Potential for Waste Reduction in Activated Sludge Systems: Evaluation of the Initial Conditions of a Rapid Test with Rhamnolipid Biosurfactant

Verônica M. F. Alexandre*[1], Melissa L. E. Gutarra[2], Denise M. G. Freire[3], Magali C. Cammarota[4]

[1]Department of Biochemical Engineering, Federal University of Rio de Janeiro, Avenida Athos da Silveira Ramos, 149, Bloco E, Sala 203, Cidade Universitária, CEP 21941-909, Rio de Janeiro, Brazil
e-mail: vero.marinho@gmail.com

[2]Campus Xerém, Federal University of Rio de Janeiro, Estrada de Xerém, 27, Xerém, CEP 25245-390, Duque de Caxias, Brazil
e-mail: gutarra@gmail.com

[3]Department of Biochemistry, Federal University of Rio de Janeiro, Avenida Athos da Silveira Ramos, 149, Bloco A, Sala 549-1, Cidade Universitária, CEP 21941-909, Rio de Janeiro, Brazil
e-mail: freire@iq.ufrj.br

[4]Department of Biochemical Engineering, Federal University of Rio de Janeiro, Avenida Athos da Silveira Ramos, 149, Bloco E, Sala 203, Cidade Universitária, CEP 21941-909, Rio de Janeiro, Brazil
e-mail: christe@eq.ufrj.br

ABSTRACT

Adding chemicals that alter the microbial metabolism and reduce cell growth, without affecting organic matter removal, is an alternative technology to reduce sludge production. This study was intended to determine the best initial conditions to conduct a rapid test (4 h), which evaluates the potential of chemicals to reduce microbial growth and chemical oxygen demand removal. A commercial biosurfactant was used as model product and a central composite design (face centered) was performed using substrate/inoculum ratio and rhamnolipid/inoculum ratio as independent variables, and cellular yield coefficient, substrate consumption rate, and specific oxygen uptake rate for exogenous respiration as response variables. Lower values of substrate/inoculum ratio permitted larger reductions of cellular yield coefficient with lower rhamnolipid concentrations. The best condition was 1.06 g chemical oxygen demand/g total suspended solids and 25 mg rhamnolipid/L or 25 mg rhamnolipid/g total suspended solids, which achieved a reduction of 50-75% in cellular yield coefficient.

KEYWORDS

INTRODUCTION

In Wastewater Treatment Plants (WWTPs), biological processes ensure the removal of biodegradable organic matter, at lower costs than physical-chemical processes [1], and are considered the main treatment step [2]. Among the available biological processes,

---

* Corresponding author

activated sludge system is one of the most commonly used due to its high efficiency and versatility [3]. However, secondary sludge, formed as a result of microbial growth, is an inevitable waste that needs to be treated and disposed of, and this sludge is the main environmental and economic issues in wastewater treatment [4]. In conventional activated sludge systems, each ton of applied organic matter [quantified as Chemical Oxygen Demand (COD)] can produce up to 800 kg of primary and secondary sludge [quantified as Total Suspended Solids (TSS)], while in extended aeration systems, total sludge production is 500 kg TSS/ton COD applied [5]. Despite the relatively small volume produced, which is around 1% of the wastewater volume treated, the cost of sludge management is significant and can reach up to 60% of the total operational costs in a WWTP [6], with values between 350 and 750 EUR/ton of dry matter [7].

The main objective of Directive 2008/98/EC of the European Commission is to minimize negative effects that the generation and management of wastes can cause to the environment and human health. Therefore, prevention is the first action in the waste management hierarchy. Similarly, in Brazil, law No. 12,305/2010, known as the National Policy on Solid Waste (NPSW), proposes the following as waste management hierarchy: no generation, minimization, reuse, recycle, treatment, and final disposal. Figure 1 shows available strategies for excess sludge management. When it is seen as a by-product, waste management hierarchy does not apply, because it is no longer seen as a waste. Thus, eco-innovation should be used to recover its material or energy [8].

Excess sludge

Waste

Product

Waste Management Hierarchy

Eco-innovation

- No generation
- Reduced generation
- Reuse
- Recycle
- Treatment
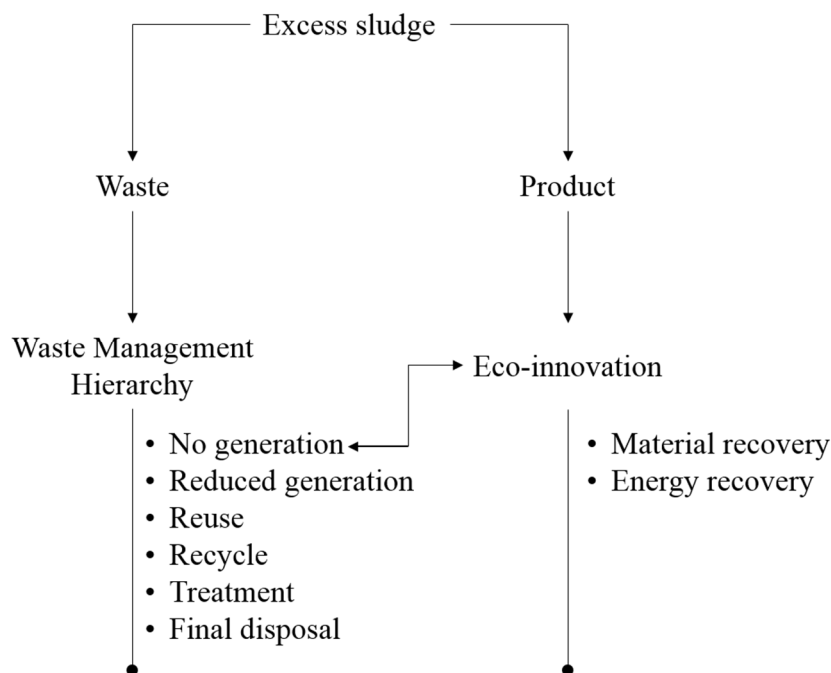- Final disposal

- Material recovery
- Energy recovery

Figure 1. Sludge management strategies

In Europe, the most common options for material and energy recovery are agricultural use and incineration, respectively [8]. Nonetheless, some countries have considered environmental risks associated with the agriculture use of sludge. Switzerland, for example, has banned this practice since 2005 [8]. For energy recovery through incineration, the moisture content of the sludge requires a very efficient dewatering stage, and even then, in some cases, the energy balance may still be unfavourable [4, 5].

