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Preozonation of Primary-Treated Municipal Wastewater for Reuse in Biofuel Feedstock Generation

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The results of a laboratory scale investigation on ozone pretreatment of primary-treated municipal wastewater for potential reuse in fermentation processes for the production of biofuels and bio-based feedstock chemicals were presented. Semi-batch preozonation with 3.0% (w/w) ozone at 1 L min^{-1} *resulted into a considerable inactivation of the indigenous heterotrophic bacteria in the wastewater with less than 0.0002% comprising the ozone-resistant fraction of the microbial population. The disinfection process was modeled using first-order inactivation kinetics with a rate constant of* 4.39×10^{-3} s^{-1}. *Chemical oxygen demand (COD) levels were reduced by 30% in 1-h experiments. COD depletion was also modeled using a pseudo-first-order kinetics at a rate constant of* 9.50×10^{-5} s^{-1}. *Biological oxygen demand* (BOD_5) *values were reduced by 60% up to 20 min of ozonation followed by a plateau and some slight increases attributed to partial oxidation of recalcitrant materials. Ozone also had no substantial effect on the concentration of ammonium and phosphate ions, which are essential for microbial growth and metabolism. Preliminary tests indicated that oleaginous microorganisms could be cultivated in the ozonated wastewater, resulting in relatively higher cell densities than in raw wastewater and comparable results with autoclave-sterilized wastewater. This process could potentially produce significant quantities of oil for biofuel production from municipal wastewater streams.* © 2010 American Institute of Chemical Engineers Environ Prog, 30: 666–674, 2011

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INTRODUCTION

Municipal wastewater treatment remains a daunting task worldwide due to the rapid increase in population and demand for industrial products resulting into high wastewater volumes and treatment costs. In the United States alone, around 16,583 operational wastewater facilities were treating a total of 34 billion gallons of municipal wastewaters daily within the last 4 yr [1]. With this high volume, the total nationwide cost for wastewater treatment and collection was estimated to be \$189.2 billion annually [2]. One proposed strategy to mitigate the environmental impacts and treatment costs of these high-volume wastewater streams and enhance environmental sustainability is through wastewater reclamation and reuse for a vari-

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ety of applications. Some examples include industrial cooling and washing water, nonpotable domestic water, irrigation in urban areas (gardens, parks, golf course, etc.), restoration of natural water courses [3], and irrigation for agriculture activities [4]. Another potential application that is under investigation by our group is to reuse municipal wastewaters as cultivation media for microorganisms involved in the production of biofuels and/or biobased fuel feedstocks. In addition to producing high-value products, this process could degrade the organic pollutants in the wastewater that make up its biochemical oxygen demand (BOD_5) as well as its nutrient (N and P polyionic species such as nitrate, nitrite, ammonium, and phosphate) levels. Examples of these microorganisms are bioethanol-producing yeasts, and more recently, *oleaginous* yeasts, bacteria, and algae capable of accumulating lipids that could be used for the production of biodiesel [5].

However, as with all municipal wastewater reutilization projects, certain pretreatment schemes must be implemented, particularly in reducing the pathogenic risk and microbial toxicity of these wastewater streams [3]. For reusing primary-treated (i.e. screening, grit removal, sedimentation) wastewater in fermentation processes involving specific microbial strains, the main pretreatment objectives are to eliminate the indigenous microbial population in the wastewater that may compete with the pure strains for substrates in the media, improve wastewater quality and biodegradability, and reduce toxicity. Among the various water and wastewater treatment methods available, ozone has been selected for this study because of its well-documented strong disinfecting power against a wide variety of bacteria, viruses, and other pathogenic organisms [6–9]. Compared with chlorination, ozone pretreatment requires shorter contact times, produces less harmful residuals due to its rapid decomposition, and could be generated onsite, resulting into fewer shipping and handling problems. When compared with ultraviolet (UV) radiation, ozonation results into less regrowth of inactivated microorganisms after the treatment process [8]. Moreover, ozone has also been shown to reduce the BOD_5, chemical oxygen demand (COD), total organic carbon (TOC), total solids, turbidity, and improve the color of domestic wastewater because of its strong oxidizing power, hence improving the quality of the wastewater stream [6]. In most cases, ozone has also been shown to improve the biodegradability of domestic wastewaters by oxidizing recalcitrant materials in the wastewater into more biodegradable matter thus decreasing the COD while the BOD_5 remained constant or increased slightly [6, 10–12]. Dissolved oxygen levels in wastewaters have also been shown to increase to saturation levels following ozonation since ozone molecules are relatively unstable and decompose to oxygen quickly in aqueous media [13]. This robustness and versatility of ozone in reacting with virtually every component of the wastewater can be attributed to two different routes through which ozone reacts with organic and inorganic compounds in the wastewater: by direct molecular attack, or by the action of hydroxyl radicals which are produced by the decomposition of ozone molecules in aqueous solutions. The latter mechanism has been shown to be more unselective with rate constants ranging from 10^8 to 10^{10} M^{-1} s^{-1} [14]. Although capital and treatment costs and power requirements for ozonation are relatively higher than those of chlorination and UV, the benefits appear to outweigh the extra costs, which will eventually decrease with continued research to improve current technologies [6].

