) (2004) 193.

8. *Kumar, S., Viswanathan, L.,* Enz. Microb. Technol. **13** (2) (1991) 179.
9. *Maynard, H. E., Ouki, S. K., Williams, S. C.*, Water Res. **33** (1) (1999) 1.
10. *Mara, D. D., Pearson, H. W.,* Water Res. **33** (2) (1999) 591.
11. *Travieso, L., Leon, M.,* Microalgae growths on distillery waste. In Proceedings of I Botanical Symposium and II National Meeting of Algology. La Habana, Havana University, 1985.
12. *Agunwamba, J. C.,* Water Res. **35** (5) (2001) 1191.
13. *Koura, A., Fethi, F., Fahde, A., Lahlou, A., Ouzzani N.*, Urban Water **4** (4) (2002) 373.
14. *Ferreira, F. L. A., Lucas, J., Amaral, L. A.*, Bioresour. Technol. **90** (2) (2003) 101.
15. *Fiedler, E., Juanico, M. O., Shelef G.*, Ecol. Eng. **20** (2) (2003) 121.
16. *Beran, B., Kargi, F.,* Ecol. Model. **181** (1) (2005) 39.
17. *Lavoie, A., de la Noüe, J.*, Water Res. **19** (1985) 1437.
18. *Pearson, H. W., Mara, D. D., Mills, S. W., Smallman, D. J.*, Water Sci. Technol. **19** (12) (1987) 131.
19. *Travieso, L., Benítez, F., Sánchez, E.,* Ciencia y Técnica en la Agricultura. Veterinaria **9** (1) (1988) 7.
20. *Oswald, W. J.,* J. Sanit. Eng. Division **81** (1955) 321.
21. *Valderrama, L. T., Del Campo, C. M., Rodriguez, C. M., de-Bashan, L. E., Bashan Y.*, Water Res. **36** (17) (2002) 4185.
22. *Tesmer, M. G., Joyce T. W.,* Tappi **63** (9) (1980) 105.
23. *Pearson, H. W., Mara, D. D., Mills, S. W., Smallman, D. J.,* Water Sci. Technol. **19** (12) (1987) 131.
24. *Azis, M. A., Ng, W. J.,* Water Sci. Technol. **28** (7) (1993) 71.
25. *Dilek, F. B., Taplamacýodlú, H., Tarlan, E.,* Appl. Microbiol. Biotechnol. **52** (1999) 585.
26. *Endo, H., Shirota, M.,* Proc. Int. Ferment. Technol. Symp. **4** (1972) 533.
27. *Ogawa, T., Aiba, S.*, Biotechnol. Bioeng. **23** (1981) 1121.
28. *Caizares, R. O., Rivas, L., Montes, C., Domínguez, A. R., Travieso, L., Benítez F.,* Bioresour. Technol. **47** (1994) 89.
29. *Follman, H., Märkl, H., Vortmeyer, D.,* Ger. Chem. Eng. **1** (1978) 335.
30. *Nameche, Th., Vasel, J. L.,* Water Res. **32** (10) (1998) 3039.
31. *APHA, WCPF, AWWA*, Standard Methods for the Examination of Waters and Wastewaters 18th Ed., American Public Health Association (APHA), Washington D.C., 1997.
32. *Travieso, L., Sánchez, E., Borja, R., Benítez, F., León, M., Colmenarejo, M. F.*, Environ. Technol. **25** (2004) 565.
33. *Travieso, L., Sánchez, E., Borja, R., Benítez, F., Raposo, F., Rincón, B., Jiménez, A. M.*, Ecol. Eng. **27** (2) (2006) 100.
34. *Hughes, D. E., Stafford, D. A.,* CRC Crit. Rev. Environ. Control 6 (1976) 233.