

Improving the Biodegradation of Organic Pollutants with Ozonation *during* Biological Wastewater Treatment

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Abstract

Pre-ozonation to enhance the biodegradability of a certain compound prior to biological treatment is often recommended. One shortcoming of such an approach is wasting ozone on other compounds that are already biodegradable. This paper investigates an approach of ozonating a recalcitrant substance *during* biological treatment. Two parallel bench-top activated sludge processes were fed a synthetic wastewater containing typical biodegradable substances and also methylene blue at 5 mg/L. Ozone was applied continuously and directly *into* one of the activated sludge units at 17 mg/L. The methylene blue was removed by 95% in the ozonated process compared with just 40% removal in the non-ozonated control. The latter removal was demonstrated to be mainly due to biosorption. The oxidation reaction products, mainly organic acids, were degraded biologically in the same process unit together with the other biodegradables in the feed stream. A concern that ozone, as a powerful disinfectant, could inhibit or kill the bacteria was proven to be incorrect. The reaction rate with organic substances depleted the dissolved ozone at such a rate that the disinfective effect on the treatment bacteria (and protozoa) was minimal, affecting mainly filamentous bacteria.

Key Words

Ozone, activated sludge, biodegradability, dye, methylene blue, byproducts, filamentous bulking

Introduction

Six billion humans produce millions of tons of organic wastes every day. A large proportion of this ends up in wastewaters. The wastewaters are collected in sewers from residential and industrial sources for processing in wastewater treatment plants mainly in central facilities, usually operated by local authorities. Typically, the principal wastewater treatment process is biological – aerated bacterial cultures – in the form of activated sludge. Some industries have

their own activated sludge plants. The biodegradable organic substances are easily removed by biological treatment processes and thereby prevent subsequent oxygen depletion in surface waters. Many organic substances of synthetic origin are difficult to degrade and are not effectively removed during biological treatment. Such substances may persist in the environment and bio-accumulate and can be detrimental to the water environment and degrade the value of water for downstream users. Some of these cause discoloration of water or pose toxic threats to water life and downstream users (Hallberg, 1987). The removal of synthetic substances is therefore important, but involves more advanced and expensive technologies than biological treatment. Improving on present technology is required as it could make treatment more feasible and cost effective. Two of the most commonly found groups of synthetic substances that are recalcitrant to degradation are the agrochemicals, particularly pesticides, and textile dyes. The aim was to develop methodology to improve the removal of these substances during wastewater treatment. This particular project addressed the removal of dyes, using methylene blue as a test substance.

Many organic substances can be oxidized to a more degradable form by ozonation (Sontheimer et al., 1978). Ozonation is one of the more effective technologies to oxidize a large spectrum of pesticides and dyes (Narkis and Schneider-Rotel, 1980; van Leeuwen, 1980 and 1987; Rice and Browning, 1981 and Zimmerman and Richard, 1990). These are generally not oxidized to CO₂ and H₂O (Glaze *et al.*, 1993) but to new byproducts. Non-degradable substances found in wastewater can be converted to a biodegradable form by chemical oxidation and then degraded biologically. Some of these may be toxic (Laplanche *et al.*, 1974) but generally, most of the byproducts will be more biodegradable, such as aldehydes, ketones, organic acids and generally smaller molecules (Lopez et al., 1998). Ozonation of wastewater before biological treatment has been found to increase the removal of organic material by the subsequent biological treatment (van Leeuwen *et al.*, 1981; Hu and Yu, 1994; and Rivas *et al.*, 2000). *However, most ozone is wasted when applied before biological treatment, since it oxidizes biodegradable substances also – even more readily than non-biodegradables.*

A better approach is to *first* effect biological treatment and *then* ozonate to make the remaining recalcitrant organic material biodegradable and subsequently remove these products biologically. This would require another biological treatment process after ozonation, however. Recycling the ozonated water to the prior biological process circumvents having a second biological process, but this has its limitations, as recycling increases the total throughput rate (and consequently impacts on the settling process to retain the biomass) and only part of the newly degradable compounds can be recycled continuously. Clearly, a better approach is still required.

Activated sludge processes are usually fully mixed, which implies that the effluent quality will be the same as that of the liquid within the process. The liquid phase needs to be ozonated to degrade unwanted dissolved substances. It would therefore make sense to apply the ozone *directly into* the activated sludge process. There it can react with the less degradable organic materials as the degradable ones have been largely removed already. The degradable ozonation products can then also be removed biologically. The only possible weakness in the argument is the fact that ozone is also a powerful disinfectant, which could inhibit or inactivate the bacterial culture.

