The Aerobic Biodegradation Kinetics of Plant Tannins in Industrial Wastewater

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Dedicated to Prof. Dr. Đurđa Vasić-Rački on occasion of her 60th birthday

This paper describes an experimental determination of the biodegradation rate for tannins present in industrial wastewater, after the extraction of chestnut chips. Experiments were performed in a laboratory aerobic reactor (Armfield) by using biomass from an existing industrial wastewater treatment plant. The outlet tannins concentration was determined under various processing conditions. Simultaneously, an optical microscope was used to monitor the mix of microbiological cultures in the biomass. On the basis of data obtained in experiments, non-linear regression was used to perform parametric analysis of various kinetic models, which took into account inhibition, as quoted in literature (Haldane, Edwards, Aiba, Luong). The statistical analysis, based on the P-criterion, F-criterion, adjusted coefficient of determination, Kolmogorov-Smirnov test and root mean squared error, showed that the biodegradation of plant tannins in industrial wastewater under selected conditions for aerobic digestion, can be most successfully described statistically by the Aiba's kinetic model.

Key words:
Plant tannins, substrate inhibition, kinetic models, statistic analysis, aerobic industrial wastewater digestion

Introduction

Tannins are natural, water-soluble polyphenol compounds, of varying molar mass.[1] They are classified into two groups in regard to their structures and properties – hydrolysable and condensed tannins.[2] Hydrolysable tannins are composed of esters of gallic or ellagic acid with a glucose core,[3] while condensed tannins are polymers of flavonoids.[4] They differ from most other natural phenol compounds[5] by their ability for the precipitation of alkaloids, gelatine and other proteins from solutions and have, therefore, been used for a long time in the leather industry for tanning animal skins. Tannins have a toxic effect in a natural environment and, hence, act as a prevention against various micro-organisms and bacteria.[6] Over the last few decades their use has become more widespread being used in a wide range of special products, with the priority of protecting and preserving a healthy environment.

The most important commercially available tannins are extracted from various plant cultures or saplings.[2] Among them are plant tannins obtained by extraction from wood chips of the sweet chestnut tree. Despite the use of state-of-the-art and innovative technology, during this production method, in addition to the desired extract (plant tannins), an unwanted side product – wastewater, results. In order to keep this process aligned with the principles of sustainable development, careful wastewater management is urgently required, preventing any adverse impact on the environment.

Wastewater from the timber industry contains large amounts of various phenol compounds, from simple monomers to high molecular polyphenol polymers (plant tannins). Past studies have shown the possibility of utilising chemical,[7–9] as well as biochemical[2, 10–12] methods, for the degradation of tannins. The latter are a big challenge when planning industrial anaerobic and aerobic wastewater treatment plants, notably because of the well-known inhibition and toxic effects of phenol compounds.[13,14] The impact of the tannins' structure on their toxicity was described by *Scalbert*.[15] *Field* and *Lettinga*,[16] published studies on the degradability of tannins by continuous anaerobic treatment, while *Makkar*, *Singh* and *Kamra*[17] searched for the biodegradability of tannins in oak tree leaf sample. In the existing studies on the biodegradability of tannins, we have not found any data on the experimental determination of kinetics for the degradation of plant tannins in the aerobic biological treatment of industrial wastewater.

Biological wastewater treatment plants operate on the principle of organic substance conversion in

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a bioreactor by using various micro-organisms.[18] The rate of an organic substance (substrate) degradation from wastewater is an important criterion in the planning, development and dimensioning of a biological reactor. In the bioreactor, the kinetics of those chemical reactions inhibited by substrate can be described using special kinetic models.[19,20] The rate of substrate degradation is determined by experiments and depends on the types of micro-organisms, the inhibition effect of the substrate, and other conditions in the bioreactor. Similar factors are also decisive in the selection of an appropriate kinetic model.

The purpose of this research was to determine the most statistically appropriate kinetic model for describing the rate of plant tannins' degradation under the selected aerobic processing conditions (temperature, mass concentration of biomass, air volume flow rate) on the basis of experimental measurements.

