Electronic Journal of Biotechnology ISSN: 0717-3458 Vol.6 No.3, Issue of December 15, 2003

Received June 26, 2003 / Accepted December 5, 2003

RESEARCH ARTICLE

Optimisation of the solids suspension conditions in a continuous stirred tank reactor for the biooxidation of refractory gold concentrates

Ramón González[§]
Escuela de Ingeniería Bioquímica
Pontificia Universidad Católica de Valparaíso
Av. Brasil 2147, Valparaíso, Chile
Tel: 1 515 294 1516
Fax: 1 515 294 2689
E-mail: ramong@iastate.edu

Juan C. Gentina*
Escuela de Ingeniería Bioquímica
Pontificia Universidad Católica de Valparaíso
Av. Brasil 2147, Valparaíso, Chile
Tel: 56 32 273647
Fax: 56 32 273803
E-mail: jgentina@ucv.cl

Fernando Acevedo
Escuela de Ingeniería Bioquímica
Pontificia Universidad Católica de Valparaíso
Av. Brasil 2147, Valparaíso, Chile
Tel: 56 32 273644
Fax: 56 32 273803
E-mail: facevedo@ucv.cl

Financial support: FONDECYT 1980335 and DGIP-UCV 203.780. One of them (RG) received a AGCI fellowship from the Chilean government.

Keywords: agitation, bioreactor, biooxidation, slurry.

Present address: § Department of Chemical Engineering, Iowa State University, Ames IA 50011-2230, USA.

Abbreviations:
C: Distance from the bottom of the reactor to the impeller;
CCR: Central composite rotatable experimental design;
CSTR: Continuous stirred tank reactor;
D: Impeller diameter;
H_L: Fluid height in the tank;
H_T: Total tank height;
h: Distance from bottom of the cylindrical section of the tank to the mpeller;
J: Baffle width;
N: Agitation rate;
N_P: Power number, $P/\cdot\rho\ N^3\cdot D^5$;
P: Ungassed agitation power;
RSM: Response surface methodology;
rpm: Revolutions per minute;
T: Tank diameter;
vvm: Volumes of air per volume of fluid per minute;
ρ: Fluid density;
3MPU: Three-blade marine propeller pumping up;
6MFU: Six-blade mixed flow turbine pumping up.

The large-scale biooxidation of gold concentrates is usually carried on in continuous stirred tank reactors (CSTR). Attaining homogeneous slurries is a difficult task, as solids tend to stratify in the tank. The objective of this work was to determine the optimal conditions of agitation in a CSTR so to obtain the best solids suspension. The experiments were performed in a 5 litre glass tank operated with 3 litres of 6% w/v slurry. The impellers (pitched blade turbine or marine propeller) were placed at heights of 6.7 to 13.4 cm from the bottom and operated at 370 to 1040 rpm, with specific aeration rates of 0.3 to 3.7 vvm. A statistical experimental design was used which allowed the derivation of a model representing response surfaces of the exit and mean solids concentration as a function of the impeller type, impeller distance from the bottom and aeration and agitation rates. During the experiments no solids were deposited on the bottom and the solids concentration near the bottom was always higher than that of the top region. At the optimal conditions for each type of impeller, the marine propeller required agitation rates about 15 to 22% higher than the pitched blade turbine. Nevertheless it is concluded that the marine helix is preferable because it requires less power and produces a more homogeneous suspension.

*Corresponding author

The biooxidation of refractory gold concentrates has become the technology of choice for the pre-treatment of these types of minerals. It is extensively used in no less than eight large-scale mining operations in Australia, Africa and South America. The biooxidation is carried on in continuous stirred tank reactors (CSTR) with residence times of three to eight days (Rawlings, 1997; Acevedo, 2000; Rawlings et al. 2003). The reacting system is quite complex, as it consists of three phases: gas (air), liquid and solid (mineral particles, cells adsorbed onto the particles and planktonic cells). In order to obtain an efficient steady state operation the contents of the reactor should be homogeneous, so no gradients in composition, pH, redox potential, or temperature exist and no solids accumulation is produced.