In Brazil, every facility must present to the environmental agency a Waste Management Plan, which must contain targets and procedures for minimizing waste generation. Therefore, studies regarding sludge management must be suitable for Brazil. The sludge produced in biological treatment of industrial wastewater is significant [7]

and may contain toxic and recalcitrant substances. Some municipal WWTPs also receive industrial wastewater, which can transfer undesirable contaminants to the sludge and, as a consequence, to the environment [9]. Therefore, although agriculture use of sewage sludge is allowed by law in Brazil, illegal discharges of industrial wastewater into sewage systems represent an environmental risk of this practice. In Brazil, as well as in Latin America in general, incineration for energy recovery is not a common practice, especially due to high costs of the process and the need for skilled labour [10]. In addition, the distance between sludge generation and incineration points can be a limiting factor due to transportation, because it can increase costs and release secondary emissions [11].

Hence, the use of excess sludge as a by-product might not currently be the ideal strategy for Brazil. Sludge management consists of a set of operations with high energy consumption [12]. For sustainability in WWTP, technologies capable of preventing sludge generation may be more appropriate and they would still fall under eco-innovation (Figure 1), because they would promote economic value to the process, due to cost reduction and significantly reduced environment impacts [13]. The benefits obtained with this strategy are resource and area savings, as the volume of sludge treated would be lower.

Among the technologies able to reduce sludge generation in activated sludge systems is the addition of products that alter microbial metabolism in the aeration tank, reducing cellular growth without affecting removal of organic matter. The advantage is that only a product dosing tank is necessary, which makes the technology very attractive [6]. On the other hand, there is the problem of microbial adaptation to the added product, which leads to the loss of its effectiveness [14]. Therefore, the search for new products with prolonged effect is necessary, and so is the understanding of all phenomena involved in the process [7].

The existence of a rapid test to evaluate the potential of a product to reduce microbial growth is extremely important, because it helps in the decision about which product should be used and which experimental conditions lead to better results. A very widespread test found in literature [14-19] uses inoculum from an activated sludge Sequencing Batch Reactor (SBR), in which the feed consists of a synthetic wastewater. For the test, the product is added to a smaller reactor along with inoculum and feed, and a Control reactor is always run in parallel, in which no product is added. Initial and final concentrations of biomass and substrate are determined through mixed liquor Total or Volatile Suspended Solids (TSS or VSS) and soluble COD, respectively. Then, observed growth yield (Y) is calculated. To minimize sludge decay, reaction time should be kept low.

Despite its simplicity, this rapid test has not yet been standardized: reaction time varies from 3 [15] to 9 h [14], carbon source varies from simple substrate, like sodium acetate [17], to more complex ones, like starch [14], and S_0/X_0 ratio varies from 0.12 [17] to 2 [18]. The choice of variables can influence the result obtained with the product added to reduce microbial growth. Increasing S_0/X_0, even without the addition of any product, reduces the value of *Y* because of metabolic uncoupling in the cells [20]. The product/biomass ratio is more important than the evaluated product concentration, and in the case of metabolic uncouplers, represents the real strength that a product is capable of exerting on microbial cells [21]. Therefore, the initial condition of the test can considerably influence the results obtained.

Hence, the main objective of this study is to determine the effects of the initial condition of the rapid test on the responses. The secondary objective is to determine the best initial condition of the rapid test to evaluate the effect of a model product used to reduce cell growth – a commercial Rhamnolipid (RL) biosurfactant. Biosurfactants are very interesting molecules due to their environmental compatibility [22]. Because they have widely verified antimicrobial activity, specially rhamnolipids [22, 23], they can

inhibit the growth of some microbial species in activated sludge, thus reducing sludge production.

Technologies that aim to reduce sludge are still widely studied [24], even with all the paradigm shifts that see excess sludge as a by-product, and not a waste. However, pilot and full-scale studies are not as widespread as they should be [7, 24]. Part of this phenomenon is due to the lack of a thorough study of the technologies. When it comes to addition of products in the aeration tank, for example, it is essential to know everything about the mechanism of action, evaluating if combined effects of all variables could affect microbial growth. Therefore, this study can be used as the basis for defining a simple methodology to help choose the best product and condition for maximum growth reduction efficiency. Then, efforts can be directed towards more thorough studies, aiming at more information for the actual implementation of the technology.

MATERIALS AND METHODS

In this study, the commercial product R90 from Agae Technologies (Corvallis, Oregon, USA) was used. It is a lyophilized product with 90% purity. An analysis showed that the product contains 564 mg RL/g dry matter (223 mg rhamnose/g dry matter) and Critical Micellar Concentration (CMC) of 154 mg RL/L (61 mg rhamnose/L).

Cultivation of activated sludge

The activated sludge used in the experiments was collected from a municipal WWTP located in Rio de Janeiro, Brazil, and was kept refrigerated (4 °C) until use. The sludge was cultivated in a Sequencing Batch Reactor (SBR) to serve as inoculum in the rapid test. The 5 L working volume reactor was kept at room temperature (19-28 °C) and had initial concentration of 2,000 mg TSS/L. Compressed air was introduced into the system through a 10 cm (length) × 2 cm (base) × 2 cm (height) trapezoidal diffuser located in the bottom centre of the reactor to ensure the homogeneity of the system and appropriate Dissolved Oxygen (DO) levels (at least 4 mg/L). The medium (2.6 L) was changed twice a day, once in the morning and then 8 h later. The feed consisted of commercial medium TSB (COD 1,000 mg/L) and sodium bicarbonate ($NaHCO_3$) (0.9 g/L) to maintain pH around 7 in the reactor. To avoid significant changes in the microbial consortium, the operation was set to a 30-d maximum, and no experiments were conducted during the first week to allow adaptation of the microorganisms to the medium and working conditions. The mixed liquor was removed from the reactor to be used as inoculum in the rapid test before any medium change was done on the day (approximately 15 h after the previous change), and this led to a sludge age of 10 d.

Rapid test

The rapid test was performed in batch mode, using 1 L reactor with 0.6 L working volume, consisting of 100 mL phosphate buffer 0.1 M pH 7, appropriate volume of inoculum for the desired initial TSS, appropriate volume of feed (TSB medium) for the desired initial COD, and distilled water to complete the volume. Appropriate volume of RL solution was added in Test reactors (0.4-1.8 mL). For each Test condition, there was a similar Control reactor that did not receive any RL. All reactors operated with magnetic stirring and compressed air introduced through a porous cylindrical diffuser with 3.5 cm (height) × 1 cm (diameter) located at the bottom of each reactor. Air flow was manually controlled so that the foam was kept inside the Test reactors, which led to DO of at least 0.5 mg/L. In the Control reactors, similar air flows were used to maintain the same DO levels in all reactors. Every hour up to 4 h, 25 mL samples were taken to analyse TSS and soluble COD to determine *Y* and *k* (substrate consumption rate). Then, after 30 h, Specific Oxygen Uptake Rate (SOUR) was determined. In this study, all test results are reported

considering Control value of 1, unless noted otherwise, which is why the variables are presented without units. Thus, values greater than 1 indicate an increase in the response due to RL addition, and values lower than 1 indicate a reduction.

