Application of the advanced oxidative process on the degradation of the green leaf and purple açaí food dyes with kinetic monitoring and artificial neural network modelling

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ABSTRACT

The study evaluated the advanced oxidative processes concerning the degradation of green leaf and purple açaí dyes, as well as the prediction of data through artificial neural networks (ANNs). It was verified that percentage of degradation on the wavelengths (λ) of 215, 248, 523 and 627 nm was 5.95, 49.99, 98.17 and 95.99%, respectively, when UV/H₂O₂ action and UV-C radiation was applied. A non-linear kinetic model proposed by Chan and Chu presented a good fit to the experimental data, reaching an R² value between 0.978 and 0.999, for the studied λ. Within the ANN simulations through Statistica 6.0, the multilayer perceptron (MLP) (3-9-4) presented a better fit to the experimental data. However, higher values of R² were obtained when utilizing the sklearm package with Python language and an MLP (4-5-4) model. Assays with Staphylococcus aureus and Staphylococcus pyogenes bacteria isolates were performed and it was verified that after employing the UV/H₂O₂ process, there was a decrease in the toxicity of the solution of dyes. In evaluating S. aureus toxicity, normal growth was observed. However, for S. pyogenes bacteria, it was found that when using the UV/H₂O₂ process, toxicity was evidenced at post-treatment solution concentrations of 100, 70 and 50%.

Key words | artificial neural networks, food dyes, kinetic modelling, toxicity, UV/H₂O₂

INTRODUCTION

Progress has been made regarding the technology employed in the food industries, which allowed the introduction of chemical additives in the food production process. These additives have the function of assigning sensory characteristics to the products, like consistency, coloring and flavor, as well as acting to guarantee a greater longevity, that is, shelf life (Honorato et al. 2013). Among the most used additives by the food industry are dyes, which have the function of conferring the desired coloration to the final product, making it more attractive to consumers (Anastácio et al. 2016).

The chromophore groups present in the dye composition are responsible for conferring color to the product. However, it is known that the coloration does not fully occur, because there is a certain resistance in the dyes to fixing it in the product, which is due to the presence of another group, the auxochromes, also responsible for the color intensity (Christie 2001; Zhang et al. 2018). Therefore, the effluents generated by these industries are strongly stained and require adequate treatment (Akceylan et al. 2009; Barros et al. 2014).

Among the most used food dyes are the purple açaí and green leaf. Each of these dyes is composed of a mixture of...
other dyes; açaí purple is composed of Bordeaux red and blue bright (Marchi et al. 2005; Silva et al. 2008), containing azo and triphenylmethane chromophore groups, respectively; the green leaf is composed of tartrazine yellow and indigotine blue (Marchi et al. 2005; Santos et al. 2010), of azo and indigoid chromophore groups (Itoh et al. 1989).

Having such a rich mixture of various types of dyes, the effluents of the food industry are harmful to the environment since they have the power to modify the biological properties of water resources, due to the pigmentation. This coloration prevents the passage of light, inhibiting the photosynthesis and therefore affecting organisms dependent on the photosynthetic activity. Moreover, such matrices have a high organic load and the presence of suspended solids, making it difficult to maintain the life of some microorganisms in that environment. Thus, the occurrence of these dyes in the watercourses have been verified, even at low concentrations (Pavan et al. 2007), and the treatment of this residual water becomes necessary before its disposal in the watercourses.

Although the conventional treatments can reduce the organic load of the effluent, these have not been sufficient to promote the reduction of pigmentation, even when it is in low concentrations. Thus, more effective methods should be used, such as the advanced oxidation processes (AOPs). An AOP acts by mineralizing contaminants, including organic matter, through the formation of oxidizing species such as the hydroxyl radical (·OH) (Chakma et al. 2015). These processes occur in the presence or absence of light (ultraviolet (UV) or solar radiation). There are various types of AOP, Fenton, photo-Fenton, UV/H2O2 and ozonation being the most used (Salazar & Izário Filho 2009; Deng & Zhao 2015; Nair et al. 2016).