Materials and methods

Equipment

A continuous laboratory aerobic digester consists of a 9 L reactor vessel with a liquid feed pump, air supply, and instrumentation for monitoring and controlling the process. The cylindrical wall of the reactor is made from a porous plastic material in order to retain the suspended solids, while allowing treated water to pass through to the outer, annular chamber. Wastewater is drawn from a floor-standing feed tank by a motor driven peristaltic pump. Flow rate is accurately set by a ten-turn potentiometer. Air is supplied at a measured rate by a small compressor, and discharges into the base of the reactor via a spider-arm dispenser, designed to produce sufficient bubbling for stirring and reaction. The reactor temperature is maintained by a controller which varies electric power to an immersion heater within the vessel.

Industrial wastewater

Industrial wastewater, after the extraction of tannins from chestnut chips, was used in our study. The wastewater consisted of dissolved (plant tannins, sugars, acids) and suspended solids (wood powder). The values shown after analysis are: pH = 3.3, mass concentration of total solids $\gamma_{TS} = 6$ g L^{-1}, mass concentration of total dissolved solids $\gamma_{TDS} = 3$ g L^{-1}, and mass concentration of plant tannins $\gamma_{S0} = (1.0 \pm 0.05)$ g L^{-1}. Suspended solids were removed by sedimentation before introducing the wastewater into the reactor.

Biomass sludge

The reactor was inoculated with biomass sludge from an existing full–scale aerobic digester for treating industrial and municipal wastewater. The microscopic analysis of initial biomass showed a mixed culture of micro-organisms, with *Vorticella sp.* prevailing. Mass concentration of total solids, which indicates the biomass present in the reactor, was $\gamma_X = 5$ g L^{-1}.

Analytics

UV spectrophotometric method[21] (UV–VIS spectrophotometer, PERKIN–ELMER, model 552) was used to determine the plant tannins' mass concentration. All chemicals used, *N*,*N*-dimetylformamid (Fluka), iron (III) chloride hexahydrate (Kemika), ethanol (Riedel-de Haën) and powdered tannic acid (Riedel-de Haën), are commercially available. The mass concentration of biomass was determined gravimetrically.

Kinetic models

Aeration was used in a bench top digester in order to provide ideal mixing and, thus, identical composition of the substrate (plant tannins) within the entire volume of the reactor, and in the outflow. Thus, we were able to describe the degradation rate of plant tannins in the bioreactor (by taking into account the kinetics of biomass growth), using the model of continuously-stirred tank reactors (CSTR) in a steady-state condition:[22]

$$-r_S = \frac{1}{Y_{XS}} \mu \gamma_X = D(\gamma_{S0} - \gamma_S) \tag{1}$$

where:

r_S – biodegradation mass rate of plant tannins, kg m^{-3} d^{-1}

γ_X – mass concentration of biomass, kg m^{-3}

γ_{S0} – inlet mass concentration of plant tannins, kg m^{-3}

γ_S – outlet mass concentration of plant tannins, kg m^{-3}

Y_{XS} – yield of biomass regarding plant tannins, kg kg^{-1}

μ – specific growth rate of biomass, d^{-1}

D – dilution rate, d^{-1}.

Specific growth rate of biomass μ, excluding tannins inhibition effect, can be described by the simple Monod equation:

$$\mu = \frac{\mu_{max} \gamma_s}{K_s + \gamma_s} \tag{2}$$

where:

μ_{max} – maximum specific growth rate of biomass, d^{-1}

K_s – saturation constant for plant tannins, kg m^{-3}.

It is well-known, that the inhibition effect of plant tannins in larger concentrations results in a reduction in the specific growth rate of biomass, which contradicts the Monod equation. Some general formulas in literature,[19] named after their authors (Haldane, Edwards, Aiba and Luong), which by modifying the Monod equation take inhibition into account, are presented in Table 1.

Table 1 – *Expressions for specific growth rate of biomass calculation, including inhibition*

Author	Expression
Haldane[23]	$\mu = \dfrac{\mu_{max}\gamma_S}{K_s + \gamma_S + \dfrac{\gamma_S^2}{K_{IH}}}$
Edwards[24]	$\mu = \mu_{max}\left(\exp\left(-\dfrac{\gamma_S}{K_{IE}}\right) - \exp\left(-\dfrac{\gamma_S}{K_s}\right)\right)$
Aiba[24,25]	$\mu = \dfrac{\mu_{max}\gamma_S}{K_s + \gamma_S}\exp\left(-\dfrac{\gamma_S}{K_{IA}}\right)$
Luong[26]	$\mu = \dfrac{\mu_{max}\gamma_S}{K_s + \gamma_S}\left(1 - \dfrac{\gamma_S}{\gamma_{S,m}}\right)^n$

By taking into account the equations from Table 1, and assuming that the yield of the biomass is identical with regard to plant tannins in the entire concentration range and, that the mass concentration of the biomass in the reactor is constant, the biodegradation mass rate of tannins (Equation (1)) can be described by the expressions presented in Table 2.