Design of experiments. A Face-Centred Cube Design (FCCD, Table 1) was used to investigate the effect of the independent variables S_0/X_0 and RL/X_0 on the response variables analysed (Y, k, SOUR), fixing initial biomass concentration at 1,000 mg TSS/L.

Table 1. Coded and real values of the independent variables used in the FCCD

Variables	Levels		
	−1	0	1
S_0/X_0 [g COD/g TSS]	0.12	1.06	2.00
RL/X_0 [mg RL/g TSS]	15	25	35

Validation. In this step, twelve conditions were tested to validate the models previously obtained. Two initial biomass concentrations were also used: 1,000 and 2,000 mg TSS/L.

Analytical methods

Rhamnose was quantified by HPLC after acid hydrolysis and CMC was determined by the pendant drop technique [23]. Rhamnose values were converted to rhamnolipid content according to Santa Anna [25]. Soluble COD and TSS of the mixed liquor were determined according to standard methodologies [26]. Cell yield was calculated from the increase of TSS divided by the decrease in soluble COD in the 4 h period. The k value was determined by exponential adjustment of the experimental data in the substrate degradation curve (COD) over the 4 h. Then, 30 h from the beginning of the rapid test, the mixed liquor was transferred to a 300 mL BOD bottle equipped with a DO probe and magnetic stirrer, along with 10 mL of sodium acetate 0.3 M. Temperature was kept at 20-22 °C. The change in the DO level was monitored every 30 s for at least 15 min or until 1 mg/L, whichever occurred first [26]. SOUR was determined from DO depletion rate and TSS of the mixed liquor. SOUR was also used to determine TSB and RL consumption rates, using mixed liquor from the SBR reactor (before the first medium change of the day), TSB medium and RL solution, at different initial conditions. All data were analysed in the software Protimiza (http://experimental-design.protimiza.com.br) considering confidence level of 90%.

RESULTS AND DISCUSSION

Results obtained in each condition of the FCCD are presented in Table 2. Microbial growth (evaluated through the Y value) varied widely, regardless of the RL concentration, in some cases, it reduced up to 100% ($Y = 0$, exp. 3-5), but in other cases, it increased up to 31% ($Y = 1.31$, exp. 6). For example, the lowest RL concentration (15 mg RL/g TSS) reduced Y by 77% (exp. 1) when S_0/X_0 had the lowest value (0.12 g COD/g TSS), and increased Y by 24% (exp. 2) when S_0/X_0 had the highest value (2.00 g COD/g TSS).

Analysis of Variance (ANOVA) showed that the pure error in the models presented extremely low values (around 2%), indicating no repeatability problems [27]. For all the responses evaluated, the model adjustment explained 75-78% of the results obtained. The differences between predicted and experimental values could be explained by the error propagation in the analyses, especially for Y. In an attempt to overcome lack of fit and regression simultaneously, non-significant variables were removed from the model,

however, this made R^2 values considerably lower (as low as 30%). Therefore, the complete model was maintained, and the coefficients and respective *p*-values are presented in Table 3.

Table 2. Results obtained in the FCCD

Exp.	Independent variables		COD removal[b]	Response variables		
	S_0/X_0[a]	RL/X_0[a]		Y[b]	k[b]	SOUR[c]
1	−1	−1	0.96	0.23	0.93	1.17
2	1	−1	0.87	1.24	0.92	0.94
3	−1	1	0.77	0.00	0.77	1.21
4	1	1	0.90	0.00	0.87	1.78
5	−1	0	0.79	0.00	0.75	1.01
6	1	0	0.76	1.31	0.78	1.79
7	0	−1	1.10	1.25	1.09	1.04
8	0	1	1.22	0.75	1.28	0.90
9	0	0	0.93	0.34	0.91	0.87
10	0	0	0.88	0.51	0.86	0.87
11	0	0	0.93	0.69	0.90	1.07

Note: Values in relation to each Control experiment (Control results = 1)
[a] Coded values
[b] For 4 h period
[c] For 30 h period

Table 3. Regression obtained in FCCD

	Coef.	*p*-value	Coef.	*p*-value	Coef.	*p*-value
	Response: Y		Response: k		Response: SOUR	
Mean	0.68	0.0157	0.93	0.0000	0.97	0.0005
S_0/X_0 (L)	0.39	0.0495	0.02	0.6533	0.19	0.1105
S_0/X_0 (Q)	−0.27	0.3014	−0.22	0.0179	0.38	0.0505
RL/X_0 (L)	−0.33	0.0801	0.00	0.9397	0.12	0.2568
RL/X_0 (Q)	0.08	0.7453	0.20	0.0286	−0.05	0.7497
S_0/X_0 vs. RL/X_0	−0.25	0.2275	0.03	0.6151	0.20	0.1510

Note: (L) Linear
(Q) Quadratic
Values in bold represent significant terms of the model (90% confidence level)

The main objective of this study is to evaluate response behaviour under different initial situations. Although the models did not present a perfect fit, the qualitative analysis of the results can still be considered reliable and a reasonable quantitative result was obtained. The response surface for Y (Figure 2a) clearly shows that the effect in microbial growth depends on the initial condition of the test: the higher the value of S_0/X_0, the greater the amount of RL needed to achieve a certain percentage of growth reduction. This is due to energetic uncoupling when S_0/X_0 has a high value, leading to a lower value of Y, even when no product is added [20]. This effect was also observed in this study: Y in Control experiments was 1.27 ±0.64, 0.75 ±0.09, and 0.58 ±0.10 g TSS/g COD for S_0/X_0 0.12, 1.06, and 2.00 g COD/g TSS, respectively. As the reference values used to assess growth decreased considerably with increased S_0/X_0, the product added to maintain the same percentage of growth reduction required increased concentration to further reduce Y value of the Test. The result obtained is extremely important, because biosurfactants are expensive due to high production costs [28], which can make their use impossible in less noble applications, such as WWTPs. In a situation of substrate

deficiency, low concentrations of RL would be sufficient to significantly reduce growth and reduce the cost of applying the product. In plants that use SBR, all that is required is to maintain low S_0/X_0. In complete mix systems, considering that the influent is instantly and completely mixed with the reactor content, and that generally all biodegradable organic matter is completely consumed in the biological reactor [29], no excess substrate is expected in the reactor and a low S_0/X_0 is maintained.