The AOP that deserves special attention, as it has shown good efficiency discoloring dyes (Machado & Stülp 2010) and does not make necessary the use of a catalytic agent, is the UV/H2O2 process. This process happens combined with UV radiation that is capable of promoting the homolytic breakdown of hydrogen peroxide (standard redox potential = +1.78 V), which is a strong oxidizing agent, capable of forming the hydroxyl radical (E0 = +2.80 V) to promote oxidation of the pollutants (Vilar et al. 2017). According to studies developed by Jamal et al. (2015), Silva et al. (2014), and Zuorro et al. (2013), it is possible to prove the efficacy of the UV/H2O2 AOP for the degradation of effluents containing artificial dyes. All the results obtained, in terms of percentage of discoloration, were above 95% for the different types of dyes used in this study. The kinetics of dye degradation processes usually follow pseudo-first-order models, and sometimes depend on the H2O2 concentration used. However, despite the fact that AOPs have been showing good results, the presence of by-products, also known as intermediary reactions, can be found to be equally or more toxic than the initial impurities (Araújo et al. 2016). Therefore, toxicity tests have been carried out to evaluate the viability of AOPs according to Lima et al. (2016) and Zaidan et al. (2017a).

The artificial neural network (ANN) has been increasingly applied in AOPs (Moraes et al. 2016; Lira et al. 2017; Nascimento et al. 2018). In recent studies, Zarei et al. (2010), Dil et al. (2016), Çelekli et al. (2016) and Lenzi et al. (2016) show that the ANN has been used as an alternative to predict results of the degradation of artificial dyes, achieving linear regression coefficients (R2) between 0.98 and 0.99.

The present work had the objective of evaluating the degradation of an aqueous solution containing a mixture of green leaf and açaí purple food dyes, and evaluating the toxicity before and after the treatment. The experimental data were adjusted to the kinetic model and an ANN was applied to predict the percentage of degradation of the dyes.

**METHODOLOGY**

**Identification and quantification of the dyes**

Firstly, the wavelengths (λ) of maximum absorbance for the mixture of green leaf and açaí purple dyes (F. Trajano) were determined through a UV-Visible spectrophotometer (Thermo Scientific, model Genesys 10S). For this, a scanning in the range of λ of 190 to 1,100 nm was made. Then, analytical curves with a working range of 2 to 50 mg L−1 were constructed for each of the λ previously selected and the limits of detection (LOD) and quantification (LOQ) of the method were determined, as well as the coefficient of variation (CV), according to the Instituto Nacional de Metrologia Qualidade e Tecnologia (INMETRO 2011).

**Working solution and reagents**

A solution of the commercial dyes green leaf and purple açaí (F. Trajano) was prepared with deionized water, at a concentration of 50 mg L−1 of each dye. This work solution was
used throughout the process of evaluation of the dye degradation through AOPs. In the degradation step, the following reagents were used: hydrogen peroxide (30% w/v, Química Moderna) as oxidizing agent; $\text{H}_2\text{SO}_4$ 1 mol·L$^{-1}$ (Merck) for pH adjustment and FeSO$_4$·7H$_2$O (Vetec) as the source of iron.

**Preliminary study**

In order to identify the advanced oxidation process (AOP) that presents greater efficiency for the degradation of the dyes under study, a preliminary study was carried out using Fenton, photo-Fenton, photolysis and UV/H$_2$O$_2$ processes. The work solution was submitted to the different AOPs using a concentration of hydrogen peroxide ([H$_2$O$_2$]) equal to 50 mg·L$^{-1}$ and pH between 5 and 6 (pH of natural solution), iron concentration ([Iron]) equal to 5 mg L$^{-1}$ in a period of 60 minutes. The aliquots were irradiated in the UV-A, UV-C and sunlight reactors. A schematic drawing of the reactor with UV-A radiation is shown in Figure 1, while the UV-C and sunlight reactors were the same as used by Zaidan et al. (2017a, 2017b) and Santana et al. (2017), respectively. For each reactor the photon emissions were measured with the aid of a radiometer (Emporionet).

