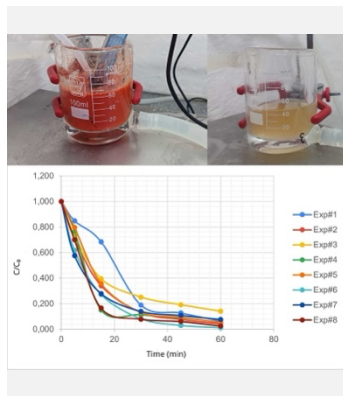


## Degradation of Carotenoids from Annatto Dye Industry Effluent by Ozonation

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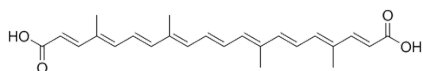
J. V. P. Batista, M. N. Esperança, A. L. de Castro Peixoto\*. Federal Institute of São Paulo (IFSP), Av. Dr. Ênio Pires de Camargo 2971, Capivari, Brazil, alcpeixoto@ifsp.edu.br



The present work sought to evaluate the degradation of norbixin, a carotenoid present in high concentration in the effluent from the annatto industry by oxidation processes. Full-factorial  $2^3$  experimental design was used to evaluate temperature, pH and ozone dose on the kinetics of carotenoid degradation. The questions that guided our research were: (1) is it possible to degrade carotenoids dissolved and dispersed in industrial annatto dye effluent by ozone-based processes with high pH? (2) would radical action be the main degradation mechanism in an ozone-based process? The study showed that: (1) ozone oxidation is a promising alternative for treating annatto dye effluents; (2) the ozone direct reaction of ozone stands out from possible radical reaction mechanisms.

### Introduction

Annatto, designated E160b, is a naturally derived food colorant sourced from the seeds of the *Bixa orellana* tree. The pigment extracted from the seed's orange-red outer layer has extensive applications in the food industry, pharmaceutical formulations, and fine chemistry. Chemically, annatto dye is characterized by its principal constituents, bixin and norbixin, along with trace amounts of other carotenoids and fatty acids. Bixin is the major pigment component, constituting approximately 70-80% of the dye's colorant properties. Conversely, norbixin, a by-product of bixin production through partial degradation, contributes to the remaining colorant fraction, accounting for 20-30% of the dye's color profile [1]. Fig. 1 illustrates the  $\beta$ -norbixin molecule.



**Figure 1.** Illustration of a carotenoid molecule ( $\beta$ -norbixin).

The present study pioneered the investigation of the efficacy of ozonation for the removal of carotenoids, quantified as norbixin salts, from real effluents generated within the annatto dye manufacturing sector. Prior to this research, the literature lacked an examination of oxidative treatment methodologies tailored to effluents from the annatto dye industry, making this work an inaugural exploration into optimizing effluent treatment processes within this niche.

### Material and Methods

50.0 mL of untreated industrial effluent was

transferred to a jacketed reactor vessel. Subsequently, an antifoaming agent was introduced, followed by the incremental addition of a 1.0 mol/L KOH solution to adjust the pH to the desired level. Following this preparatory phase, a sample of the effluent was extracted for baseline spectrophotometric quantification of carotenoids, denoted as time zero ( $t=0$  min). The ozonation process was initiated by controlled infusion of ozone into the reactor. The duration of each ozonation experiment was set to one hour. The ozonation system was operated in semi-batch mode, continuously sparging gas at 1.0 L/min through a sintered glass diffuser ( $15 \times 20$  mm). Ozone was generated using a corona discharge ozone generator (O&L3.0 RM Ozone&Life), supplied with  $>99.0\%$   $O_2$ . The temperature was maintained constant using a thermostatic bath (SL-152 Solab), and the pH was manually adjusted to remain constant using a 1 mol/L KOH solution. A full  $2^3$  factorial design was used to evaluate the effects of pH, temperature, and  $O_3$  on carotenoid degradation (Table 1). Spectrophotometric analysis was performed according to the method proposed by Reith and Gielen [2].

