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APPLICATION OF RESIDUAL YEAST AS A SOURCE OF REDOX MEDIATORS FOR THE ANAEROBIC DECOLORIZATION OF A MODEL AZO DYE

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Abstract - This work investigated the anaerobic degradation of the model azo dye Remazol Yellow Gold RNL in batch reactors using discharged residual yeast as the source of redox mediators (RM). Two yeast lysis methods (mechanical lysis and sonication) were tested and optimized to produce a riboflavin-rich yeast lysate. The reactors were operated at 25 ^{o}C for 48 hours, evaluating the effect of external carbon source (glucose) and RM (from residual yeast lysate and commercial yeast extract) addition. The results showed that color removal efficiencies for the batch reactors fed with commercial yeast extract reached 90%, whereas those fed with discharged yeast lysate reached 80% (sonication) and 73% (mechanical lysis). These values were statistically higher when compared to reactors operating without RM (48 to 66%), demonstrating that yeast extract enhances azo dye degradation in anaerobic conditions and that the residual yeast is a cheap and alternative source of carbon and of the RM riboflavin.
Keywords: Azo dye; Anaerobic digestion; Residual yeast; Riboflavin.

INTRODUCTION

The textile industry is an important economic sector for the Brazilian economy. The textile sector represented in 2013 about US$58.2 billion, which is equivalent to 5.7% of the total production value of Brazilian manufacturing industry – excluding the activities of mining and construction (IEMI, 2014). Unfortunately, the textile production increase contributes to the escalation of severe pollution problems. Textile wastewater contains a variety of chemicals such as dyes and starch, which confer color and organic matter to the effluent. Removal of dyes is a major concern when treating textile wastewater due to carcinogenic and mutagenic properties of some dyes and their degradation byproducts (Baêta *et al.*, 2012).

The main technologies used nowadays for the treatment of textile wastewater are physicochemical

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and biological (mainly aerobic) processes. Although the physicochemical techniques are effective in removing particulate organic matter, they are inefficient in degrading azo dyes and dissolved organic compounds (Kunz *et al*., 2011). As a result, destructive techniques, such as biological treatment, are normally preferred. Although azo dyes are good electron-accepting compounds in reducing environment (anaerobic), in aerobic systems oxygen is the preferential electron acceptor when compared with azo dyes, due to the presence of electrophilic functional groups in their structure, making them more resilient to conventional biological aerobic treatment (Alvarez *et al*., 2010).

Anaerobic digestion has been considered as one of the best technologies for color removal from textile effluents (Georgiou *et al.*, 2004). Removal efficiencies of an anaerobic reactor can reach up to 80% during the treatment of azo dye solutions (Méndez-Paz *et al*., 2005; Dos Santos *et al*., 2006). However, the electron transfer between different species of the anaerobic consortia and the final acceptor (azo dyes) can be limited and be a major drawback for the treatment of textile effluents in anaerobic reactors.

According to Dos Santos (2005a), anaerobic treatment efficiency can be enhanced by using redox mediators, which act by improving electron transfer between the donor (source of carbon) and acceptor (azo dye) (Dos Santos, 2005b), thereby increasing the decolorization rates. Several studies (Rau *et al*., 2002; Cervantes *et al*., 2001; Brady, 2003) have shown the ability of vitamins such as riboflavin and other purified substances such as quinones (e.g., sulfonated antraquinone – AQS) to act as redox mediators. Field *et al.* (2002) has shown that reduced flavins, such as riboflavin, were responsible for the direct chemical reduction of azo dyes (non-enzymatic), instead of acting as a vitamin.

Saccharomyces cerevisiae and *Ashbya gossypii* are normally employed for industrial riboflavin production, since riboflavin extraction is more cost-effective than its chemical synthesis (Santos *et al.*, 1995). Côrrea *et al.* (2009) evaluated the use of yeast extract as a source of riboflavin to improve the color removal in anaerobic digestion, and found that the efficiencies increased by 30%. Baêta *et al*. (2012) also performed studies that showed increased color removal efficiencies in the presence of commercial yeast extract as a source of riboflavin, but in a large-scale treatment plant, its use would raise the costs of treatment.