A 52% reduction in sludge disposal was observed in activated sludge SBR with the addition of 19 mg RL/g VSS, using non-purified RL produced through fermentation with *Pseudomonas aeruginosa* (PA1), using glycerol PA as only carbon source [30]. However, the mechanism of action has still not been demonstrated. One possibility is the natural antimicrobial effect due to biosurfactant interaction with the lipid fraction of cellular membranes, which could affect its integrity [31, 32], inhibiting the growth of some species in the activated sludge consortium. Nonetheless, this does not explain the different results obtained in this study, because the initial microbial population was essentially the same in all conditions evaluated.

Another possibility is that, in the concentrations evaluated, RL only acted as an additional substrate to the medium, and probably had a lower consumption rate than the constituents of TSB medium. Therefore, when the substrate was deficient in the reactor (low S_0/X_0), cells used RL to obtain the energy necessary for metabolism over the test period. If the consumption rate was lower, cells could not obtain immediate energy, which was why no significant growth occurred during the 4 h period. Surface response for k (Figure 2b) showed that when S_0/X_0 was low, k could be up to 30% lower than Control experiments.

Assuming that substrate consumption was proportional to oxygen consumption [33], SOUR was determined under different conditions to verify whether TSB medium and RL were consumed at different rates. When biomass concentration was 100 mg TSS/L, SOUR values for TSB (COD 100 mg/L) and RL (COD 100 mg/L ≡ 27 mg RL/L) were 0.73 and 0.26 mg O_2/g TSS min., respectively, meaning that RL was consumed at a rate 64% lower than TSB. When biomass concentration was 1,000 mg TSS/L, the same as in FCCD, SOUR values for TSB medium (COD 1,000 mg/L) with and without RL (25 mg RL/L) were 0.80 and 0.93 mg O_2/g TSS min., respectively. This means that RL reduced substrate consumption by 14%. This value is consistent with the reductions in k obtained in the FCCD and, therefore, it can be stated that the consumption rate of RL was lower than the consumption rate of TSB medium.

The conditions that lead to 100% growth reduction observed with lower values of S_0/X_0 (0.12 g COD/g TSS, exp. 3 and 5) is not of interest, because it would result in total loss of biomass in the activated sludge reactor, since there would be no cellular renovation. As can be seen in Figure 2 (a and b), when S_0/X_0 increased up to the central point, growth reduction was still significant (25-75%) and values of k in Test were not far from Control experiments (between 15% lower and 10% higher than Control). COD removal (Table 2) was 24% lower to 22% higher than Control conditions. However, considering the region around the central point, COD removal in Test conditions were, on average, 10% lower than Control. Considering the error in the COD analysis, reduction in k and COD removal was too small to represent a problem. Therefore, ideally S_0/X_0 cannot be so low, and should be restricted to a maximum value of 1.06 g COD/g TSS (central point of FCCD).

Oxygen consumption rate can also help analysis about the mechanism of action of RL. SOUR data after a 30 h reaction time indicated that only the quadratic term of S_0/X_0 was significant in the model (Table 3). In most cases, RL was consumed by 30 h of reaction, because no more intense foaming was observed. Thus, the product no longer had major influence in the process. Depending on the region, SOUR was 20% lower and up to 80% higher than Control (Figure 2c). In general, SOUR was higher than Control when

significant growth reduction was observed. When there is energetic uncoupling in cellular metabolism, oxygen consumption rate increases without any association with growth [15, 34]. In addition, fatty acids (especially long-chain fatty acids) can act as metabolic uncouplers when cells are submitted to certain physiological conditions, and part of this is due to the interaction with cellular membranes [35]. Therefore, based on SOUR results and the fact that RL also interacts with cellular membranes [31, 32], it can be hypothesized that RL also acts as metabolic uncoupler, thus reducing sludge production in activated sludge. However, more tests need to be performed to prove this hypothesis.
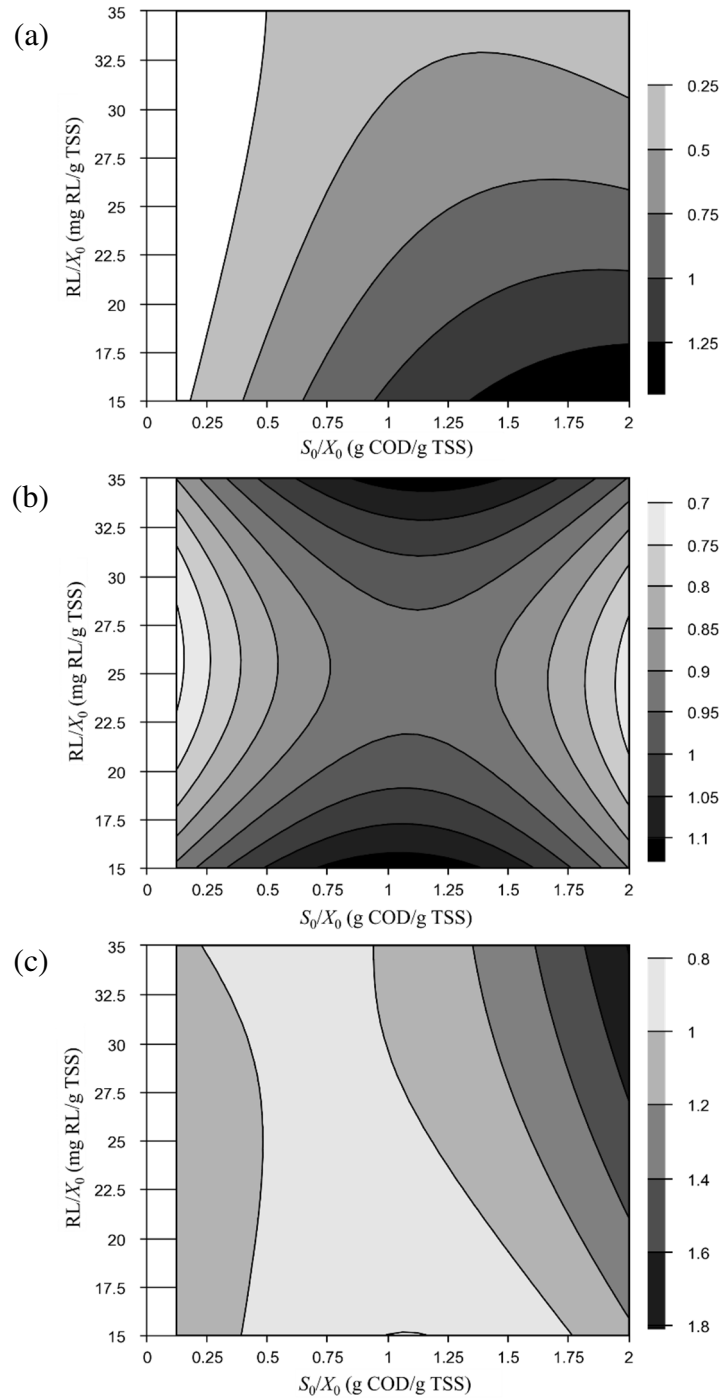