**Hydrogen peroxide evaluation and pH study**

In possession of the results from the preliminary study, the best [H$_2$O$_2$] was evaluated for the AOP that presented a higher degradation percentage. The working solution was irradiated for 60 min, varying [H$_2$O$_2$]: 30, 35, 40, 45, 50, 55, 60, 65 and 70 mg·L$^{-1}$. After, the influence of pH on the AOP study was evaluated by varying the pH of the working solution in three ranges 3–4, 4–5 and 5–6, using the same period of time.

**Modelling**

**Kinetic modelling**

Along with the best experimental conditions (reactor, [H$_2$O$_2$] and pH), a kinetic study was performed, irradiating 1 L of the working solution. For this, aliquots were quantified and the percentages of degradation were quantified at the times of: 0, 30, 60, 75, 90, 95, 100, 110, 120 and 150
minutes. Then, the fit of the data to the kinetic model proposed by Chan & Chu (2003), shown in Equation (1), was verified.

\[
C = C_0 \left(1 - \frac{t}{\rho - \sigma t}\right)
\]  

(1)

In this equation, \(C\) is the remaining concentration of the product after the reaction time \(t\) (mg·L\(^{-1}\)), \(C_0\) is the initial concentration of the dye (mg·L\(^{-1}\)), \(t\) is the reaction time (min) and \(\rho\) and \(\sigma\) indicate the reaction kinetics (min) and the oxidative capacity of the system (dimensionless), respectively.

**ANN modelling**

For the modelling using the ANN, two different methodologies were used. The first methodology applied the sklearn package allied with the Python programming language. The strategy used a multilayer perceptron (MLP) network consisting of a layer with four neurons for the input: time (min), pH range (two inputs) and [H\(_2\)O\(_2\)]; a layer with four output neurons; and two hidden layers, with five neurons each (MLP (4-5-4)). Therefore, a learning curve of 0.001 was used, with a constant learning rate and a logistic activation function. The maximum number of interactions was 100,000.

The Statistica 6.0 software was also used to model the data obtained experimentally. The correlation was accomplished from the input data: time (min), pH, [H\(_2\)O\(_2\)]; and output: percentage of degradation at wavelengths of 215, 248, 523 and 627 nm. Sampling was performed randomly limited to between 3 and 10, and 1,000 interactions were performed for each training. The program tested 50 networks but retained only the top 10. In the end, the ANN model was chosen with the highest linear regression coefficient (\(R^2\)) among them.

**Toxicity**

Toxicity tests were carried out using strains of human pathogenic bacteria *Staphylococcus aureus* UFPEDA-02 and *Streptococcus pyogenes* ATCC-16642 obtained from the culture collection (WDCM114) of the Departamento de Antibióticos from the Universidade Federal de Pernambuco. The microbiological toxicity was performed with the initial solution (IS) and post-treatment dye solutions previously filtered on a syringe filter (0.22 \(\mu\)m). The bacteria were cultured in Muller Hinton agar plates overnight at 36 \(^\circ\)C and were subsequently fitted to a cell suspension at 10\(^6\) colony-forming units per mL (CFU·mL\(^{-1}\)). The samples, as well as the sterile ultrapure water, which was the negative control (4.5 mL), were added to a solution of the bacterial inoculum (0.5 mL) and Muller Hinton broth (1 mL) for 24 h at 36 \(^\circ\)C. After this time the post-treatment solution (PTS) was successively diluted 1:10, 1:100, 1:1,000 and 1:10,000 in sterile ultrapure water and then inoculated with a platinum loop (1 \(\mu\)L) into Petri dishes and incubated again (24 h at 36 \(^\circ\)C). The classification of bacterial growth was obtained in comparison with the growth observed in the negative control.