Residual yeast, regarded a solid waste from the fermentation process in brewery and other fermentation industries, could be a viable source of redox mediator if cheaper lysing methods are employed for extract production. Therefore, the main objective of this paper is to present results on the application of a fermentation industry residue as the source of redox mediators for the anaerobic decolorization of a model azo dye (Yellow Gold Remazol) commonly used in the textile industry. Two yeast lysis methods (mechanical lysis and sonication) were tested and optimized to produce a riboflavin-rich yeast extract.

MATERIALS AND METHODS

Pretreatment of the Yeast Biomass

The yeast biomass was collected from a brewery fermentation vat where the production process was already over. The first step to produce yeast lysate from the residual yeast biomass was clarification, consisting of a sequence of centrifugations and ethanol solution (10% M/M) addition. The final solution of this process, hereby called whole cells clarified solution (WCCS), was further submitted to distinct cell lysis processes aiming at the release of riboflavin.

Experimental Design

A screening procedure was initially performed to determine which variables and their levels resulted in the best experimental condition for residual yeast lysis. For this, two factorial designs at two levels (2^3 and 2^2), both with triplicates at the central point, for sonication and blender, respectively, were carried out to optimize the best experimental conditions for riboflavin release in the screening step. The effects of variables were calculated at the level of significance of 0.05 by using the software PASW Statistics 18®, and a response surface methodology (MATLAB R2011a®) was used to explore the relationship between the variables.

Cell Lysis Methods

Sonicator: The WCCS, as described above, was prepared according to each experimental condition described by the factorial experimental design, and the variables studied during sonic disruption were the cell density in g L^{-1} (x_1), duty cycle in % (x_2) and contact time in minutes (x_3). A *Branson 250* sonicator was used with a titanium¾" probe; the solutions were prepared at 4 ºC and maintained in ice bath to prevent over heating.

Mechanical Lysis: The mechanical lysis was performed using the blender method (rotating blades). A bench scale industrial blender (448 W) was employed to grind and disperse the WCCS. The solutions were prepared according to each experimental condition described by the factorial experimental design, and the variables studied during the mechanical disruption were the cell density in g L^{-1} (x_1) and the contact time in minutes (x_2).

Batch Experiments

The batch experiments were carried out in triplicate with solutions of the model azo dye Remazol Yellow RNL Gold (50 mg.L^{-1}) ($C_{16}H_{18}N_4O_{10}S_3$; CAS 12226-47-0; molecular weight of 522,54 g mol^{-1}) using amber glass flasks (20 mL) duly sealed with rubber stoppers and aluminum caps. This work evaluated the azo dye without hydrolysis, as normally happens for this type of dye in the industry surveyed, since the dye is readily soluble in water. Anaerobic conditions were ensured by purging nitrogen gas into each flask for about 2 min. The flasks were then incubated in a shaker at 25 °C for 48 hours using anaerobic sludge (~1,000 mg.L^{-1} of volatile suspended solids - VSS) as inoculum. The sludge was collected from a demo scale UASB reactor installed at the Centre for Research and Training in Sanitation (CePTS) UFMG/COPASA, located at the Arrudas WWTP, in Belo Horizonte - Brazil. The incubation conditions are detailed in Table 1.

Analytical Procedures

Riboflavin Quantification: Riboflavin quantification analyses were carried out in a high performance liquid chromatography (HPLC) system (Shimadzu – LC- 20A) equipped with a fluorescence detector (SPD – 20A) setting the wavelengths at λ_{ex} = 450 nm and λ_{em} = 525 nm, with an analysis time of 20 min per sample, according to the validated method of Bueno Solano *et al.* (2010). The chromatographic separation was performed on a Phenomenex Luna 5mM C18 (150 mm × 4.6 mm and 5 mM) column at 25 ºC, using 0.005 M ammonium acetate and methanol (72:28 v / v) as mobile phase under isocratic flow (1.0 mL min^{-1}) with an injection volume of 30 µl. A calibration curve was generated in the range of 0.005 to 0.2 mg.L^{-1} and resulted in very good linearity (r^2 = 0.9996).