Figure 2. Response surfaces obtained in FCCD: response values in relation to each Control experiment (Control results = 1) for Y (a); k (b) and SOUR (c) (the x and y axes of each graph show the actual values of each variable studied)

Increased oxygen consumption can be considered a disadvantage to the process, because it represents additional cost of aeration. More studies need to be performed to evaluate if the increased aeration compensates for sludge reduction, keeping in mind all pillars involved: economic, environmental, social, and political [36], not just economic.

The observations found in this study can be extended to products other than biosurfactants. A survey of results found in literature with application of 2,4-dinitrophenol (DNP) in activated sludge systems [16, 34, 37-39] shows that, even though it is a chemically stable product with a well-known and widespread application, results obtained are extremely variable, with growth varying from −56 to 50% [16, 39] in relation to Control. This variation occurs not only due to differences in concentration or in product/biomass ratio. In some cases, a combined effect between DNP/X_0 and S_0/X_0 can be assumed. In one study [38], for the same DNP concentration added in an activated sludge SBR at different operation times, DNP/X_0 showed 57% correlation with sludge reduction, while differences in S_0/X_0 showed a 97% correlation.

In the validation step, results in Test experiments are shown in Table 4.

Table 4. Results obtained in the validation of the model

S_0/X_0 [a]	RL/X_0 [b]	FCCD [c]	Model [d]	Validation [e] 1,000	Validation [f] 2,000
		Response: Y			
−0.745	−1	—	0.46	0.76	0.19
−0.745	0	—	0.24	0.30	0.00
−0.745	1	—	0.18	0.00	0.35
0	−1	1.25	1.09	0.87	0.08
0	0	0.52 ±0.17	0.68	0.47	0.42
0	1	0.75	0.43	0.00	0.43
		Response: k			
−0.745	−1	—	1.02	0.89	0.66
−0.745	0	—	0.79	0.64	0.54
−0.745	1	—	0.97	0.58	0.39
0	−1	1.09	1.13	0.99	1.46
0	0	0.89 ±0.02	0.93	1.16	1.10
0	1	1.28	1.13	1.25	0.87
		Response: SOUR			
−0.745	−1	—	1.02	1.00	1.37
−0.745	0	—	1.04	1.03	1.42
−0.745	1	—	0.96	1.37	1.49
0	−1	1.04	0.80	1.01	0.91
0	0	0.94 ±0.11	0.97	0.84	1.00
0	1	0.90	1.04	0.85	1.16

Note: Standard values in relation to the respective Control experiments (Control results = 1)
[a] Coded values
[b] Coded values [for RL/X_0 −1, 0, and +1, RL concentrations are equivalent to mg RL/L: 15, 25, and 35 (1,000 mg TSS/L) and 30, 50, and 70 (2,000 mg TSS/L), respectively]
[c] Values previously obtained (Table 2), central point values are reported as average ±Standard deviation
[d] Calculated according to the model coefficients (Table 3)
[e] Values obtained at this stage of the study with initial TSS 1,000 mg/L
[f] Values obtained at this stage of the study with initial TSS 2,000 mg/L

As stated earlier, 100% growth reduction is not of interest in WWTPs. Moreover, when S_0/X_0 is very low, the error in the analyses increases significantly. Therefore, in order to validate the model, experiments were carried out with the same RL concentrations previously evaluated (15, 25, and 35 mg RL/g TSS) and S_0/X_0 0.36 (coded value −0.745) and 1.06 g COD/g TSS (central point of the FCCD). In this region, growth was significantly lower (25-75%, Figure 2a) without major losses in substrate consumption rate (maximum of 10% lower than Control, Figure 2b).

Control experiments showed good repeatability, with k and SOUR values statistically equal to those obtained in the previous step (*t*-test, 90% confidence level), considering S_0/X_0 1.06 g COD/g TSS (central point of the FCCD). Only Y values had significant differences: 0.75 ±0.09 (FCCD), 0.52 ±0.04 (validation, 1,000 mg TSS/L), and 0.61 ±0.14 g TSS/g COD (validation, 2,000 mg TSS/L). This occurred due to higher error propagation in Y analysis, however, the differences are acceptable and do not compromise the analysis.