Research conducted in the 1980's showed that ozonation within the activated sludge process does not kill all bacteria and improves biomass settling (Légeron et al., 1980, Doré, 1984).

Subsequent research was specifically directed at using ozone as a selective disinfectant for use within the activated sludge process. Ozone, applied continuously within an activated sludge system, was found to be a selective disinfectant towards nuisance filamentous bacteria, but did not affect the other - mainly beneficial - bacterial species (van Leeuwen, 1988a and b and 1989). Ozone dosages ranged from 3 to 26 mg/L. This principle was applied on full-scale by the City of Pretoria (Saayman *et al.*, 1996). Collignon *et al.* (1994) found that intermittent ozonation into the sludge recirculation stream for only 30 minutes every three days was also effective in combating filamentous bacteria, reducing ozone requirements significantly.

Van Leeuwen found that the dosages required to inhibit the growth of some of the bacterial species did not affect the overall biomass production and did not detrimentally affect the effluent quality either. In fact, the ozonation resulted in improved effluent quality, but this aspect was not quantified in great detail, as this was not the main aim of the research. The dosages were probably lower than optimal for good organic removal too. Moreover, the research was conducted on municipal – mainly domestic – wastewater and (van Leeuwen, 1989) on a highly degradable industrial wastewater.

Yasui and Shibata (1994) conducted research on using ozone in a biological process, aimed at degrading excess biomass. A side-stream of the mixed liquor was ozonated to a high level and the authors were successful in reducing the biomass suspended solids concentration to the point where no excess sludge had to be disposed of. Kamiya and Hirotsuji (1998) improved on this concept with intermittent ozonation, reducing the ozone requirements considerably. A small increase in the effluent organic concentration measured as dissolved organic carbon was observed.

Contreras *et al.* (2003) modeled biomass inhibition as a function of ozone level in biomass.

The research in this paper aimed to develop a more effective and economical treatment process for wastewater from industries with a large component of non-biodegradable substances such as dyes. The specific objectives were to:

- establish boundaries for ozonation dosage rates for improving organic removal, particularly dyes, while not detrimentally affecting beneficial bacteria in activated sludge,
- establish the reaction rate of ozonolysis of a typical dye, in this case methylene blue, in industrial wastewater within an activated sludge system,
- identify the byproducts from the ozonolysis of the methylene blue,
- determine the fate of the ozonolysis byproducts,
- find an effective process configuration for introducing ozone into an activated sludge process for continuous operation conditions

Experimental

Hypothesis

Ozonation can be used to improve the biodegradability of organic substances in industrial wastewater *within* an activated sludge treatment process without detrimentally affecting the bacterial processes. This will improve the overall removal of synthetic organic substances.

Activated sludge treatment

Two bench-top activated sludge plants (See Figure 1) were operated in the laboratory. Sludge biomass from the Boone, Iowa nutrient removal wastewater treatment plant was used as a starting culture in the two activated sludge units of 6L capacity. The biomass with its associated liquor was aerated continuously through fritted glass porous diffusers. About 10% of the biomass was wasted daily to maintain a sludge age of 10 days. The plants were each fed with synthetic wastewater at 0.6L/h, using a peristaltic pump, ensuring a hydraulic retention time of 10h. The synthetic wastewater was tap water, to which a number of organic and inorganic nutrients had been added (See Table 1).