Color Analyses: For color analyses an HP 8453 UV-Visible (California, U.S.A) spectrophotometer was used previously set at the wavelength where the azo dye exhibited the maximum absorption (λ_{max} = 410 nm). All samples were centrifuged at 2,500 rpm for 20 minutes before the analyses, which were carried out with samples collected at 0h, 12h and 48h. A calibration curve was generated in the range of 1 to 100 mg.L^{-1} (r^2 = 0.9977) to allow the calculation of azo dye removal efficiency.

Table 1: Operational conditions for the batch experiments that evaluated the anaerobic decolorization of azo dye solutions.

Operational Condition*	Biomass** (gVSS.L^{-1})	Glucose (g.L^{-1})	Nutrient solution *** (L)	Residual yeast lysate (g.L^{-1})	Comercial yeast extract (g.L^{-1})	Yellow Gold Remazol**** (g.L^{-1})
B	1.0	-	0.02	0.350	-	0.05
S	1.0	-	0.02	0.350	-	0.05
C	1.0	-	0.02	-	0.350	0.05
L	1.0	-	0.02	-	-	0.05
LG	1.0	0.01	0.02	-	-	0.05
Ab	-	-	0.02	-	0.350	0.05

*Operational Conditions: B (blender), S (sonication) and C (commercial) are aimed at evaluating the removal of color using yeast lysate, residual (B and S) and commercial (C), as a source of carbon and redox mediators; L and LG aim to evaluate the degradation only by the presence of microorganisms and Ab is the abiotic control of the reactors.
**Anaerobic sludge.
***According to Mesquita *et al.* (2012)
**** Azo dye provided by a local textile industry and used without purification or pre-treatment, as per its industrial use.

RESULTS AND DISCUSSION

Riboflavin Release Optimization

Sonication: Sonication screening experiments have shown that the best levels (based on riboflavin release) of the three variables tested are 60 to 75 g L^{-1} cell density, 40 to 60% of duty cycle and an operation for 10 to 20 minutes. Analyses of variance for normality (Shapiro-Wilk) showed that the null hypotheses can be accepted, i.e., the samples come from a normally distributed population (W = 0.207; 0,212 and 0,204). The results of the factorial design (2^3) allowed an analysis of the effects of variables, calculated using the software PASW Statistic 18. The effects of the three variables (x_1; x_2 and x_3) and the interactions between them (x_{12}, x_{13}, x_{23} e x_{123}) made it possible to infer that the variables cell density (x_1), time (x_2) and the interaction between duty cycle and time (x_{23}) statistically influenced ($P > 0.05$) the final response of the system (riboflavin release). These results allowed the construction of an approximation model that could capture interactions between those variables, Equation (1), were Y is the amount (mg g^{-1}) of riboflavin released.

$$y(x_1, x_2, x_3) = 0.0890 + 0.00027\, x_1 + 0.00014\, x_2 + 0.00014 x_1 x_2 \quad (1)$$

This model allowed us to build response surface curves (RSM) using the software MATLAB (R2011a - v. 7.12) as shown in Figures 1 and 2.

The data provided by the RSM allowed the optimization of riboflavin release during sonication (65 g L^{-1} of yeast biomass, 60% of duty cycle for 10 minutes of lysis) so that an amount of 9 µg of riboflavin per gram of yeast could be obtained. This value represents 33% of the riboflavin present in a commercial yeast extract, which is harvested by a much more costly process.

Blender: The results of the screening experiments showed that the best levels for the two variables tested are 80 to 100 g L^{-1} of cell density (x_1) and 15 to 30 minutes (x_2). The analyses of variance test for normality (Shapiro-Wilk) showed that the null hypotheses can be accepted and that the sample comes from a normally distributed population (W = 0.086 and 0.085). The results of the experimental design (2^2) show that only the variable x_1 (cell density) influenced riboflavin release ($P > 0.05$). Variable x_2 and the interaction between them (x_{12}) were not statistically significant ($P < 0.05$) and hence were excluded from the model. The optimized variables, 85 g L^{-1} of yeast biomass and 17 min of blending, led to the release of 21 µg of riboflavin per gram of yeast, which represents 60% of the riboflavin amount observed in a commercial yeast extract.