The differences between values predicted by the model and obtained experimentally for all responses evaluated in experiments were high, as can be observed in Figure 3, but most of these differences could be justified by the fact that the models only explain 75-78% of the results. Considering X_0 1,000 mg TSS/L and S_0/X_0 1.06 g COD/g TSS, errors varied from 11 to 31%. The error increases significantly (reaching 93%) when comparing the results obtained in experiments with X_0 2,000 mg TSS/L, probably because in this case RL concentration exceeds the range evaluated in the FCCD. This indicates that the effect of RL is not only dependent on RL/X_0 ratio, but also on RL concentration. Therefore, to validate the model, only experiments with 1,000 mg TSS/L should be considered. With the differences observed, the models can be considered validated.
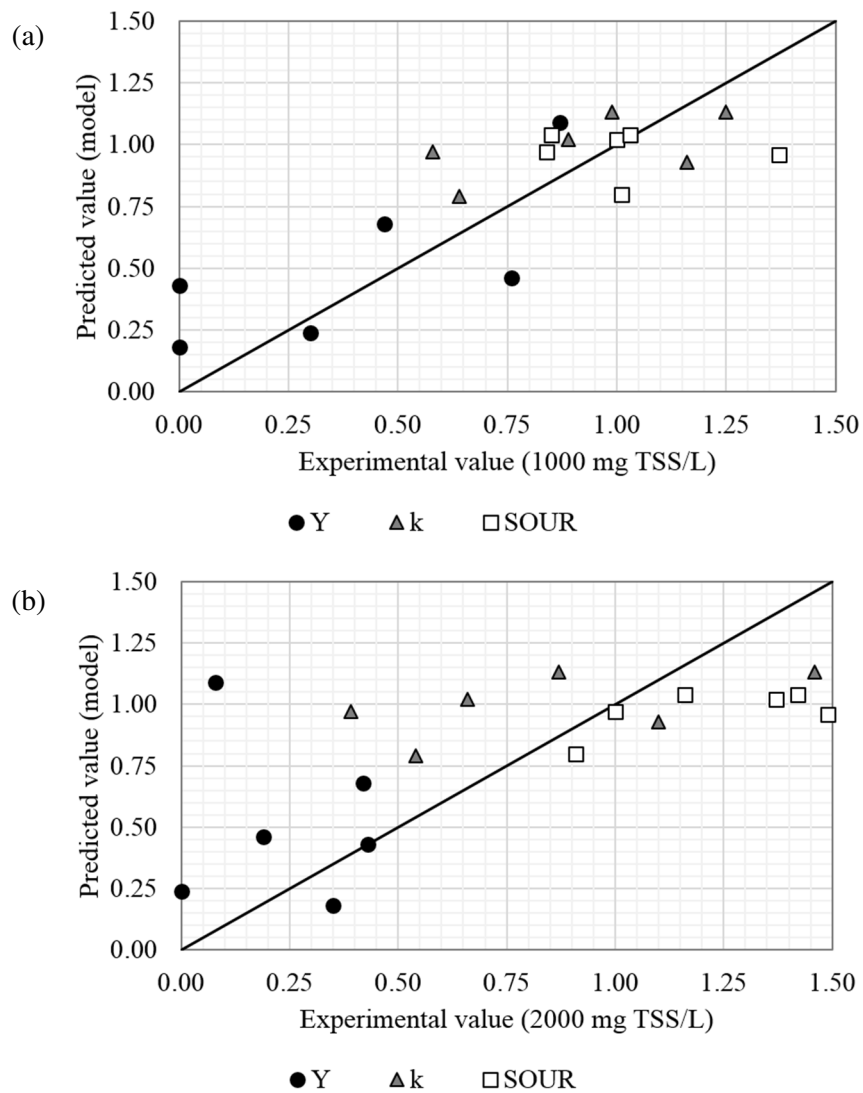

Figure 3. Predicted and experimental values for all variables analysed in the validation step [all results are from Tests with initial biomass concentration of: 1,000 mg TSS/L (a) and 2,000 mg TSS/L (b)]

In Figure 4a, a high correlation ($R^2 = 0.9683$) is observed between RL/X_0 and growth reduction. The higher the RL/X_0 and, in this case, RL concentration, the higher the growth reduction. However, when X_0 increases from 1,000 to 2,000 mg/L, to keep the same RL/X_0 ratio, RL concentration doubles. Figure 4b shows the effect of RL concentration on microbial growth considering all conditions evaluated. RL reduced growth up to a concentration between 35 and 50 mg RL/L. From 50 mg RL/L, the effect on growth reduction was attenuated and Y increased, probably due to RL consumption as substrate.
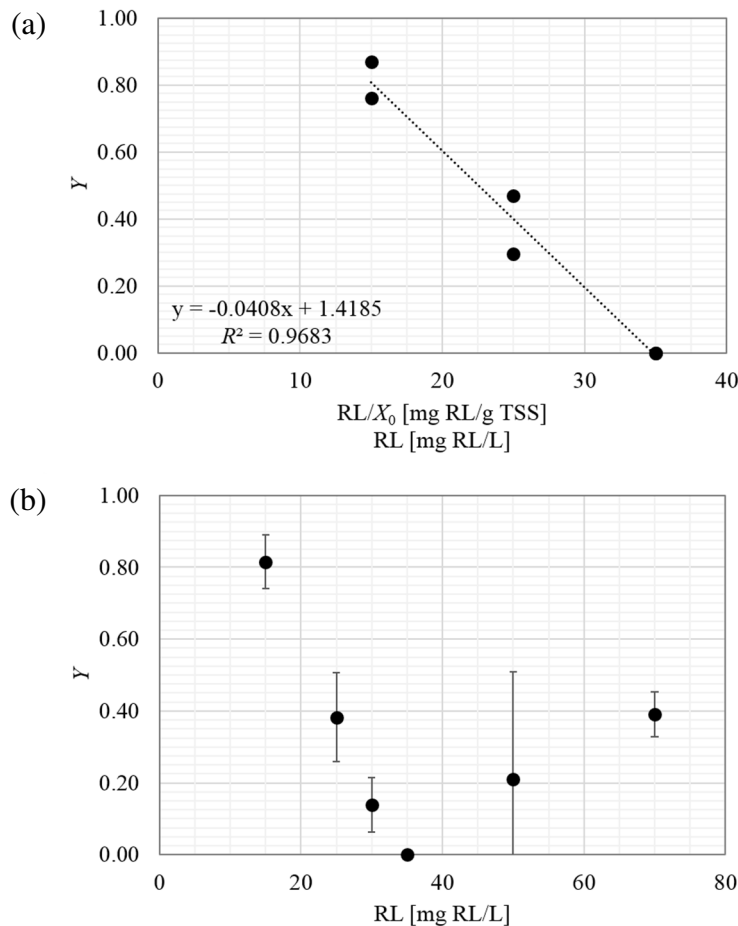

Figure 4. Effect of the RL concentration on growth reduction [Y results are reported considering Control value of 1: results of conditions with initial biomass concentration of 1,000 mg TSS/L (a) and results of conditions with initial biomass concentration of 1,000 and 2,000 mg TSS/L (b)]

One hypothesis that can justify the effect of RL on sludge reduction in activated sludge systems is its action on cellular membranes. Even in concentrations lower than CMC, surfactants are able to interact with membranes, so monomers, and not micelles, are responsible for this effect [40]. However, interactions between surfactant molecules do not occur only at CMC, in concentrations below CMC, vesicles and micelles are already observed, and above CMC, only micelles are present [41]. Small aggregates, like vesicles, have been observed in RL [42, 43]. In the conditions of this study, addition of 50 mg RL/L should already be able to form aggregates, reducing the number of monomers and, therefore, the antimicrobial effect.

CONCLUSION

The design of experiments conducted with the variables S_0/X_0 and RL/X_0 found that the effect of microbial growth reduction by a certain product depends on the initial

condition employed in the rapid test. For commercial rhamnolipid R90 used as model product, low S_0/X_0 values allow significant growth reductions with lower concentrations. Considering the maintenance of organic matter removal, evaluated by substrate consumption rate, the design of experiments pointed to S_0/X_0 of 1.06 g COD/g TSS. SOUR results indicate that RL could act as metabolic uncoupler. Therefore, the study indicated that the initial condition of the test is capable of affecting all responses analysed. If an initial condition is fixed, the real effect of the product might not be evaluated, leading to wrong conclusions.