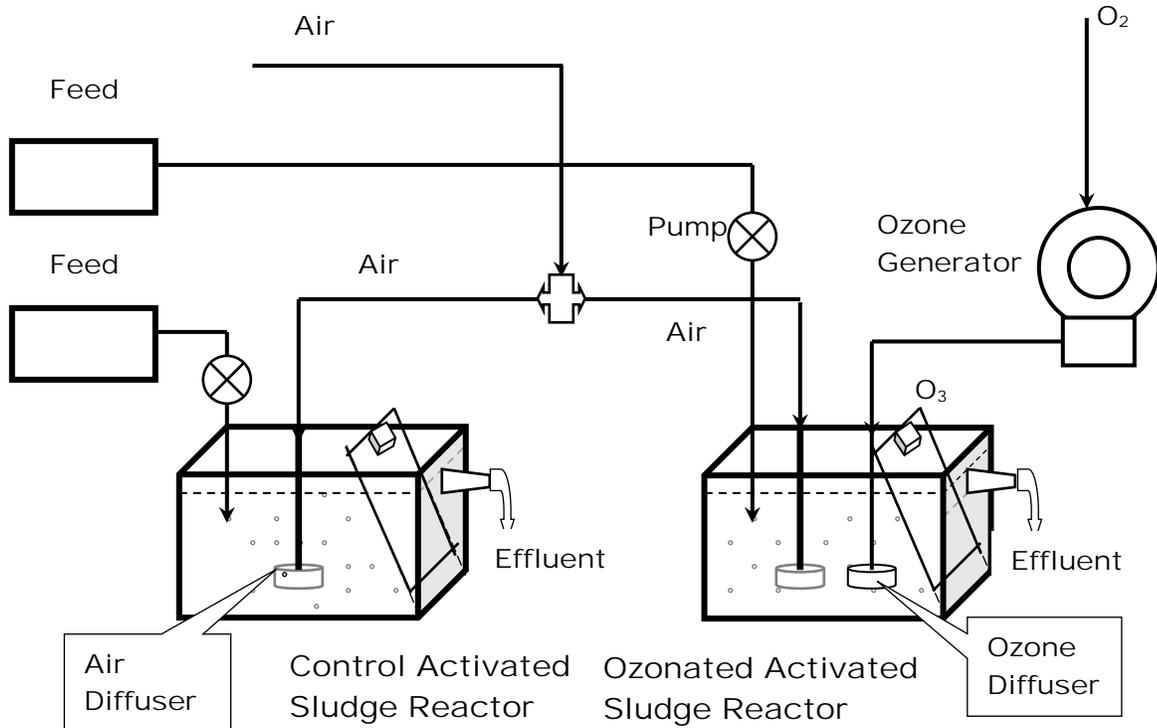


Figure 1 Bench scale activated sludge investigation

One of the units was ozonated through a fritted glass porous diffuser, using an oxygen-fed Ozonology ozone generator. Ozone was dosed at 10mg/h, i.e. 16.7 mg/L based on the flow rate.

Table 1: Synthetic feed composition

Synthetic Wastewater Nutrients	Stock Concentrations, mg/L
Urea	37.6
Nutrient broth	60
Potato starch	6
Non-fat dried milk	60
Dietary fiber	10
Sodium acetate	20
Sodium bicarbonate	10

Potassium phosphate(K ₃ PO ₄ .H ₂ O)	3
Ferrous sulphate (FeSO ₄ .7H ₂ O)	0.5
Methylene blue	5

Limited optimization of ozone dosage to remove color had indicated that this dosage removed the color substantially, without upsetting the biological treatment system. Dosage rates were controlled through a variable control valve and through a rotameter. Ozone dosages were determined by gas phase concentrations by the standard iodometric absorption method and flow rates. Methylene blue removal was studied as well as the effect of ozonation on the microbial composition of and the overall effluent quality.

Water analyses

Effluent samples were monitored on a daily basis for the chemical oxygen demand and spectrophotometric absorbance of the methylene blue at 660 nm (APHA et al., 1995).

Microbial investigations

Biomass samples were subjected to visual inspections by light microscopy under 100x to 400x magnification to observe floc structure and the presence and abundance of typical microorganisms. Identification of filamentous bacteria and protozoa were based on visual characteristics using the manual of Eikelboom and van Buijssen (1986).

Physical biomass characterization

Mixed liquor samples were tested for mixed liquor suspended solids concentrations as well as the sludge volume index. These are important variables to adjudicate the health of the biomass, growth rates and sludge settleability. The methodology here will be as prescribed by the American Water Works Association and others in Standard Methods (1995).

Biosorption

Biosorption is adsorption onto organic substances without biodegradation (Aksu et al., 1998). Methylene blue, for instance, adsorbs onto activated sludge. The extent of this biosorption was determined with adsorption isotherms for the methylene blue on biomass suspensions inactivated with 5 mg/L of mercuric chloride. Increasing quantities of the dead biomass were added to a 5 mg/L of methylene blue solution, shaken for 1.5h and remaining methylene blue measured spectrophotometrically at 660 nm.

Biodegradability

Extant respirometric tests (Ellis et al., 1996) were conducted, using adapted biomass from the activated sludge experiments. The biomass was first oxygenated to saturation and then allowed to consume this oxygen by endogenous respiration, i.e. no source of organic material was added. The effect of then adding methylene blue in low concentrations on the biomass respiration was monitored. An increase in respiration rate would be an indication of some biodegradability of the substance; a decrease indicative of toxic effects.