The attainment of this goal is not an easy task and requires careful consideration of the reactor design and operation, with special reference to agitation (Brucato and Brucato, 1998; Nienow and Bujalski, 2002).

In a CSTR agitation increases the rate of mass and heat transfer operations and provides the required degree of mixing of the reactor contents. Insufficient agitation may lead to limitations in the transfer operations and the appearance of regions of insufficient nutrient content or inadequate temperature or pH. As a result, the overall productivity of the process will decline (Namdev et al. 1994).

The minimum stirrer speed for the suspension of solids that avoids deposition on the bottom of the tank vessels, also known as critical speed, has been studied from a theoretical and experimental standpoint (Zwietering, 1958; Oldshue, 1983; Tatterson, 1996; Armenante et al. 1998). The negative effect of aeration on solids suspension was discussed by Oldshue, 1983, pointing out that gas bubbling tends to disturb the flow patterns established by the impellers. It has been shown that increasing aeration rates produce an increase in the required critical speed, while pulp density has a minor effect (Acevedo and Aroca, 1986). Axial flow impellers are known to be superior to radial flow ones for solids suspension. Lately a number of papers have appeared on this topic(Armenante et al. 1998; Myers and Bakker, 1998; Hebrard et al. 1999; Rieger, 2000; Dohi et al. 2001; Nienow and Bujalski, 2002) and on agitation in the biooxidation of gold concentrates (Oolman, 1993; Dew et al. 1997; Spencer et al. 1997; Greenhalgh and Ritchie, 1999; Harvey et al. 1999; Acevedo, 2000).

All physical reactor design variables influence its performance regarding the quality of mixing and the formation and maintenance of homogeneous slurries. In particular, consideration should be given to the type and number of impellers and the geometrical ratios between significant dimensions such as tank diameter, liquid height, baffle width, distance between impellers and impeller diameter (Oldshue, 1969; Greenhalgh and Ritchie, 1999; Harvey et al. 1999). Regarding operation variables, aeration and agitation rates are the most pertinent. The most adequate aeration must be carefully determined so to provide oxygen and carbon dioxide as demanded by the cell population, but avoiding excessive flow rates that can lead to impellers flooding and cause unnecessarily high operating costs. On the other hand, an intense agitation must be provided, but too high agitation rates should be avoided to prevent attrition and metabolic stress in the bacterial population (Toma et al. 1991; Acevedo, 2000; Enfors et al. 2001; Canales et al. 2002). So the problem of optimisation of the agitation conditions is complex and should be addressed using a statistically based experimental design.

Central composite rotatable (CCR) experimental designs have been used in the optimisation of biotechnological processes (Kiran et al. 2001; Wen and Chen, 2001; Li et al. 2002; Sheeja and Murugesan, 2002). The two most desirable characteristics of any experimental design are orthogonality and rotatability. Orthogonality ensures that the main effect and interaction estimates of interest are independent of each other. In other words, the more orthogonal the design is, the more independent information can be extracted from the results regarding the effects of interest. On the other hand, rotatability addresses how to extract the maximum amount of unbiased information from the design. Orthogonality and rotatability depend on the number of centre points in the design and on the so-called axial distance α, which is the distance of the star points from the centre of the design. The objective of this work was to determine the optimal conditions of agitation in a stirred tank so to obtain the best solids suspension for the biooxidation of refractory gold concentrates. Two impeller designs were compared in respect of the exit and mean solids concentrations at different impeller locations and aeration and agitation rates.