REFERENCES

1. Ebrahimi, M., Kazemi, H., Mirbagheri, S. A. and Rockaway, T. D., An Optimized Biological Approach for Treatment of Petroleum Refinery Wastewater, *J. Environ. Chem. Eng.*, Vol. 4, No. 3, pp 3401-3408, 2016, https://doi.org/doi:10.1016/j.jece.2016.06.030

2. Paul, E. and Liu, Y. (eds.), *Biological Sludge Minimization and Biomaterials/bioenergy Recovery Technologies*, John Wiley & Sons Inc., New Jersey, USA, 2012.

3. Von Sperling, M., Activated Sludge and Aerobic Biofilm Reactors (Vol. 5), in: *Biological Wastewater Treatment Series*, p 336, IWA Publishing, London, UK, 2007.

4. Syed-Hassan, S. S. A., Wang, Y., Hu, S., Su, S. and Xiang, J., Thermochemical Processing of Sewage Sludge to Energy and Fuel: Fundamentals, Challenges and Considerations, *Renew. Sustain. Energy Rev.*, Vol. 80, pp 888-913, 2017, https://doi.org/10.1016/j.rser.2017.05.262

5. Andreoli, C. V., von Sperling, M. and Fernandes, F. (eds.), Sludge Treatment and Disposal (Vol. 6), in: *Biological Wastewater Treatment Series*, p 244, IWA Publishing, London, UK, 2007.

6. Foladori, P., Andreottola, G. and Ziglio, G., *Sludge Reduction Technologies in Wastewater Treatment Plants*, IWA Publishing, London, UK, 2010.

7. Ginestet, P. (ed.), *Comparative Evaluation of Sludge Reduction Routes*, IWA Publishing, London, UK, 2007.

8. Kacprzak, M., Neczaj, E., Fijałkowski, K., Grobelak, A., Grosser, A., Worwag, M., Rorat, A., Brattebo, H., Almås, Å. and Singh, B. R., Sewage Sludge Disposal Strategies for Sustainable Development, *Environ. Res.*, Vol. 156, pp 39-46, 2017, https://doi.org/10.1016/j.envres.2017.03.010

9. Pasda, N., Panichsakpatana, S., Limtong, P., Oliver, R. and Montange, D., Evaluation of Bangkok Sewage Sludge for Possible Agricultural Use, *Waste Manag. Res.*, Vol. 24, No. 2, pp 167-174, 2006, https://doi.org/10.1177/0734242X06063347

10. González Martínez, T., Bräutigam, K.-R. and Seifert, H., The Potential of a Sustainable Municipal Waste Management System for Santiago de Chile, Including Energy Production from Waste, *Energy. Sustain. Soc.*, Vol. 2, No. 1, pp 1-14, 2012, https://doi.org/10.1186/2192-0567-2-24

11. Metcalf, L. and Eddy, H., *Wastewater Engineering: Treatment and Resource Recovery*, McGraw Hill Higher Education, New York, USA, 2014.

12. Lacroix, N., Rousse, D. R. and Hausler, R., Anaerobic Digestion and Gasification Coupling for Wastewater Sludge Treatment and Recovery, *Waste Manag. Res.*, Vol. 32, No. 7, pp 608-613, 2014, https://doi.org/10.1177/0734242X14538308

13. James, P., The Sustainability Cycle: A New Tool for Product Development and Design, *J. Sustain. Prod. Des.*, pp 52-57, 1997.

14. Tian, Y., Zhang, J., Wu, D., Li, Z. and Cui, Y., Distribution Variation of a Metabolic Uncoupler, 2,6-dichlorophenol (2,6-DCP) in Long-term Sludge Culture and their Effects on Sludge Reduction and Biological Inhibition, *Water Res.*, Vol. 47, No. 1, pp 279-288, 2013, https://doi.org/10.1016/j.watres.2012.10.008

Alexandre, V. M. F., *et al.*
Potential for Waste Reduction in Activated ...

Year 2019
Volume 7, Issue 4, pp 702-715

15. Chen, G. H., Mo, H. K. and Liu, Y., Utilization of a Metabolic Uncoupler, 3,3',4',5-tetrachlorosalicylanilide (TCS) to Reduce Sludge Growth in Activated Sludge Culture, *Water Res.*, Vol. 36, No. 8, pp 2077-2083, 2002, https://doi.org/10.1016/S0043-1354(01)00426-2

16. Chen, G. W., Yu, H. Q. and Xi, P. G., Influence of 2,4-dinitrophenol on the Characteristics of Activated Sludge in Batch Reactors, *Bioresour. Technol.*, Vol. 98, No. 4, pp 729-733, 2006, https://doi.org/10.1016/j.biortech.2006.04.001

17. Fang, F., Hu, H.-L., Qin, M.-M., Xue, Z.-X., Cao, J.-S. and Hu, Z.-R., Effects of Metabolic Uncouplers on Excess Sludge Reduction and Microbial Products of Activated Sludge, *Bioresour. Technol.*, Vol. 185, pp 1-6, 2015, https://doi.org/10.1016/j.biortech.2015.02.054

18. Feng, X.-C., Guo, W.-Q., Chen, C., Yang, S.-S., Jin, W.-B., Ren, N.-Q., Zheng, H.-S., Du, J.-S. and Liu, B., Treatability Study of 3,3′,4′,5-tetrachlorosalicylanilide (TCS) Combined with 2,4,6-trichlorophenol (TCP) to Reduce Excess Sludge Production in a Sequence Batch Reactor, *Bioresour. Technol.*, Vol. 143, pp 642-646, 2013, https://doi.org/10.1016/j.biortech.2013.05.119

19. Yang, X.-F., Xie, M.-L. and Liu, Y., Metabolic Uncouplers Reduce Excess Sludge Production in an Activated Sludge Process, *Process Biochem.*, Vol. 38, No. 9, pp 1373-1377, 2003, https://doi.org/10.1016/S0032-9592(03)00019-0

20. Liu, Y., Bioenergetic Interpretation on the S_0/X_0 Ratio in Substrate-sufficient Batch Culture, *Water Res.*, Vol. 30, No. 11, pp 2766-2770, 1996, https://doi.org/10.1016/s0043-1354(96)00157-1

21. Liu, Y., Effect of Chemical Uncoupler on the Observed Growth Yield in Batch Culture of Activated Sludge, *Water Res.*, Vol. 34, No. 7, pp 2025-2030, 2000, https://doi.org/10.1016/S0043-1354(99)00386-3

22. Soberón-Chávez, G. (ed.), Biosurfactants: From Genes to Applications (Vol. 20), in: *Microbiology Monographs*, Springer, Berlin, Germany, 2011.