Table 2 – *Kinetic models for biodegradation mass rate of tannins*

Author	Expression
Haldane	$-r_S = \dfrac{r_{max}\gamma_S}{K_s + \gamma_S + \dfrac{\gamma_S^2}{K_{IH}}} = D(\gamma_{S0} - \gamma_S)$
Edwards	$-r_S = r_{max}\left(\exp\left(-\dfrac{\gamma_S}{K_{IE}}\right) - \exp\left(-\dfrac{\gamma_S}{K_s}\right)\right) = D(\gamma_{S0} - \gamma_S)$
Aiba	$-r_S = \dfrac{r_{max}\gamma_S}{K_s + \gamma_S}\exp\left(-\dfrac{\gamma_S}{K_{IA}}\right) = D(\gamma_{S0} - \gamma_S)$
Luong	$-r_S = \dfrac{r_{max}\gamma_S}{K_s + \gamma_S}\left(1 - \dfrac{\gamma_S}{\gamma_{S,m}}\right)^n = D(\gamma_{S0} - \gamma_S)$

From the mass balance of tannins in a steady-state at various dilution rates of industrial wastewater and the selected processing conditions, it is possible to determine those kinetic parameters which describe the biodegradation mass rate of plant tannins, by using non-linear regression.

Experimental work

Experiments were conducted in a laboratory aerobic reactor. By keeping the mass concentration of the biomass constant (γ_X = 5.0 g L^{-1}) we were changing the volume flow rate of the industrial wastewater and measuring the outflow mass concentration of the plant tannins. All other operating conditions of the laboratory aerobic reactor (temperature ϑ = (25 ± 1) °C, the initial mass concentration of the biomass γ_X = 5.0 g L^{-1}, the inlet mass concentration of plant tannins γ_{S0} = (1.0 ± 0.05) g L^{-1}, and the air volume flow rate $q_{V,a}$ = 1.6 L min^{-1}) remained unchanged in all experiments.

Individual experiments were conducted so that on the first day the biomass sludge from the existing aerobic wastewater treatment plant was accustomed to its new environment by aeration at constant volume air flow rate. The gravimetric method was used to determine the mass concentration of biomass, by adjusting it to the desired value (γ_X = 5.0 g L^{-1}), if required. The second day continuous introduction of the wastewater to the aerobic reactor started. The inflow and outflow of the aerobic reactor was sampled daily and the mass concentration of plant tannins determined. The experiment was conducted under given operating conditions until the steady-state conditions were established, i.e. until the outlet mass concentration of the plant tannins was constant. Simultaneously, with the analytic determination of plant tannins outlet mass concentration, the microscope was used to monitor changes in the morphology of the microbiological cultures in the biomass. In order to ensure identical initial microbiological composition of the biomass, only one experiment was conducted, with a single batch of biomass sludge at a time. The fresh biomass sludge was used for new measurements at higher flows of wastewater.

Results

The experimentally determined outlet mass concentrations of plant tannins at increasing industrial wastewater dilution rates together with the corresponding biodegradation mass rates of tannins are presented in Table 3. Confidence intervals (*CF*) are also calculated. The dilution rate *D*, is the quotient

Table 3 – *Mass concentration and biodegradation mass rate of plant tannins at steady state versus dilution rate*

D/d^{-1}	γ_S/kg m^{-3}	$-r_S$/kg m^{-3} d^{-1}	CF/%
0.00 ± 0.00	0.000 ± 0.000	0.0000 ± 0.0000	± 0.0
0.11 ± 0.01	0.030 ± 0.005	0.1067 ± 0.0112	± 10.5
0.17 ± 0.01	0.050 ± 0.005	0.1615 ± 0.0128	± 7.9
0.22 ± 0.01	0.110 ± 0.010	0.1958 ± 0.0143	± 7.3
0.28 ± 0.01	0.180 ± 0.010	0.2296 ± 0.0165	± 7.2
0.33 ± 0.01	0.400 ± 0.020	0.1980 ± 0.0188	± 9.5
0.38 ± 0.01	0.700 ± 0.025	0.1140 ± 0.0215	± 18.8