**RESULTS AND DISCUSSION**

**Identification and quantification of the dyes**

For the identification of the dye mixture under study, green leaf and purple açaí, four wavelengths (\(\lambda\)) were detected: 215 and 248 nm (aromatic groups), 523 and 627 nm concerning the chromophore groups.

After identifying the characteristic \(\lambda\), the analytical curves were constructed, obtaining correlation coefficients (\(r\)) higher than 0.96, indicating a good linearity of the method (ANVISA 2003; INMETRO 2011). The LOD and LOQ values varied between 1.85 and 2.78 mg·L\(^{-1}\) and 2.22 and 3.82 mg·L\(^{-1}\), respectively. The CV values were less than 5%, indicating that the methodology used is accurate, as CV values were less than 5% (ANVISA 2003).

**Preliminary study**

A preliminary study was carried out according to the proposed methodology. The efficiency of the AOP was measured in terms of degradation percentage of the aqueous solution of the dyes, and the emission of photons of each reactor was obtained through a radiometer: UV-A (1.82 \(\times\) 10\(^{-4}\) W·cm\(^{-2}\)), UV-C (1.44 \(\times\) 10\(^{-3}\) W·cm\(^{-2}\)) and sunlight (5.35 \(\times\) 10\(^6\) W·cm\(^{-2}\)).

The best result was verified while using UV/H\(_2\)O\(_2\) action with the UV-C reactor, reaching a color removal of 91.05% (\(\lambda = 523\) nm) and 92.06% (\(\lambda = 627\) nm). Regarding the
degradation of the aromatic groups, 17.94% ($\lambda = 215$ nm) and 43.24% ($\lambda = 248$ nm) were obtained. This same process using UV-A radiation achieved a color removal percentages lower than 13.68% and 57.57% ($\lambda = 215$ nm) and 49.89% ($\lambda = 248$ nm). When using the sunlight reactor, no degradation of the studied groups was observed, so it was decided to continue the study using UV-C radiation, since this one was able to remove the color of the solution under study more efficiently.

The photo-Fenton process achieved its optimum result when using sunlight radiation, obtaining 82.99% ($\lambda = 523$ nm) and 78.21% ($\lambda = 627$ nm) of degradation, while the photolysis and Fenton processes promoted an inefficient degradation of the chromophore groups, being less than 34%.

Concentration of hydrogen peroxide evaluation and pH study

The UV/H$_2$O$_2$ process was used to define the concentration of H$_2$O$_2$ that best fits the degradation process. It was noticed that the best condition was with H$_2$O$_2$ equal to 40 and 45 mg·L$^{-1}$, since the concentrations of hydrogen peroxide diverged around 2% for all $\lambda$, which was confirmed by the analysis of spectral scan performed for the two [H$_2$O$_2$], as can be seen in Figure 2.

It can be verified through the analysis of Figure 2 that an overlap of the spectra of the two [H$_2$O$_2$] under discussion occurred, indicating that there are no significant differences between the percentages of degradation obtained. Therefore, the [H$_2$O$_2$] of 40 mg·L$^{-1}$ was chosen, considering the cost associated with this reagent. Then, the influence of the pH of the solution in the degradation process was evaluated, being analyzed for three different pH ranges: 3–4, 4–5 and 5–6.

It was found that a higher degradation was obtained in a pH range of 5–6, achieving 5.95% ($\lambda = 215$ nm), 49.99% ($\lambda = 248$ nm), 98.17% ($\lambda = 523$ nm) and 95.66 ($\lambda = 523$ nm).

Modelling

Kinetic modelling

After the best operating condition for discoloration (UV-C radiation, [H$_2$O$_2$] of 40 mg·L$^{-1}$ and natural solution pH (5–6)) was determined, the kinetic study was performed. The kinetic monitoring was achieved by withdrawing aliquots of the working solution at the times of: 30, 60, 75, 90, 100, 110, 120 and 150 min. Figure 3(a) shows the kinetic monitoring of the degradation of the chromophore groups and the aromatic groups over time, based on the adjustment to the model proposed by Chan & Chu (2005), and Figure 3(b) shows the residual values of the percentage of degradation over time.