MATERIALS AND METHODS

Equipment

The agitation studies were performed in a 5 L acrylic reactor with round bottom operated with 3 L of refractory gold concentrate slurry. Pulp concentration was 6% w/v and particle size ranged from 35 to 75 mm. The concentrate (Minera El Indio, La Serena, Chile) contained 42 g Au/ton, 440 g Ag/ton, 42.8% pyrite and 40.7% enargite as the main constituents. The CSTR was equipped with a one-impeller agitator, annular air sparger, four baffles and overflow exit. Figure 1 shows a schematic representation of the reactor and Table 1 presents the main geometrical ratios and

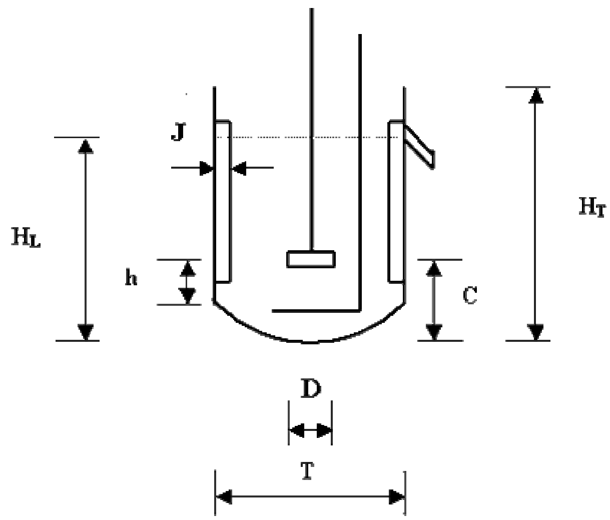

Figure 1. Schematic representation and main dimensions of the 5 L continuous stirred tank reactor.

Table 1. Geometrical ratios and operation conditions of the 5-L continuous stirred reactor.

Geometrical Ratio	
H_L/T	1.64
H_T/T	2.14
h/T	0.43
J/T	0.11
C/T	0.48-0.96
D/T pitched-blade turbine	0.75
D/T marine propeller	0.57
Operation conditions	
Aeration rate	0.3-3.7 vvm
Agitation rate	370-1040

Table 2. Codified values of the variables for the experimental design.

Run	Aeration rate	Agitation rate	Impeller height
1	-1 (1 vvm)	-1 (500 rpm)	-1 (8 cm)
2	1 (3 vvm)	-1	-1
3	-1	1 (900 rpm)	-1
4	1	1	-1
5	-1	-1	1 (12 cm)
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-α (0.3 vvm)	0 (700 rpm)	0 (10 cm)
10	α (3.7 vvm)	0	0
11	0 (2.0 vvm)	-α (370 rpm)	0
12	0	α (1040 rpm)	0
13	0	0	-α (6.7 cm)
14	0	0	α (13.4 cm)
15	0	0	0
15a	0	0	0
15b	0	0	0

operation conditions of the reactor. According to previous studies (Acevedo and Aroca, 1986; Myers and Bakker, 1998), two types of impellers were used: a pitched-blade turbine (0.105 m diameter) pumping up (6MFU) and a marine propeller (0.080 m diameter) pumping up (3MPU). Independent liquid and solids feeds were used. Solids were fed by means of an adapted syringe pump (Model 352, Sage Instruments, Cambridge, MA); a Cole-Parmer (Chicago, IL) peristaltic pump was used for the incoming liquid. All runs were performed at 33ºC and pH 1.5.

Solids content was measured by dried weight. Exit composition was determined from samples taken from theliquid surface; mean solids were estimated as a weighed mean of samples taken from different heights of the CSTR. It was considered that complete suspension of solids was attained when particles remained on the bottom of the tank for less than 2 seconds, as observed with a mirror placed under the bottom of the reactor.