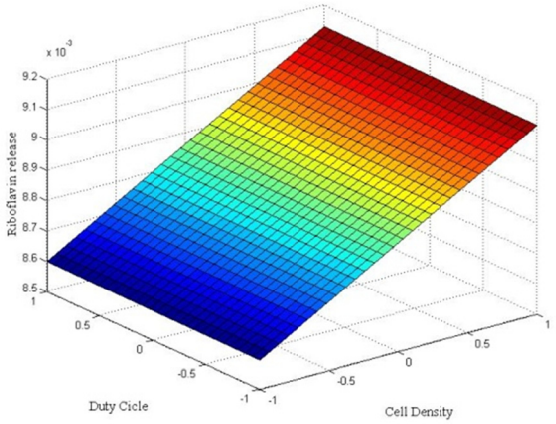

Figure 1: Response surface for the interaction between the cell density (x_1) and the duty cycle (x_2) on the yeast lyses by sonication methodology.

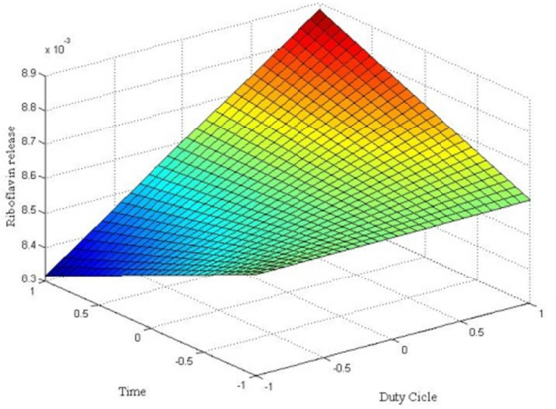

Figure 2: Response surface for the interaction between the duty cycle (x_2) and time (x_3) on the yeast lyses by sonication methodology.

Color Removal Experiments

Figure 3 shows the change in color removal efficiency in the six experimental conditions. The highest color removal efficiency (90%) was achieved during the experimental condition C, when using 350 mg L^{-1} of commercial yeast extract as a source of carbon and redox mediators. The experimental conditions B and S, using the residual lysates from the blender and sonication technique, respectively, as source of carbon and redox mediator, led to 80 and 73% of color removal by the end of the experiment (48h).

The comparison of color removal efficiencies in the presence (B, S and C) and absence (L and LG) of

redox mediators clearly shows that both the commercial yeast extract and the residual yeast lysate led to an increase (from 11 to 53%) in color removal. These results prove that the use of residual yeast lysate or commercial yeast extract (source of the redox mediator riboflavin) enhanced degradation of the azo dye Yellow Gold Remazol in anaerobic conditions. Van der Zee and Cervantes (2009) point out that the use of redox mediators increases the rate of azo dye degradation in anaerobic conditions, thereby favoring the applicability of such technology in full-scale textile wastewater treatment. However, the use of commercial yeast extract in full-scale treatment plants would raise treatment costs. Figure 3 shows that the use of residual yeast lysate, a cheaper source of redox mediator (riboflavin) led to removal efficiencies only 12% lower compared to the commercial yeast extract, reaching up to 80% with a low cost (Blender) lysis technique. The use of residual biomass and a low cost lysis technique would allow the use of residual yeast lysate in full-scale treatment plants as a source of carbon and redox mediators.

The flask LG, incubated with glucose (10 mg L^{-1}) and without yeast lysis products, led to an increase of 27% in color removal compared to the flask L, incubated without yeast lysis and glucose. These results show that the nutritional and substrate limitations influenced the degradation rates of the azo model Remazol Yellow Gold. This seems to indicate that the soluble microbial products (SMP's) and the aromatic amines, byproducts of azo dye degradation, are not good sources of carbon and energy or even might be toxic to the anaerobic sludge, thereby limiting the rate of the anaerobic process.

This behavior can also be observed in Figure 3, where it is seen that, in the first 12h, nearly 70% of the azo dye is removed in the flasks incubated with redox mediators, compared with 50% in the experimental condition (L) without redox mediator and carbon source. When the flasks were incubated without redox mediator and with glucose as carbon source, there was up to 80% of color removal. These results indicate that the commercial yeast extract and the residual yeast lysate are more complex sources of carbon, demanding a longer time for the hydrolysis of such compounds (e.g., proteins).