The analysis of Figure 3(a) shows that the experimental data fitted well with the kinetic model proposed by Chan & Chu (2003). There is also a rapid degradation of the chromophore groups with $\lambda$ equal to 523 and 627 nm, since the decay rate of the concentration of these groups is much higher in the first minutes.

It can be stated from the analysis of Figure 3(b) that the degradation of green leaf and purple açaí dyes employing the UV/H$_2$O$_2$ process presented a good kinetic adjustment for the proposed model, since for all $\lambda$ analyzed, the $R^2$ values were greater than 0.90 (Figure 3(c)). Paulino et al. (2015) also obtained values of $R^2$ between 0.989 and 0.999, strengthening the usage of this model for kinetic monitoring of dyes. Finally, the results of each of the parameters of the kinetic model, whose values obtained are presented in Figure 3(c), were evaluated.

It is known that the maximum discoloration capacity is represented by the value of the constant 1/$\sigma$, which is obtained when the time goes to infinity. Therefore, according to the data in Figure 3(c), it can be inferred that the oxidation capacity of the process for the characteristic $\lambda$ of the color is much higher than the values obtained for the $\lambda$ characteristic of aromatic groups, since there is a difficulty to degrade them. Thus, it is necessary to study the toxicity of the solution after treatment (Paulino et al. 2015).

Artificial neural network

Among all the simulations from the Statistica 6.0 software, it was verified that the RBF model was not accurate since the
R² values were lower than 0.9 (INMETRO 2014). The best result was found when applying the MLP model with nine neurons in the hidden layer (MLP 3-9-4). The hidden activation layer and output activation layer were Tanh and Logistic, respectively, with SOS (sum of squares) as an error function. The R² obtained for the training, test and validation were, respectively, 0.957, 0.976 and 0.897. The error obtained for training was 0.016 and for the test was 0.017. The values for the input, concealment and exit layer weights are shown in Table 1.

From the experimental data, the adjustment for the ANN models was verified: MLP (4-5-4) and MLP (3-9-4). Figure 4 shows the linear regression for the models used.

The analysis of Figure 4 revealed that R² values between 0.979 and 0.997 were obtained for MLP (4-5-4) model and between 0.901 and 0.922 for MLP (3-9-4) model. These results indicate a good fit of the experimental data when using MLP (4-5-4) model.

It can also be stated that the MLP (3-9-4) model fitted well to the experimental data, but with a greater dispersion, especially for the characteristic wavelengths of the aromatic groups. The results obtained for the ANN MLP (4-5-4) are in agreement with the literature, as the values of R² ranged

![Figure 3](image-url)  
(a) Kinetic monitoring of the degradation of green leaf and purple açaí dyes, (b) residual graph of the percentage of degradation over time and (c) conversion data and kinetic parameters for the oxidation reaction (X: percentage degradation).

Table 1 | Weight for the inputs, concealment and exit layer

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Time (min)</th>
<th>pH</th>
<th>[H₂O₂]</th>
<th>Bias input layer</th>
<th>215 nm</th>
<th>248 nm</th>
<th>523 nm</th>
<th>627 nm</th>
</tr>
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<tr>
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<td>2.665</td>
<td>0.954</td>
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<td>0.020</td>
<td>1.156</td>
<td>-0.209</td>
<td>0.227</td>
<td>0.800</td>
</tr>
<tr>
<td>2</td>
<td>-0.767</td>
<td>-0.199</td>
<td>0.476</td>
<td>0.068</td>
<td>1.325</td>
<td>1.198</td>
<td>-0.153</td>
<td>-0.259</td>
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<tr>
<td>3</td>
<td>1.084</td>
<td>0.447</td>
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<td>0.629</td>
<td>1.540</td>
<td>-0.233</td>
<td>0.126</td>
</tr>
<tr>
<td>4</td>
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<td>0.241</td>
<td>0.102</td>
<td>-1.310</td>
<td>1.330</td>
<td>0.678</td>
<td>0.827</td>
<td>-0.402</td>
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<tr>
<td>5</td>
<td>0.462</td>
<td>-0.291</td>
<td>0.120</td>
<td>-0.838</td>
<td>-0.659</td>
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<td>1.528</td>
<td>-0.204</td>
</tr>
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<td>6</td>
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<td>-0.020</td>
<td>-0.036</td>
<td>0.365</td>
<td>0.972</td>
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<td>7</td>
<td>0.798</td>
<td>2.304</td>
<td>0.748</td>
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<td>-0.475</td>
<td>-0.666</td>
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<tr>
<td>8</td>
<td>-0.549</td>
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<td>-0.034</td>
<td>0.877</td>
<td>-0.254</td>
<td>0.323</td>
<td>1.012</td>
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<td>0.377</td>
<td>0.423</td>
<td>-0.528</td>
<td>-0.393</td>
<td>-0.601</td>
</tr>
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</table>
from 0.97 to 0.99 (Yonar & Kilic 2014; Eskandarloo et al. 2016; Thivaharan et al. 2016).

