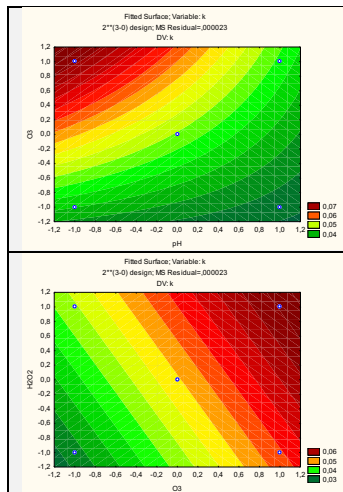


# Degradation of Carotenoids from Annatto Dye Industry Effluent by Ozonation

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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. A full-factorial  $2^3$  experimental design was used to evaluate temperature, pH, and ozone dose about the kinetics of carotenoid degradation. The questions that guided our research were: (1) Can ozone-based processes at high pH degrade carotenoids in annatto dye wastewater? (2) would radical action be the primary 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 principal 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.

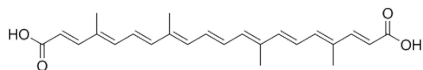


Figure 1. Illustration of carotenoid molecule ( $\beta$ -norbixin).

This study first explored the effectiveness of ozonation in removing carotenoids, measured as norbixin salts, from actual effluent from the annatto dye industry. Prior to this research, the literature did not examine oxidative treatment methodologies tailored to effluents from the annatto dye industry, making this work an inaugural exploration for 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 is introduced. When necessary, H<sub>2</sub>SO<sub>4</sub> or KOH was used to adjust the pH to the desired level. The pH of the raw effluent had a value of 1.90. Following this preparatory phase, a sample of the effluent was extracted for baseline spectrophotometric quantification of carotenoids, denoted as time zero (t=0 min). At t=0, the auxiliary oxidant H<sub>2</sub>O<sub>2</sub> was added. No new additions of H<sub>2</sub>O<sub>2</sub> were made throughout the experiment. The ozone-based 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 × 20 mm). Ozone was generated using a corona discharge ozone generator (O&L3.0 RM Ozone&Life), supplied with >99.0% O<sub>2</sub>. The temperature was maintained at 20 °C by using a thermostatic bath (SL-152 Solab). A complete  $2^3$  factorial design was used to evaluate the effects of the pH, O<sub>3</sub> dose, and H<sub>2</sub>O<sub>2</sub> initial concentration on carotenoid degradation (Table 1). Spectrophotometric analysis was performed according to the method proposed by Reith and Gielen [2]. It is a standard methodology adopted by the United Nations Food and Agriculture Organization (FAO). All experiments were performed in triplicates.

## Results and Discussion

The pH of the effluent, as received from the annatto dye industry, was 1.90. In this study, we evaluated the acidic range of the degradation process because industry generates effluents with acidic characteristics. The degradation percentage and pseudo-first-order kinetic constants were studied as response variables (Table 1a). For the % degradation, only the linear O<sub>3</sub> factor was statistically significant (data not shown). However, considering the degradation kinetics, all linear factors, pH, O<sub>3</sub> dose, and initial H<sub>2</sub>O<sub>2</sub> concentration were significant (Table 1b). For this response variable, the pH × O<sub>3</sub> interaction was also significant (Table 1b). The O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> parameters had a positive effect on the reaction speed. Thus, faster reactions were achieved by increasing the O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> concentrations.

On the other hand, pH had a negative effect. An excess of H<sup>+</sup> increases the efficiency of the process. Protonation of carotenoids favors the attack of organic molecules by oxidants. Under the conditions studied, no evidence exists of ·OH

generation by the ozone and hydrogen peroxide reaction. Cleavage of chromophoric groups in the effluent occurred through direct oxidation by ozone and hydrogen peroxide.

We must also consider that we have not yet reached an optimal oxidant concentration. The high doses of O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> required by the process were due to the high content of carotenoids and dissolved salts in the effluent. For example, the presence of chlorides causes impracticable ·OH radical attack. Finally, Table 1b indicates that the direct reaction of ozone is much more significant than that of hydrogen peroxide and the pH × O<sub>3</sub> interaction. The effect of O<sub>3</sub> on the degradation rate is approximately twice as significant as that of H<sub>2</sub>O<sub>2</sub> and pH × O<sub>3</sub>. In terms of the design process application, the use of H<sub>2</sub>O<sub>2</sub> can be disregarded in the treatment of effluents from the annatto industry. Greater efforts should be directed towards adjusting the ozone dose and pH of the reaction medium for further optimization studies.

**Table 1.** (a) Full factorial 2<sup>3</sup> design was applied to study carotenoid degradation in annatto dye industrial effluents. Left: Uncoded values. Right: coded values. (b) Pareto chart of pseudo-first-order carotenoid degradation kinetics. 95% confidence level (α=0.05). The experiments were performed in triplicate, including the central point (CP) condition.

(a)						(b)	
Run	pH	O <sub>3</sub> (mg min <sup>-1</sup> )	H <sub>2</sub> O <sub>2</sub> (g L <sup>-1</sup> )	Deg. %	k (min <sup>-1</sup> )	Pareto Chart of Standardized Effects; Variable: k 2 <sup>**</sup> (3-0) design; MS Residual=.0000109 DV: k	
1	2.5(-1)	8.0(-1)	1.572(-1)	87.4	0.0354	(2)O3	8,838179
2	5.5(+1)	8.0(-1)	1.572(-1)	84.0	0.0310	(1)pH	-7,04276
3	2.5(-1)	18.0(+1)	1.572(-1)	96.6	0.0586	(3)H2O2	4,54199
4	5.5(+1)	18.0(+1)	1.572(-1)	90.4	0.0379	1b2	-4,22138
5	2.50(-1)	8.0(-1)	4.716(+1)	92.2	0.0426	2b3	2,404583
6	5.5(+1)	8.0(-1)	4.716(+1)	86.0	0.0338	1b3	-1,67786
7	2.5(-1)	18.0(+1)	4.716(+1)	97.4	0.0805		
8	5.5(+1)	18.0(+1)	4.716(+1)	94.5	0.0485		
CP	(0)	(0)	(0)	94.8	0.0492		
CP	(0)	(0)	(0)	95.6	0.0513		
CP	(0)	(0)	(0)	95.8	0.0497		

## Conclusions

Initially, we sought to verify the action of hydroxyl radicals on apocarotenoid degradation, which is favored by the reaction of ozone with hydrogen peroxide. However, owing to the effluent's high complexity and the presence of dissolved salts, statistical analysis suggested that the direct action of ozone corresponded to the primary reaction mechanism. A strongly acidic medium favored the direct action of ozone on the kinetics of dye degradation.

## Acknowledgments

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## References

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- [2] *Annatto: A Seed with the History of Brazil*, P.R.N. Carvalho. Editor, Campinas, Evidência, 2020.