Ozonation byproducts

Separate ozonation tests were conducted on a pure solution of methylene blue of 5 mg/L in unbuffered and phosphate buffered (at pH 6) deionized distilled water to establish reaction rates as well as to determine the type of ozonolysis products formed. The ozone dosages ranged from 4.5 to 5 mg/L and were dosed in a 500 mL reaction vessel with a fritted glass porous diffuser. Off-gas was captured in a subsequent reaction vessel containing a 0.5% solution of potassium iodide. Analysis of the products of degradation by ozone and bacteria was conducted using samples from the small activated sludge units and other samples as prepared in the Environmental Engineering Laboratory. These samples were analyzed using a combination of gas chromatography, a GOW-MAC Series 580 with an injector and detector at 140C and the column at 100C, using helium as a carrier.

Electrospray ionization

Electrospray ionization (Fenn et al., 1990) was performed on a Finnigan TSQ700 triple quadrupole mass spectrometer (Finnigan MAT, San Jose, CA) fitted with a Finnigan ESI interface. Samples were introduced into the electrospray interface through an untreated fused-silica capillary with a 50 μm i.d. and 190 μm o.d. A myoglobin and MRFA mixture was used for tuning and routine calibration of the instrument. The mixture was made in the following manner: stock solutions of myoglobin and MRFA were prepared by dissolving 0.9 mg of horse skeletal muscle myoglobin in 1 ml of a solution of 50:50 methanol: water containing 1% acetic acid, and 0.6 mg of MRFA was dissolved in 1 ml of a solution of 50:50 methanol: water containing 1% acetic acid. The calibration and tuning standard was made from the two stock solutions: 50 μl of myoglobin and 12 μl of MRFA were diluted with 538 μl of a 50:50 methanol:water solution in a polypropylene vial. The tuning mixture was infused into the mass spectrometer at a rate of 3 $\mu\text{l}/\text{min}$ on a Harvard Apparatus (model 22, South Natick, MA) syringe pump.

Results

The results are presented as below.

Effluent quality

COD removal

The COD removal in both the ozonated and unozonated processes was variable, but averaged about 80.5% in the ozonated and 79.6% in the unozonated control – no significant difference.

Methylene blue removal

The methylene blue removal showed significant variation in the biological process. Fresh biomass was much more effective in the removal of the dye than seasoned biomass. Once stabilized, the ozonated process was able to remove on average 95% of the methylene blue against about 40% in the case of the unozonated control process.

Biomass characteristics

The biomass concentration in both processes varied considerably due to difficult control over the biomass inventory. The average mixed liquor suspended solids was maintained at 2 g/L. The

sludge settleability differed markedly. The average sludge volume index of the unozonated sludge was 150 mL/g against that of the ozonated sludge of 120 mL/g.

Microscope photos revealed that the ozonated biomass was more compact and had far fewer filamentous bacteria and apparently more protozoa (Figures 2 and 3). While the latter fact is not conclusive, the conclusion can be drawn that ozonation did not in any way discourage protozoa. Note also the stronger blue color of the non-ozonated activated sludge, due to higher biosorption. The main type of filamentous bacteria appeared to be Type 0041 or 0675, both characterized by massive attached growths. The protozoa were mainly of the genii *Chilodonella* and *Paramecium*.

Biodegradability of methylene blue

The extant respirometric test indicated that the biodegradability of methylene blue in an unacclimatized culture was minimal with only a slight change in the slope of the dissolved oxygen depletion rate. The contribution of biodegradation was therefore expected not to be of great significance, but may explain the additional 7.5% removal observed above the 32.5% predicted.