Table 2 shows the composition of the dissolved fraction resulting from brewery yeast biomass lysis (chemical lysis by addition of NaCl). It is possible to infer that there is a high amount of proteins in the yeast extract and lysates (61.5 and 46.4%), which might require longer contact times for their hydrolysis. As shown in Figure 3, yeast extract and the yeast lysate are good sources of carbon; however, longer contact times might be required for hydrolyzing such compounds.

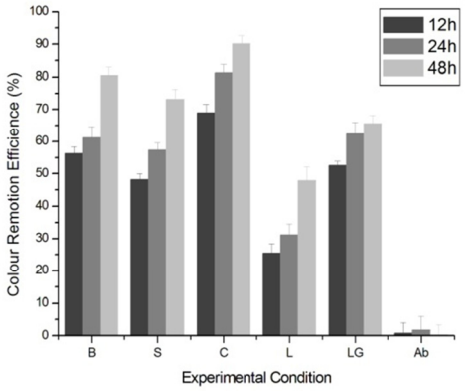

Figure 3: Results from the batch experiment degradations of the azo dye Remazol Yellow Gold under anaerobic conditions.

Table 2: Chemical composition of the brewery yeast lysate obtained after chemical lysis (NaCl addition).

Compound	Biomass (%)	Lysate (%)	Extract (%)	Cell Wall (%)
Protein	48.74	46.45	61.54	32.70
Ribonucleic Acid	5.70	7.80	6.90	1.83
Lipids	3.33	3.30	1.89	4.54
Ash	8.55	8.83	12.50	4.43
Total Fiber	24.40	25.03	2.70	55.04
Soluble Fiber	22.52	24.76	2.70	31.59
Insoluble Fiber	1.88	0.27	0.00	23.45

Font: Adapted from Sgarbieri *et al.* (1999)

Technical Viability Aspects

Table 3 shows a comparison between the lysis methods evaluated in this study and the most common industrial process employed to produce yeast extract, i.e., the chemical lysis by NaCl addition. It can be seen that the amount of riboflavin achieved by the industrial process, 50 μg g^{-1}, is twice the riboflavin released by the mechanical lysis method tested. However, mechanical lysis is simpler and cheaper when compared with sonication and the osmotic lysis typically used at large scale. The mechanical lysis method demands 1% of the time needed for the chemical lysis by NaCl addition (industrial process) and does not require the addition of a chemical that contributes to increase the production costs. In addition, the training support and technological complexity are low in the mechanical lysis when compared to the classical yeast extract production process, which consists of a series of centrifugations and the incubation of the yeast biomass in a

Table 3: Comparison between the lysis method evaluated and the industrial process.

Lysis Methods	Variables				Riboflavin release ($mg\ g^{-1}$)
	Cell Density ($g\ L^{-1}$)	Time (hours)	Temperature (ºC)	Duty Cicle (%)	
Mechanical	100	0.25	25**	-	0.026 ± 0.004
Sonication	65	0.33	Ice bath	60	0.011 ± 0.009
Industrial*	-	24 to 32	40 to 60	-	0.050***

* Industrial process of osmotic lysis (NaCl addition) described by Oliveira (2008).
** Ambient temperature.
*** Value described by Himedia®.

saline solution in a temperature-controlled shaker (Oliveira, 2005). Compared to the sonication, the mechanical lysis is also simpler, requiring less contact time for lysis and not demanding a temperature control, which would reduce energy costs. Since the mechanical lysis led to twice as much riboflavin when compared to sonication, it should be the method selected for further economic viability analysis.

CONCLUSION

The screening experimental designs allowed the identification of the main variables affecting riboflavin release during two (sonication and blending) residual yeast treatment techniques. Under optimized conditions cell lysis by sonication (65 g L^{-1}; 60% and 10 min.) and the blending process (85 g L^{-1} and 17 min.) led to riboflavin contents of 9 and 21 mg/g, respectively. Although these values represent only 33 and 60%, respectively, of the riboflavin amount in a commercial yeast extract, the anaerobic decolorization assays indicated that the residual yeast from a brewery industry could be adequately used after simple lysis treatment. This would be a cheap and sustainable source of riboflavin and carbon which increased by 11% the anaerobic degradation of a model azo dye.

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