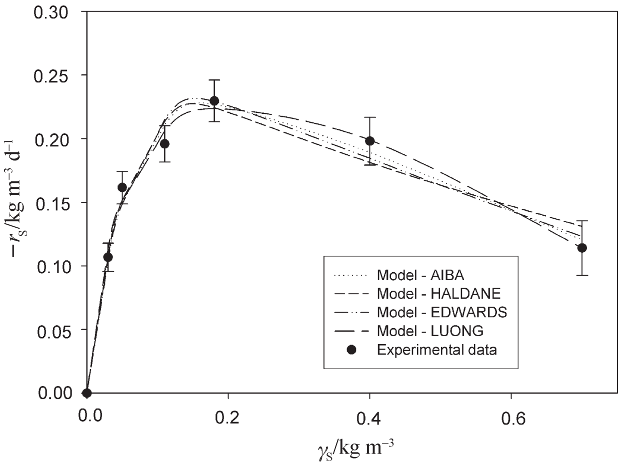


Fig. 1 – *Experimental and model-based values of biodegradation mass rates of plant tannins in relation to their mass concentration*

of wastewater volume flow rate $q_{V,\mathrm{w}}$, and the active volume of the reactor V. The biodegradation mass rate of plant tannins r_S, in the aerobic treatment of industrial wastewater, was calculated on the basis of experimentally determined values by using equation (1) as the product between dilution rate D, and the difference between the inlet and outlet mass concentrations of plant tannins.

The specific convex-shape of the curve, which presents the biodegradation mass rate of the plant tannins in the aerobic treatment of industrial wastewater in relation to mass concentration of plant tannins (Fig. 1), confirmed previous findings from research on the inhibition effect of plant tannins. The simple Monod kinetic model is unsuitable for describing such shape of the curve, hence we used a kinetic model with inhibition taken into account.

Testing of the proposed kinetic models with inhibition (Haldane, Edwards, Aiba, Luong) was performed by non-linear regression of experimental data, where we used commercially available software SigmaPlot®9.0.[27] Graphic comparison between the experimental and model-based values of plant tannins biodegradation rates in relation to their mass concentration is presented in Fig. 1. As it is shown in Fig. 1 the predictions of biodegradation mass rate of tannins with different models are within the range of the experimental errors.

At lower mass concentrations of tannins (γ_S < 0.18 kg m^{-3}) their increase results in increased biodegradation mass rate. At higher concentrations of plant tannins the inhibition effect of tannins occurs, resulting in lower biodegradation mass rate. Graphic analysis has shown satisfactory correlation between the experimental and model-based values. At lower mass concentrations of tannins, deviation between graphic profiles of the models is minimal, while the difference is more distinguishable at higher concentrations.

Graphic analysis could not determine the most appropriate kinetic model for the biodegradation mass rate of tannins and, hence, in addition to parametric analysis, whereby we determined the values of the kinetic parameters, we later also carried out a statistical analysis. The statistically most suitable kinetic model was selected after consecutive elimination of statistically unsuitable one. The elimination was based on various statistical criteria (statistical P-criterion (P), adjusted coefficient of determination, (R_{adj}^2), statistical F-criterion (F), root mean squared error ($RMSE$), and Kolmogorov-Smirnov test (K-S)). Similar algorithm of elimination was used in the research by *Carrera* and others[16]. Results of both analyses are given in Table 4.

In the statistical elimination of unsuitable kinetic models by inhibition, we first used the probability statistical P-criterion, P. The lower the P value, the higher the probability that the value of the underlying kinetic parameter is not zero. It is usually true that if $P < 0.05$, we may use the independent variable to predict the value of the dependent variable. Table 4 shows that Haldane's (K_s and K_{IH}) and Luong's ($\gamma_{S,m}$ and n) kinetic models do not correspond to this criterion. The biodegradation mass rate of tannins cannot be predicted by using these two kinetic models and, hence, they were eliminated from further analysis.

In continuation, the following statistical criteria were used simultaneously for elimination: R_{adj}^2, F, K-S and $RMSE$. The adjusted coefficient of determination (R_{adj}^2) is a criterion for successful match between experimental and model-based values, whereby the number of independent variables, reflecting the level of availability, was also taken into account. By approaching (R_{adj}^2) to the value 1, the appropriateness of the equation linking the independent and dependent variables increases.