Experimental design

Experimental data, obtained from CCR experimental design, was fitted to a second order polynomial function. Response surface methodology (RSM) was used, which allowed the building of models, evaluation of the effects of factors, and searching optimum conditions. In this work, RSM (with a three-level CCR experimental design) was applied to study the effect of three factors (aeration rate, stirrer speed and impeller height) on solids suspension in a CSTR. Two response variables were measured: pulp density at the exit and inside the reactor. A design with five codified levels, including stars points, was used (Table 2). A value of α = 1.68 and two replicates in the centre chosen in this work ensure the orthogonality and rotatability of the experimental design. The quality of obtained model was measured using the coefficient of determination (R^2), the significance of each parameter through an F-test (calculated p-value), and the lack of fit of the model. Coefficients with a p-value lower than 0.01 were considered significant. First partial derivatives were calculated to obtain optimal values.

RESULTS AND DISCUSSION

The results obtained for all runs of the experimental design are given in Table 3. Although no solids accumulated on the bottom, complete homogenisation was not possible. At all conditions exit solids concentration was lower than mean and bottom concentrations.

These results were well represented by response surfaces and mathematical models for each of the four cases: exit and mean solids content for the 6MFU and 3MPU impellers.

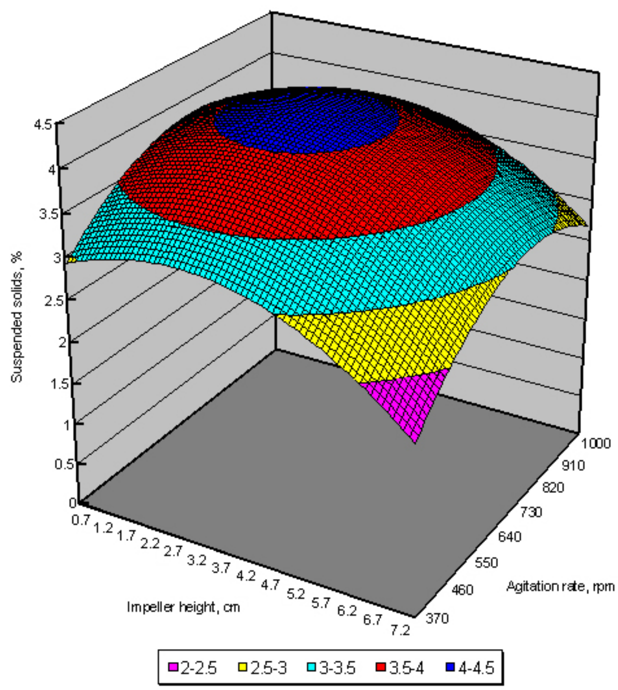

Figure 2. Solids concentration in the exit stream as a function of impeller position and agitation rate in the case of the 3MPU impeller, 6% w/v pulp density, 2.0 vvm.

Figure 2 and Figure 3 show the response surfaces of the exit and mean solids concentration as a function of impeller height and agitation rate for the case of the 3MPU impeller

at 2.0 vvm. Analogue surfaces were obtained for other aeration rates and for the 6MFU impeller.

These two figures show the existence of optimum values for impeller speed and impeller height that maximize the suspension of the solids. The effect of aeration rate was not consistent and only contributed to the curvature of the response surface of the mean solids concentration in the 3MPU impeller (Table 4). The optimum values for impeller speed and height could be related to the co-existence of two phenomena: the radial flow and the axial flow (up-flow) produced by the operation of the impellers. A *pumping zone* (associated with the axial flow) and a *re-circulation zone* (associated with the radial flow) were identified by following the trajectory of air bubbles during the operation of the reactor without mineral (*i.e.* air-water system). While the increase in size and strength of the *pumping zone* favour the suspension of solids, the increase in the strength of the *re-circulation zone* could be detrimental for solids suspension depending on its size and position. For example, if the size of the *re-circulation zone* is smaller than the total height of the reactor (which is the case in our system) this zone could isolate the bottom and surface of the reactor, and negatively affect the suspension of the solids. An increase in the impeller speed will result in stronger *pumping* and *re-circulation zones*, producing positive effects in the suspension of the solids up to a certain level.