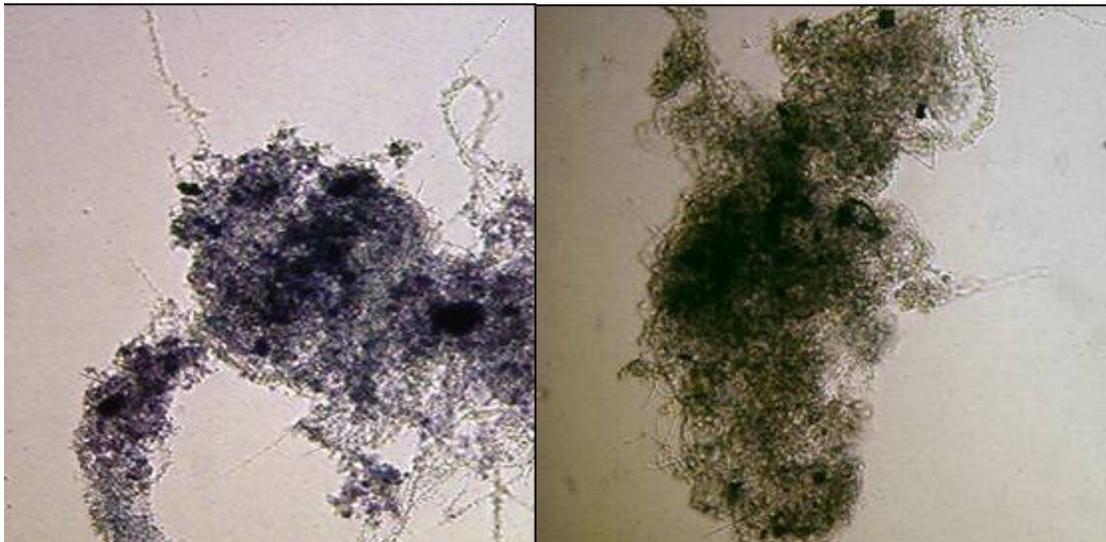


Figure 2 Non-ozonated (l) and ozonated (r) at 200x showing the latter more dense

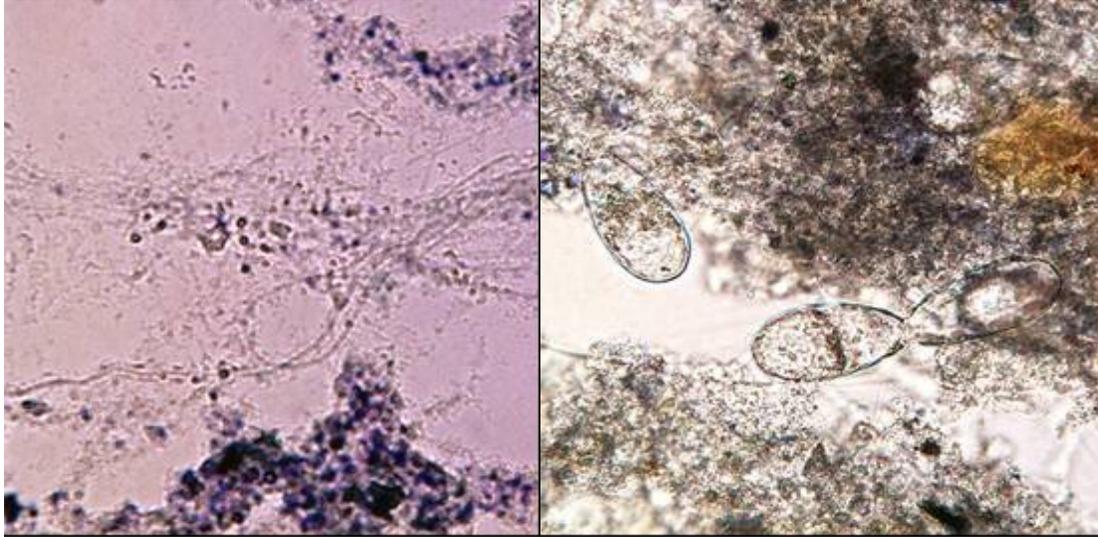


Figure 3 Non-ozonated sludge (l) showing excessive filaments and ozonated sludge(r) 400x, showing abundant protozoa

Ozonation byproducts

Ozonation of an unbuffered solution of 5 mg/L methylene blue (0.0133mM) in deionized water led to a drop in pH from about 7 to 4.1. The increase in the concentration of H^+ ions was therefore 0.08mM, or about 6 acids (if fully dissociated) formed per mol of the dye. That may be due, in part, to the formation of an aryl sulfonic acid by cleavage of the central ring. Formic acid may also be formed. Gas chromatography confirmed that the main byproduct of the reaction was formic acid. This compound is known to be highly biodegradable. Ion chromatography will be used to identify other possible acid anions. Electrospray ionization made it possible to provisionally identify some more byproducts.

1. While the molecular mass of MeBl is 319, this includes a chloride ion, the remaining ion has a molecular mass of 284, as observed in the spectra after partial ozonation.
2. Three peaks were identified and involved the $NHCH_3$ portion of the molecule. These are all deficient of chlorine anion.
3. The 2 peaks at 179 and 189 are not directly understood; further investigation using MS/MS would probably yield some answers.
4. Negative ion mode ESI analysis will have to be done to identify carboxylic acids anions.