Table 4 – *Parametric and statistical analysis of proposed kinetic models with the inhibition*

Model	Parametric analysis		Statistical analysis					
	parameter	value	*CF* (%)	R^2_{adj}	*RMSE*	*F*	*P*	*K–S*
Haldane	r_{max}/kg m^{-3} d^{-1}	0.545 ± 0.194	± 35.6	0.958	0.0159	70	0.049	0.212
	K_s/kg m^{-3}	0.119 ± 0.065	± 54.2				0.138	
	K_{IH}/kg m^{-3}	0.234 ± 0.129	± 55.1				0.146	
Edwards	r_{max}/kg m^{-3} d^{-1}	0.319 ± 0.045	± 14.1	0.967	0.0141	89	0.002	0.214
	K_s/kg m^{-3}	0.066 ± 0.013	± 19.7				0.007	
	K_{IE}/kg m^{-3}	0.732 ± 0.168	± 23.0				0.012	
Aiba	r_{max}/kg m^{-3} d^{-1}	0.481 ± 0.081	± 16.8	0.980	0.0109	151	0.004	0.146
	K_s/kg m^{-3}	0.096 ± 0.027	± 28.1				0.023	
	K_{IA}/kg m^{-3}	0.557 ± 0.086	± 15.4				0.003	
Luong	r_{max}/kg m^{-3} d^{-1}	0.358 ± 0.061	± 17.0	0.985	0.0095	132	0.001	0.206
	K_s/kg m^{-3}	0.063 ± 0.019	± 30.2				0.046	
	$\gamma_{S,m}$/kg m^{-3}	0.972 ± 0.338	± 34.8				0.064	
	n/1	0.832 ± 0.666	± 80.0				0.300	

The variance ratio, *F* (statistical F-criterion)[27,28] estimates the contribution of independent variable in predicting the value of the dependent variable. The *F* value is proportional to the contribution –larger the value of *F* larger the contribution. In the case where $F = 1$, no correlation will exist between the independent and dependent variables.

The Kolmogorov-Smirnov test, *K-S,* is used as a relative indicator of interpolation. In the case where *K-S* is large (maximum value of *K-S* = 1), interpolation will be satisfactory and, inversely, it is unsatisfactory if *K-S* is small (minimum value of *K-S* = 0). The minimal permitted value of *K-S*, where interpolation, is still satisfactory, is *K-S* = 0.05.

Statistically, the most favourable interpolation must also have a low value of root mean squared error (*RMSE*). In the best case *RMSE* = 0. The comparison between the two remaining kinetic models (Edwards and Aiba) has shown that Aiba's has bigger values of R_{adj}^2 and *F,* and smaller values of *K-S* and *RMSE*, therefore, it is statistically the most suitable for describing the degradation mass rate of plant tannins in the aerobic biological treatment of industrial wastewater.

A comparison between the values of *RMSE* and R_{adj}^2 for Aiba's and Luong's kinetic models indicated that the latter might be more suitable but was previously eliminated on the basis of statistical P-criterion. It was also corroborated that the Luong's kinetic model is statistically less suitable by a relatively wide confidence interval (*CF*), high *K-S* value, and lower *F* value. The results show the significance of statistical analysis when used with a larger number of statistical criteria. The correct selection of their sequence is also important.

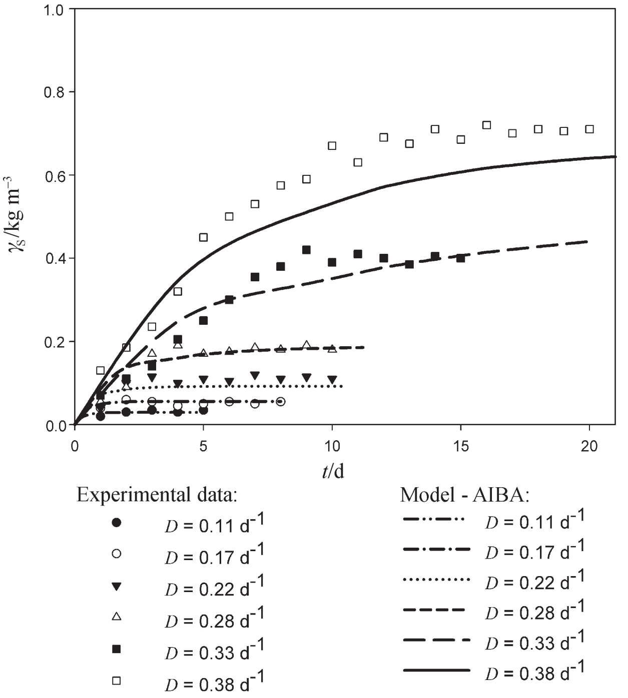
On the basis of all the findings we can summarise that the degradation mass rate of plant tannins in the aerobic biological treatment of industrial wastewater under selected conditions ($\vartheta = (25 \pm 1)$ °C and $\gamma_X = 5.0$ g L^{-1}) can be statistically the most successfully described by using the Aiba's kinetic model:

$$-r_S = \frac{r_{max}\gamma_S}{K_s + \gamma_S}\exp\left(-\frac{\gamma_S}{K_{IA}}\right) = D(\gamma_{S0} - \gamma_S) \quad (3)$$

with the following parameter values: maximum biodegradation mass rate of plant tannins r_{max} = (0.481 ± 0.081) kg m^{-3} d^{-1}, saturation constant of plant tannins K_s = (0.096 ± 0.027) kg m^{-3}, and Aiba's inhibition coefficient K_{IA} = (0.557 ± 0.086) kg m^{-3}.

On the basis of the selected model for biodegradation mass rate of plant tannins the approaching to the steady state at different dilution rates can be easily predicted. A comparison between experimental and model-based values of tannins outlet mass concentration profiles (daily basis) is shown in Fig. 2. At low dilution rates ($D < 0.28$ d^{-1}), deviations are minimal, while noticeable differences appear at higher ones.

The number of bioreactor working volumes which has to be exchanged before the steady state conditions were reached, can be estimated from Fig. 2.



F i g . 2 – *Approaching to the steady state – comparison between experimental and model-based mass concentrations of plant tannins for different dilution rates*

Conclusion

The design of a competitive production process for obtaining natural plant tannins by extraction from sweet chestnut woodchips should take into account that the treatment of wastewater is a side product of production. Key importance should be given to knowledge of the biodegradation equation rate of tannins, which serves as a tool for adequate planning of wastewater treatment plants.

The research was limited to the study of tannins' degradation in the aerobic biological treatment of industrial wastewater. We used the biomass sludge of a mixed microbiological composition from an existing industrial wastewater treatment plant. Experimental data obtained by a series of experiments in the laboratory's aerobic reactor were fitted to various kinetic models (Haldane, Edwards, Aiba and Luong) by non-linear regression. All the listed models take inhibition into account in their algorithm. By parametric and statistical analysis of the proposed models, we have determined the statistically most suitable kinetic model and the values of its parameters. Aiba's kinetic model was statistically the best at describing the biodegradation mass rate of plant tannins. On the basis of microscopic observations, we have established that under selected operating conditions before reaching the steady-state condition, the most developed micro-organism in all experiments was *Vorticella sp*. We can conclude that this kind of micro-organism had the greatest impact on tannins degradation.

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Nomenclature

CF – confidence interval, %
D – dilution rate, d^{-1}
F – statistic F-criterion (variance ratio), 1
CSTR – continuously-stirred tank reactors
K-S – Kolmogorov-Smirnov test, 1
K_s – saturation constant of plant tannins, kg m^{-3}
K_{IA} – Aiba's inhibition coefficient, kg m^{-3}
K_{IE} – Edwards's inhibition coefficient, kg m^{-3}
K_{IH} – Haldane's inhibition coefficient, kg m^{-3}
n – Luong's coefficient, 1
P – statistic P-criterion, 1
R_{adj}^2 – adjusted coefficient of determination, 1
r_{max} – maximum biodegradation mass rate of plant tannins, kg m^{-3} d^{-1}
r_S – biodegradation mass rate of plant tannins, kg m^{-3} d^{-1}
RMSE – root mean squared error, /
$q_{V,w}$ – wastewater volume flow rate, L d^{-1}
$q_{V,a}$ – air volume flow rate, L d^{-1}
Y_{XS} – yield of biomass regarding plant tannins, kg kg^{-1}
V – volume, L
γ_S – mass concentration of plant tannins, g L^{-1}
γ_{S0} – inlet mass concentration of plant tannins, g L^{-1}
$\gamma_{S,m}$ – mass concentration of plant tannins above which $\mu = 0$, kg m^{-3}
γ_{TS} – mass concentration of total solids, g L^{-1}
γ_{TDS} – mass concentration of total dissolved solids, g L^{-1}
γ_X – mass concentration of biomass, g L^{-1}
μ – specific growth rate of biomass, d^{-1}
μ_{max} – maximum specific growth rate of biomass, d^{-1}
ϑ – temperature, °C