This explains the existence of optimum impeller speeds that equilibrate positive and negative effects and maximise the solids suspension. The optimal position of the impeller also allows to maximise the positive effect of the pumping capacity of the impeller (*pumping zone*) and to minimise the negative effects of the radial flow (*re-circulation zone*). Therefore, the change in strength of competing *pumping* and *re-circulation zones* (due to the change in impeller speed and position) appear to be a reasonable explanation for the existence of optimum values for impeller height and position that maximise the solids suspension.

The mathematical models derived from the experimental results and their determination coefficients are shown in Table 4. Only the significant effects, as determined by F and p values from the analysis of variance, are included (Appendix).

In equations 1 to 4 of Table 4, "h" represents the impeller position (impeller height) in the cylindrical section of the tank, so it does not include the 6 cm of height of the round bottom.

Table 5 summarises the optimal values obtained at 2.0 vvm for the aeration and agitation conditions that maximise solids suspension measured at the exit and as mean or overall content inside the reactor fed with 6% w/v of mineral concentrate. It can be appreciated that although the 3MPU requires higher agitation rates, the optimal values of the impeller position and the agitation rate are relatively similar for both cases. This is an important feature, as it allows to operate in a condition near the optimum values for both variables. On the contrary, the optima differ

7**Table 3. Exit and mean solids concentration for each impeller at the different operation conditions.**

	Pitched-blade turbine (6MFU)		*Marine propeller (3MPU)*	
Run	**Exit, % w/v**	**Mean, % w/v**	**Exit, % w/v**	**Mean, % w/v**
1	2.98	4.96	3.13	4.32
2	4.10	4.42	3.84	4.78
3	3.41	5.15	3.57	4.76
4	3.72	5.82	3.85	5.75
5	2.02	3.82	2.95	3.98
6	2.43	3.96	3.26	4.35
7	4.03	4.93	3.35	4.43
8	3.56	5.43	3.47	4.98
9	3.63	5.43	3.86	4.15
10	2.80	5.52	4.28	4.86
11	2.39	3.43	3.18	4.64
12	3.74	4.88	3.56	5.15
13	4.16	5.26	3.95	5.06
14	3.64	4.39	2.73	4.08
15	3.29	5.35	4.01	5.35
15a	3.25	5.32	3.88	5.40
15b	3.35	5.42	3.95	5.45

Table 4. Mathematical models representing the response surfaces for the exit and mean solids composition for each impeller location.

Impeller	Model	R^2
3MPU, exit	$\%w/v = - 0.825 + 0.547\ (vvm) + 0.392\ (h) - 5.8\cdot10^{-6}\ (rpm)^2 - 0.059\ (h)^2$	0.9272
3MPU, mean	$\%w/v = 0.23 + 1.29\ (vvm) + 0.006\ (rpm) + 0.57\ (h) - 0.30\ (vvm)^2 - 4.14\cdot110^{-6}\ (rpm)^2 - 0.07(h)^2$	0.9960
6MFU, exit	$\%w/v = 2.18 + 0.005\ (rpm) - 1.25\ (h) - 0.001\ (vvm)(rpm) - 0.092\ (vvm)(h) + 0.0009\ (rpm)(h) - 3.5\cdot10^{-6}\ (rpm)^2 + 0.074\ (h)^2$	0.8915
6MFU, mean	$\%w/v = 0.84 + 0.014\ (rpm) – 0.046\ (h) + 0.001\ (vvm)(rpm) - 1.1\cdot10^{-5}\ (rpm)^2 - 0.046\ (h)^2$	0.9807

Table 5. Optimal agitation rate and impeller height that maximise exit and mean solids content[a].