### Results and Discussion

It was possible to achieve degradation above 90% under all experimental conditions. The constant kinetic response variable proved to be more effective in the statistical evaluation of the proposed factors. At 60 min, there was practically no difference in the % of carotenoid degradation among the studied experiments. It was quantified using a pseudo-first-order chemical kinetics model (Table 1). The degradation process was evaluated

using an analysis of variance (95% confidence). Among the studied parameters, temperature, pH, and ozone concentration were statistically significant. The only relevant interaction occurred between the temperature and ozone. The lack of interaction between pH and ozone parameters indicates that the degradation mechanism of hydroxyl radicals is irrelevant. This could be explained by the high concentration of dissolved salts in the effluent. The concentration of chlorides, for example, was on the order of g/L. However, the interaction between ozone and temperature ( $F=19$ , Table 1) indicated the direct reaction of ozone as the main mechanism, following the Arrhenius law. Based on these factors, it was possible to obtain a mathematical model to predict the kinetic constants (Eq. 1). This refined mathematical model satisfactorily predicted carotenoid degradation kinetics with  $R^2=0.93$ . As shown in Eq. 1, while temperature and ozone positively influence the degradation kinetics, the influence of pH was negative. Therefore,  $OH^-$  excess reduces the efficiency of the oxidative process. The formation of

$\cdot OH$  is favored under alkaline conditions. However, some pollutants can be degraded more efficiently by molecular ozone than by hydroxyl radicals.

Ozone is a gas with a high affinity for species of molecules with a high electron density (such as carotenoids). The conjugated double bonds create single and alternating bonds (Fig. 1), which allow the molecules to displace electrons, resulting in a high electron density in their molecules, making them susceptible to reaction with ozone through direct reaction, which causes loss of coloration. The mechanism governing carotenoid degradation is probably the Criegee mechanism, whereby the reactive component, ozone in this case, initiates the cleavage of unsaturations within the molecular structure of carotenoids (Fig. 1), consequently producing degradation products. These products may exhibit distinct chemical and physical properties compared to the parent pollutant, which is often characterized by reduced chromaticity or even colorlessness.

**Table 1.** (a) Full factorial  $2^3$  design applied to study carotenoid degradation in effluents from the annatto dye industry. Left: Uncoded values. Right: coded values. (b) ANOVA for pseudo-first-order carotenoid degradation kinetics. Degrees of Freedom (DF). Adjusted sums of squares (Adj SS) for different components of the regression model. Adjusted mean square of error (Adj MS). 95% confidence level ( $\alpha=0.05$ ).

Run	(a)					(b)					
	Temp. (°C)	pH	O <sub>3</sub> (mg/min)	k (min <sup>-1</sup> )	k (min <sup>-1</sup> )	Source	DF	Adj SS	Adj MS	F	p
1	10(-1)	8.00(-1)	8.00(-1)	0,0505	0,0484	Model	4	0.002175	0.000544	38.95	0.000
2	30(+1)	8.00(-1)	8.00(-1)	0,0549	0,0537	Linear	3	0.001909	0.000636	45.59	0.000
3	10(-1)	11.00(+1)	8.00(-1)	0,0423	0,0317	pH	1	0.000658	0.000658	47.14	0.000
4	30(+1)	11.00(+1)	8.00(-1)	0,0398	0,0464	O <sub>3</sub>	1	0.000509	0.000509	36.43	0.000
5	10(-1)	8.00(-1)	18.00(+1)	0,0513	0,0504	T	1	0.000743	0.000743	53.21	0.000
6	30(+1)	8.00(-1)	18.00(+1)	0,0744	0,0805	Interactions	1	0.000266	0.000266	19.04	0.001
7	10(-1)	11.00(+1)	18.00(+1)	0,0429	0,0408	O <sub>3</sub> *T	1	0.000266	0.000266	19.04	0.001
8	30(+1)	11.00(+1)	18.00(+1)	0,0590	0,0586	Error	11	0.000154	0.000014		
						Lack-of-Fit	3	0.000051	0.000017	1.34	0.328
						Pure Error	8	0.000102	0.000013		
						Total	15	0.002328			

$$k = 0,0516 + 0,0068 \cdot T - 0,0064 \cdot pH + 0,0056 \cdot O_3 + 0,0041 \cdot T \cdot O_3 \quad (1)$$

## Conclusions

Initially, we sought to verify the action of hydroxyl radicals on apocarotenoid degradation, which is favored by the reaction of ozone with hydroxyl anions (KOH). However, owing to the high complexity of the effluent and the presence of dissolved salts, statistical analysis suggested that the direct action of ozone corresponded to the main reaction mechanism.

## Acknowledgments

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## References

- [1] J.F. Reith, J.W. Gielen, *Journal of Food Science*, 36 (1971) 861.
- [2] *Annatto: A Seed with the History of Brazil*, P.R.N. Carvalho. Editor, Campinas, Evidência, 2020.