23. Araujo, L. V. de, Guimarães, C. R., Marquita, R. L. da S., Santiago, V. M. J., de Souza, M. P., Nitschke, M. and Freire, D. M. G., Rhamnolipid and Surfactin: Anti-adhesion/antibiofilm and Antimicrobial Effects, *Food Control*, Vol. 63, pp 171-178, 2016, https://doi.org/10.1016/j.foodcont.2015.11.036

24. Wang, Q., Wei, W., Gong, Y., Yu, Q., Li, Q., Sun, J. and Yuan, Z., Technologies for Reducing Sludge Production in Wastewater Treatment Plants: State of the Art, *Sci. Total Environ.*, Vol. 587-588, pp 510-521, 2017, https://doi.org/10.1016/j.scitotenv.2017.02.203

25. Santa Anna, L. M. de M., Production and Utilization of Biosurfactants in the Bioremediation of Oil Impacted Soils, *M.Sc. Thesis*, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2005.

26. American Public Health Association (APHA), Standard Methods for the Examination of Water & Wastewater, APHA, AWWA, WEF, Washington D. C., USA, 2005.

27. Manson, R. L., Gunst, R. F. and Hess, J. L., *Statistical Design and Analysis of Experiments with Applications to Engineering and Science*, John Wiley & Sons, Inc., New Jersey, USA, 2003.

28. Varjani, S. J. and Upasani, V. N., Critical Review on Biosurfactant Analysis, Purification and Characterization using Rhamnolipid as a Model Biosurfactant, *Bioresour. Technol.*, Vol. 232, pp 389-397, 2017, https://doi.org/10.1016/j.biortech.2017.02.047

29. Henze, M., van Loosdrecht, M. C. M., Ekama, G. A. and Brdjanovic, D. (eds.), *Biological Wastewater Treatment: Principles, Modelling and Design*, IWA Publishing, London, UK, 2008.

30. Alexandre, V. M. F., de Castro, T. M. S., de Araújo, L. V., Santiago, V. M. J., Freire, D. M. G. and Cammarota, M. C., Minimizing Solid Wastes in an Activated Sludge System

Alexandre, V. M. F., *et al.*
Potential for Waste Reduction in Activated ...

Year 2019
Volume 7, Issue 4, pp 702-715

Treating Oil Refinery Wastewater, *Chem. Eng. Process. Process Intensif.*, Vol. 103, pp 53-62, 2016, https://doi.org/10.1016/j.cep.2015.10.021

31. Ballot, F., Bacterial Production of Antimicrobial Biosurfactants, *M.Sc. Thesis*, University of Stellenbosch, Stellenbosch, South Africa, 2009.

32. Sen, R. (ed.), Biosurfactants (Vol. 672), in: *Advances in Experimental Medicine and Biology*, Landes Bioscience and Springer Science + Business Media, LLC, New York, USA, 2010.

33. Von Sperling, M., Wastewater Characteristics, Treatment and Disposal (Vol. 1), in: *Biological Wastewater Treatment Series*, IWA Publishing, London, UK, 2007.

34. Mayhew, M. and Stephenson, T., Biomass Yield Reduction: Is Biochemical Manipulation Possible without Affecting Activated Sludge Process Eficiency?, *Water Sci. Technol.*, Vol. 38, No. 8-9, pp 137-144, 1998, https://doi.org/10.1016/S0273-1223(98)00687-8

35. Di Paola, M. and Lorusso, M., Interaction of Free Fatty Acids with Mitochondria: Coupling, Uncoupling and Permeability Transition, *Biochim. Biophys. Acta - Bioenerg.,* Vol. 1757, No. 9-10, pp 1330-1337, 2006, https://doi.org/10.1016/j.bbabio.2006.03.024

36. Poulsen, T. G. and Hansen, J., Strategic Environmental Assessment of Alternative Sewage Sludge Management Scenarios, *Waste Manag. Res.*, Vol. 21, No. 1, pp 19-28, 2003, https://doi.org/10.1177/0734242X0302100103

37. Chen, G. W., Yu, H. Q., Xi, P. G. and Xu, D. Q., Modeling the Yield of Activated Sludge in the Presence of 2,4-dinitrophenol, *Biochem. Eng. J.*, Vol. 40, No. 1, pp 150-156, 2008, https://doi.org/10.1016/j.bej.2007.12.008

38. Alexandre, V. M. F., Reduction in Sludge Disposal in Activated Sludge Systems Treating Oil Refinery Wastewater, *M.Sc. Thesis*, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2013.

39. Aragón, C., Quiroga, J. M. and Coello, M. D., Comparison of Four Chemical Uncouplers for Excess Sludge Reduction, *Environ. Technol.*, Vol. 30, No. 7, pp 707-714, 2009, https://doi.org/10.1080/09593330902894372

40. Xia, W. J. and Onyuksel, H., Mechanistic Studies on Surfactant- Induced Membrane Permeability Enhancement, *Pharm. Res.*, Vol. 17, No. 5, pp 612-618, 2000.

41. Lichtenberg, D., Characterization of the Solubilization of Lipid Bilayers by Surfactants, *BBA – Biomembr.*, Vol. 821, No. 3, pp 470-478, 1985, https://doi.org/10.1016/0005-2736(85)90052-5

42. İkizler, B., Arslan, G., Kipcak, E., Dirik, C., Çelenk, D., Aktuğlu, T., Helvacı, Ş. and Peker, S., Surface Adsorption and Spontaneous Aggregation of Rhamnolipid Mixtures in Aqueous Solutions, *Colloids Surfaces A Physicochem. Eng. Asp.*, Vol. 519, pp 125-136, 2017, https://doi.org/10.1016/j.colsurfa.2016.06.056

43. Sánchez, M., Aranda, F. J., Espuny, M. J., Marqués, A., Teruel, J. A., Manresa, Á. and Ortiz, A., Aggregation Behaviour of a Dirhamnolipid Biosurfactant Secreted by *Pseudomonas Aeruginosa* in Aqueous Media, *J. Colloid Interface Sci.*, Vol. 307, No. 1, pp 246-253, 2007, https://doi.org/10.1016/j.jcis.2006.11.041