	Pitched-blade turbine (6MFU)		Marine propeller (3MPU)	
	Exit	**Mean**	**Exit**	**Mean**
Solids, % w/v	5.02	5.26	4.15	5.6
Agitation rate, rpm	630	760	770	860
Impeller height, cm	13.0	8.7	9.1	9.0

[a] Aeration rate: 2.0 vvm, feed concentration: 6% w/v

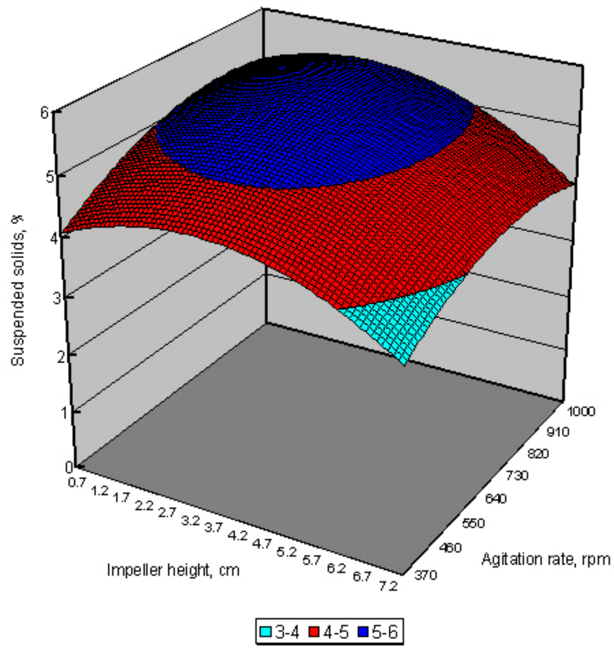

Figure 3. Mean solids concentration in the tank as a function of impeller position and agitation rate in the case of the 3MPU impeller, 6% w/v pulp density, 2.0 vvm.

significantly in the case of 6MFU. Moreover, the higher agitation rate required for the propeller does not imply higher power consumption, because of its smaller diameter (0.08 m as compared with 0.105 m of the turbine) and lower power number.

The Power Number under turbulent condition is 1.1 for 6MFU and 0.38 for 3MPU (Perry and Green, 1984). Considering the impellers diameters and the agitation rates, the ungassed power consumption for 6MFU would be of 19 to 34 W and that of 3MPU would be only of 3 to 4 W. The effect of aeration on these values would be similar for both impellers.

It is considered that the results obtained in this work can be extrapolated to a system inoculated with oxidizing micro organisms since their biomass will be negligible compared to that of the suspended mineral, so it will not change the hydrodynamic properties of the slurry.

It is concluded that under the experimental conditions used in this work the best solids suspension is obtained with 2.0 vvm, C/T = 0.64 and a marine propeller pumping up operated at 770-860 rpm.

REFERENCES

ACEVEDO, F. The use of reactors in biomining processes. *Electronic Journal of Biotechnology* [on line], 2000, vol. 3, no. 3, p. 184-194. Available from internet: http://ejbiotechnology.ucv.cl/content/vol3/issue3/full/4/. ISSN 0717-3458.

ACEVEDO, F. and AROCA, G.. Studies on the agitation and power characteristics of mineral slurries. In: LAWRENCE, R.W.; BRANION, R.M. and EBNER, H.G., eds. *Fundamental and Applied Biohydrometallurgy*. Amsterdam, Elsevier Science Publishers, 1986, p. 255-261.

ARMENANTE, P.M.; NAGAMINE, E.U. and SUSANTO, J. Determination of correlations to predict the minimum agitation speed for complete solid suspension in agitated vessels. *Canadian Journal of Chemical Engineering*, 1998, vol. 76, no. 3, p. 413-419.

BRUCATO, A. and BRUCATO, V. Unsuspended mass of solid particles in stirred tanks. *Canadian Journal of Chemical Engineering*, 1998, vol. 76, no. 3, p. 420-427.

CANALES, C.; ACEVEDO, F. and GENTINA, Juan Carlos. Laboratory-scale continuous bio-oxidation of a gold concentrate of high pyrite and enargite content. *Process Biochemistry*, 2002, vol. 37, no. 10, p. 1051-1055.