It is worth emphasizing that the present study considered only an aqueous solution of the mixture of leaf green and açaí purple dyes, and it is necessary to carry out future studies to test the validity of the ANN model proposed in real matrices, since these are more complex due to the presence of other contaminants.
Toxicity

For the same treatment conditions described in the kinetic study, toxicity tests were performed. After the incubation time (24 h at 36 °C) of the aliquots containing 1 μL of the samples, the viable cell counts (CFU·mL \(^{-1}\)) were obtained in comparison to the initial inoculum. Then, the percentage of bacterial growth was determined for each of the bacteria under study (Table 2).

According to Table 2, it was observed that S. aureus presented a behavior similar to the negative control for the solutions before and after treatment, except for 100% PTS. This result indicates that there was normal bacterial growth and the treatment employed did not influence the growth of the bacterium in question after the dilution process was carried out.

However, for S. pyogenes, it was verified that the solution from the treatment using UV/H\(_2\)O\(_2\) showed toxicity in the PTS concentrations of 100, 70 and 50%. Nevertheless, normal growth was observed when the PTS concentration was 10% and higher growth was noticed for PTS 5% and PTS 1%. The presence of toxicity may have been evidenced, since, as observed in the kinetic study, even after the treatment there are intermediate compounds that may present a higher toxicity than the initial compound.

Conclusion

The obtained results indicate that UV/H\(_2\)O\(_2\) action is a viable method to degrade an aqueous solution containing purple açaí and green leaf dyes when applying UV-C radiation for 60 minutes, achieving a degradation percentage of 5.95, 49.99, 98.17 and 95.99% for \(\lambda\) equal to 215, 248, 523 and 627 nm, respectively. It was noticed that the [H\(_2\)O\(_2\)] interferes in the process, concluding that 40 mg·L\(^{-1}\) showed the best results. However, as pH did not present a significant influence on the removal of the color, it was chosen to work with the natural solution pH. A good fit of the Chan and Chu model was verified as obtained values of \(R^2\) for the chromophore and aromatic groups were above 0.99. Also, the ANN modelling managed to predict and describe the degradation process since the obtained \(R^2\) values, especially for MLP (4-5-4), were above 0.97. The toxicity results pointed out that for both bacteria (S. aureus and S. pyogenes), the pre-treatment solution presented higher toxicity than the post-treatment.

Acknowledgements

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References


Table 2 Viability of Staphylococcus aureus and Streptococcus pyogenes after the treatment

<table>
<thead>
<tr>
<th>Samples</th>
<th>Staphylococcus aureus</th>
<th>Streptococcus pyogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (not treated)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IS</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PTS 1%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>PTS 5%</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>PTS 10%</td>
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<tr>
<td>PTS 50%</td>
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<td>-</td>
</tr>
<tr>
<td>PTS 70%</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PTS 100%</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+ normal growth; ++ significant growth; – no growth.

IS, initial solution; PTS, post-treatment solution.