Biosorption

Freshly killed biomass exhibited adsorption behavior that could best be described by the Freundlich isotherm as in Figure 4. Based on a mixed liquor suspended solids (biomass concentration) of 2.0 g/L, i.e. 12g of biomass per unit, the net daily biomass production was 10% of this, i.e. 1.2 g of biomass per day.

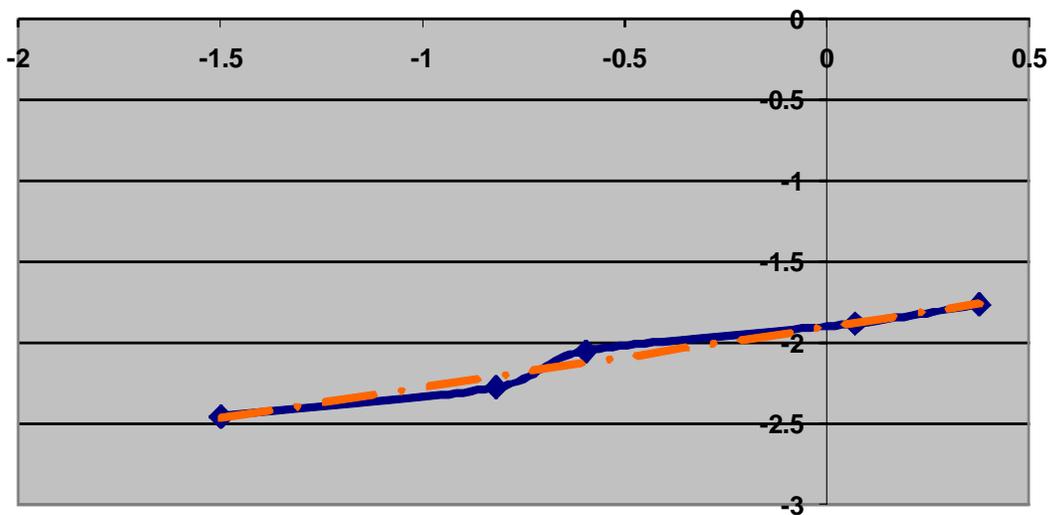


Figure 4: Freundlich isotherm for the biosorption of methylene blue on activated sludge

The expected equilibrium methylene blue concentration on the biomass at the mean non-ozonated effluent concentration of 3 mg/L would be 19.5 mg/g biomass. This would result in a removal of 23.4 mg of methylene blue per day from a total feed of $5\text{mg/L} \times 0.6\text{ L/h} \times 24\text{h/d} = 72\text{mg/d}$, which corresponds with a removal of 32.5%. Similarly, for the ozonated process, with an average effluent concentration of 0.25 mg/L, the predicted equilibrium concentration on the biomass would be 7 mg/g and the biosorptive removal would be only 8.4 mg/d from the 72 mg/d feed or 11.7%.

Conclusions

The research addressed untried areas of research in an activated sludge process with *internal* ozonation for the purpose of improving the selective removal of organic compounds. Earlier research work using ozone within an activated sludge process was to control the microbial composition or even density; the actual concept of chemical oxidation to aid organic removal *within* a biological process is new and untried. Also, while it is well known that ozonation can oxidize non-degradable organic substances to degradable byproducts, the concept of doing this within a biological process is bold. The biomass was largely unharmed by the ozone.

Ozone can be applied directly into an activated sludge process in such dosages as to oxidize synthetic organic compounds to biodegradable products. While ozonation leads to some bacterial selection, a healthy biomass can be maintained for the removal of a variety of organic pollutants, including byproducts from the ozonation of methylene blue.

The research aimed to develop a more effective and economical treatment process for wastewater from industries with a large component of non-biodegradable substances, such as dyes. This was

successful. The ozone demand, and subsequently energy requirements for such an integrated process, will be lower than for separate biological and ozone treatment. A single process will not only substantially reduce the ozone requirements, but having a single process unit also reduces other aspects of the capital cost of the system. There will be substantial savings in reactors and footprint requirements - no need for separate ozone contacting equipment, pumping and further biological treatment. This integrated process will simplify pollution control in the textile industries and potentially be beneficial in many other industrial and combined municipal wastewater treatment applications.

The potential benefits make this a worthwhile endeavor. There are many industries and local authorities with such industries within their boundaries that could make good use of such an integrated process for pollution control, e.g. textile and paper mills, agricultural chemicals manufacturers and specific food processing industry.

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