DEW, D.W.; LAWSON, E.N. and BROADHURST, J.L. The BIOX® process for biooxidation of gold-bearing ores or concentrates. In: RAWLINGS, D.E., ed. *Biomining. Theory, Microbes and Industrial Processes*. Berlin, Springer Verlag, 1997, p. 45-80.

DOHI, N.; MATSUDA, Y.; ITANO, N.; MINEKAWA, K.; TAKAHASHI, T. and KAWASE, Y. Suspension of solid particles in multi-impeller three-phase stirred tank reactors. *Canadian Journal of Chemical Engineering*, 2001, vol. 79, no. 1, p. 107-111.

ENFORS, S.O.; JAHHIC, M.; ROZKOV, A.; XU, B.; HECKER, M.; JÜRGEN, B.; KRÜGER, E.; SCHWEDER, T.; HAMER, G.; O'BEIRNE, D.; NOISOMMIT-RIZZI, N.; REUSS, M.; BOONE, L.; HEWITT, C.; MCFARLENE, C.; NIENOW, A.; KOVACS, T.; TRÄGARDH, C.; FUCHS, L.; REVSTEDT, J.; FRIBERG, P.C.; HJERTAGER, B.; BLOMSTEN, G.; SKOGMAN, H.; HJORT, S.; HOEKS, F.; LIN, H.Y.; NEUBAUER, P.; VAN DER LANS, R.; LUYBEN, K.; VRADEL, P. and MANELIUS, A. Physiological responses to mixing in large scale bioreactors. *Journal of Biotechnology*, 2001, vol. 85, no. 2, p. 175-185.

GREENHALGH, P. and RITCHIE, I. Advancing reactor designs for the gold bioleaching process In: *Biomine '99, Conference Proceedings*. (23rd – 24th August, 1999, Perth, Australia). Australian Mineral Foundation. p. 52-60.

HARVEY, P.I.; BATTY, J.D.; DEW, D.W.; SLABBERT, W. and VAN BUUREN, C. Engineering considerations in bioleach reactor design. In: *Biomine '99, Conference Proceedings*. (23rd – 24th August, 1999, Perth, Australia). Australian Mineral Foundation. p. 88-97.

HEBRARD, G.; BASTOUL, D.; ROUSTAN, M.; COMTE, M.P. and BECK, C. Characterization of axial liquid dispersion in gas-liquid and gas-liquid-solid reactors. *Chemical Engineering Journal*, 1999, vol. 72, no. 2, p. 109-116.

KIRAN, K.R.; MANOHAR, B. and DIVAKAR, S. A central composite rotable design analysis of lipase catalyzed synthesis of lauroyl lactic acid at bench-scale level. *Enzyme and Microbial Technology*, 2001, vol. 29, no. 2-3, p. 122-128.

LI, C., BAI, J., CAI, Z. and OUYANG, F. Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. *Journal of Biotechnology*, 2002, vol. 93, no. 1, p. 27-34.

LÓPEZ, R. *Diseño Estadístico de Experimentos*. La Habana, Cuba, Editorial Científico-Técnica, 1988, p. 171-192.

MYERS, K.J. and BAKKER, A. Solids suspension with up-pumping pitched-blade and high-efficiency impellers. *Canadian Journal of Chemical Engineering*, 1998, vol. 76, no. 3, p. 433-440.

NAMDEV, P.K.; DUNLOP, E.H.; WENGER, K. and VILLENEUVE, P. Role of turbulence in fermentations. In: GALINDO, E. and RAMÍREZ, O.T., eds. *Advances in Bioprocess Engineering*. Dordrecht, Kluwer Academic Publishers, 1994, p. 149-156.

NIENOW, A.W. and BUJALSKI, W. Recent studies on agitated three phase (gas-solid-liquid) systems in the turbulent regime. In: *UK Conference on Mixing*. (7th, 10th - 11th July, 2002, University of Bradford, Bradford, UK). Available from internet: www.brad.ac.uk/acad/rheology/Fluidmixing7.