References

1. *Spencer, C. M., Cai, Y., Martin, R., Gaffney, S. H., Goulding, P. N., Magnolato, D., Lilley, T. H., Haslam, E.,* Phytochemistry **27** (1998) 2397.
2. *Bhat, K. T., Singh, B. Sharma, O. P.,* Biodegradation **9** (1998) 343.
3. *Mueller-Harvey, I.,* Anim. Feed Sci. Techn. 91 (2001) 3.
4. *Hagerman, A. E., Butler, L. G.,* Method. Enzymol. **234** (1994) 429.
5. *Bate–Smith, E. C., Swain, T.,* Flavonoid compounds, and *Mason, H. S., Florkin, A. M.* (Eds.), Comparative biochemistry, Vol. 3A, Academic Press, New York, 1962.
6. *Field, J. A., Lettinga, G.,* Toxicity of tannic compounds to microrganisms, and *Hemingway, R. W., Laks, E.* (Eds.),

Plant Polyphenols: Synthesis, Properties, Significance, pp 673–692, Plenum Press, New York, 1992.

7. *Boye, B., Farnia, G., Sandona, G., Buso, A., Giomo, M.,* J. Appl. Electrochem. **35** (2005) 369.
8. *Svitelska, G. V., Gallios, G. P., Zouboulis, A. I.,* Chemosphere 55 (2004) 981.
9. *Navia R., Feuntes, B., Lorber, K. E., Mora, M. L., Diez, M. C.,* Chemosphere **60** (2005) 870.
10. *Archambault, J., Lacki, K., Duvnjak, Z.,* Biotechnol. Lett. **18** (1996) 771.
11. *Lekha, P. K., Lonsane, B. K.,* Advan. Appl. Microbiol. **44** (1997) 215.
12. *Field, J. A., Lettinga, G.,* Water Sci. Technol. **24** (1991) 127.
13. *Vidal, G., Diez, M. C.,* J. Environ. Manageme. **74** (2005) 317.
14. *Vidal, G., Fernadez, A. J.,* Diez, M. C., AFINIDAD **57** (2000) 318.
15. *Scalbert, A.,* Phytochemistry **30** (1991) 3875.
16. *Field, J. A., Lettinga, G.,* Met. Ions Biol. Syst. **28** (1992) 61.
17. *Makkar, H. P. S., Singh, B., Kamra, D. N.,* Lett. Appl. Microbiol. **18** (1994) 39.
18. *Gray, N. F.,* Biology of wastewater treatment, 2nd Edition, Imperial College Press, London, 2004.
19. *Carrera, J., Jubany, I., Carvallo, L., Chamy, R., Lafuente, J.,* Process Biochem. **39** (2004) 1159.
20. *Kumar, A., Kumar, S., Kumar, S.,* Biochem. Eng*.,* **22** (2005) 151.
21. ISO 9648: 1988-12-15, Sorghum – Determination of tannin content, International Organization of Standardization, Geneve, 1989.
22. *Levenspiel, O.,* Chemical reaction engineering, 3rd Edition, John Wiley, New York, 1999.
23. *Blanch, H. W., Clark, D. S.,* Biochemical Engineering, Marcel Dekker, New York, 1997.
24. *Edwards, V. H.,* Biotechnol. Bioeng. **12** (1970) 679.
25. *Aiba, S., Shoda, M., Nagatani, M.,* Biotechnol. Bioeng. **10** (1968) 845.
26. *Luong, J. H. J.,* Biotechnol. Bioeng. 29 (1987) 242.
27. SigmaPlot®9.0 User`s Guide, Systat Software, Inc., Point Richmond, CA 94804–2028, USA, 2004.
28. *Ranjit, K. R.,* A primer on the Taguchi method. Van Nostrand Reinhold, New York, 1990.