OLDSHUE, J.Y. *Fluid Mixing Technology*. New York, McGraw-Hill Publications Co., 1983, 574 p.

OLDSHUE, J.Y. Suspending solids and dispersing gases in mixing vessel. *Industrial and Engineering Chemistry*, 1969, vol. 61, no. 9, p. 79-89.

OOLMAN, T. Bioreactor design and scaleup applications in mineral bioleaching. In: TORMA, A.E.; WEY, J.E. and LAKSMAN, V., eds. *Biohydrometallurgical Technologies*, vol. I. Warrendale, PA., The Minerals, Metals and Materials Society, 1993, p. 401-415.

PERRY, R.H. and GREEN, D.W. *Perry's Chemical Engineer's Handbook*. 6th ed. New York, McGraw-Hill Book Co., 1984, p. 19-10.

RAWLINGS, D.E.; DEW, D. and DU PLESSIS, C. Biomineralization of metal-containing ores and concentrates. *Trends in Biotechnology*, 2003, vol. 21, no. 1, p. 38-44.

RAWLINGS, D.E. *Biomining: Theory, Microbes and Industrial Processes*. Berlin, Springer-Verlag, 1997, 302 p.

RIEGER, F. Effect of particle content on agitator speed for off-bottom suspension. *Chemical Engineering Journal*, 2000, vol. 79, no. 2, p. 171-175.

SHEEJA, R.Y. and MURUGESAN, T. Studies on biodegradation of phenol using response surface methodology. *Journal of Chemical Technology and Biotechnology*, 2002, vol. 77, part 11, p. 1219-1239.

SPENCER, P.A.; SATALIC, D.M.; BAXTER, K.G. and PINCHES, T. Key aspects in the design of a bacterial oxidation reactor. In: *Conference Proceedings, IBS'97–BIOMINE'97, Biotechnology comes of age*. (4th - 6th August, 1997, Sydney, Australia) Paper M14.1.

TATTERSON, G.B. *Scaleup and Design of Industrial Mixing Processes*. New York., McGraw-Hill, Inc., 1996, p. 107-201.

TOMA, M.K.; RUKLISHA, M.P.; VANAGS, J.J.; ZELTINA, M.O.; LEITE, M.P.; GALININA, N.I.; VIESTURS, U.E. and TENGERDY, R. Inhibition of microbial growth and metabolism by excess turbulence. *Biotechnology and Bioengineering*, 1991, vol. 38, p. 552-556.

WEN, Z.Y. and CHEN, F. Application of statistically-based experimental designs for the optimization of eicosapentaenoic acid production by the diatom *Nitzschia laevis*. *Biotechnology and Bioengineering*, 2001, vol. 75, no. 2, p. 159-169.

ZWIETERING, T.N. Suspending of solid particles in liquid by agitators. *Chemical Engineering Science*, 1958, vol. 8, p. 244-253.

Appendix Table A.1. Example of analysis of variante. The complete results of the analysis of variance (López, 1988) for the mean solids content in the case of marine propeller are presented in the table. Similar results were obtained for exit solids and the pitched-blade turbine.

Table A.1. Analysis of variance for mean solids concentration, 3MPU.

Effect	Sum of squares	Degrees of freedom	Mean square	F-values	p-values
A: aeration rate	0.9301	1	0.9301	372.05	0.0027
B: agitation rate	0.8206	1	0.8206	328.25	0.0030
C: impeller height	0.9063	1	0.9063	362.53	0.0027
AB	0.0630	1	0.0630	25.20	0.0375
AC	0.0351	1	0.0351	14.04	0.0644
BC	0.0136	1	0.0136	5.44	0.0448
AA	1.0400	1	1.0400	416.02	0.0024
BB	0.3101	1	0.3101	124.04	0.0080
CC	0.8886	1	0.8886	355.45	0.0028
Error	0.1452	5	0.1452	11.62	0.0812

$R^2 = 0.966046$; $p<0.01$, significant effect