Given the perceived benefits of ozone, the objective of the current study was to determine the feasibility of applying ozone as a pretreatment step for primary-treated municipal wastewater for potential reutilization as fermentation media for oil-producing oleaginous microorganisms. Semibatch ozonation experiments were conducted to determine the effect of ozone on heterotrophic bacteria that are indigenous to the municipal wastewater. Furthermore, ozonation effects on BOD_5, COD, and nutrient ion ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $P\text{-}PO_4^{3-}$) concentrations were investigated as these parameters represent the substrates available in wastewater for consumption by microorganisms for the production of biofuels. Ozone disinfection and COD oxidation kinetic models were tested to obtain kinetic parameters necessary for process scale-up and design. Afterwards, test cultivation runs using an oleaginous microorganism consortium developed at the Renewable Fuels and Chemicals Laboratory-Mississippi State University on ozonated wastewater media were conducted to determine the effectiveness of the ozonation process for the proposed reutilization scheme.

MATERIALS AND METHODS

Wastewater Sampling and Characterization

The primary-treated wastewaters tested in this study were obtained from a municipal wastewater treatment plant in Tuscaloosa, AL. Grab samples were collected from each of three operational primary clarifiers in the plant and stored in polyethylene bottles. Care was taken to ensure that representative samples were obtained and used in the ozonation experiments. Samples were taken at designated areas around the primary clarifiers at the same day of the week and time of the day throughout the duration of the study. The wastewater samples were then transported under ice to the laboratory and used in the ozonation experiments within 2 h of arrival to minimize changes in the microbial and physicochemical characteristics of the wastewater.

The raw and ozonated wastewater samples were characterized for BOD_5 and COD according to AWWA/APHA Standard Methods 5210B and 5220D, respectively [15]. Heterotrophic bacteria were enumerated by a membrane filtration method according to AWWA/APHA Standard Method 9215D. Serial dilutions were prepared using sterile 0.85% (w/v) NaCl solution. Triplicate 10 mL dilutions of the primary effluent samples were then filtered through sterile 47-mm diameter nitrocellulose membrane filters with a 0.45-μm mean pore size (Fisherbrand, Pittsburgh, PA)

Table 1. Characteristics of the primary-treated municipal wastewater used in this study.

pH	7.0 ± 0.1
COD (mg L^{-1})	396 ± 94
BOD_5 (mg L^{-1})	133 ± 48
N-NH_4^+ (mg L^{-1})	22 ± 3
N-NO_3^- (mg L^{-1})	N.D.
N-NO_2^- (mg L^{-1})	4.2 ± 0.9
P-PO_4^{3-} (mg L^{-1})	8.9 ± 1.1
Heterotrophic bacteria ($\times 10^7$ CFU mL^{-1})	3.3 ± 2.0

ND, not detected.

using a vacuum filtration assembly (Nalgene Inc., Rochester, NY). The used filters were placed aseptically in plates containing commercially available DIFCO Plate Count Agar (Fisher Scientific, Pittsburgh, PA) containing (in gL^{-1}): pancreatic digest of casein 5.0, yeast extract 2.5, dextrose 1.0, and agar 15. The plates were then incubated at 22 ± 2°C for 5 days.

The concentrations of nitrite, nitrate, ammonium, and phosphate were determined using two ion chromatography methods for the analysis of inorganic cations and anions in water and wastewater described by Dionex Corporation [16, 17]. The equipment used was an ICS-3000 ion chromatograph (Dionex Corp., Sunnyvale, CA) equipped with a 250 × 4-mm IonPac AS11-HC anion exchange analytical column and 50 × 4 mm AG11 guard column for nitrite, nitrate, and phosphate analysis; a 250 × 4 mm IonPac CS16 cation exchange analytical column and 50 × 4 mm CG16 guard column for ammonium analysis, and two suppressed conductivity detectors. Before analysis, the samples were pretreated by passing them through C_{18} Sep-Pak cartridges (Waters Corp., Milford, MA) that were preconditioned with 5 mL of methanol and 5 mL of Type II ASTM water (18.2 MΩ-cm resistivity). The samples were then filtered through 0.45-μm syringe filters (Millipore, Billerica, MA). The instrument detection limits were 0.01 mg L^{-1} for nitrite and nitrate, 0.02 mg L^{-1} for phosphate, and 0.01 mg L^{-1} for ammonium. Table 1 shows the average values and standard deviations of the wastewater characteristics of primary clarifier effluent grab samples obtained at different times for the performance of the ozonation experiments. Chemical analyses indicated no significant variations in wastewater characteristics among grab samples obtained at the same time.

Ozone Generation and Monitoring

Ozone was produced from compressed dry air by corona discharge using an Ozonology LC-1234 ozone generator (Ozonology, Inc., Northbrook, IL). The ozone concentrations in the inlet and outlet gas streams were monitored using a UV spectrophotometer ozone detector (PCI Ozone and Control Systems, West Caldwell, NJ). The residual ozone in the wastewater samples was measured using commercially available Ozone CHEMets Test Kits (CHEMetrics Inc., Calverton, VA).

Ozonation Experiments

Equal amounts of primary-treated wastewater grab samples were mixed together to create a homogenous mixture, which will now be referred to as the test wastewater. One liter of the test wastewater was then transferred into a 1-L glass reactor (Chemglass, Vineland, NJ) equipped with a gas diffuser and mixer. Gas containing 3.0% (w/w) ozone was bubbled through the test wastewater continuously at 1 ± 0.05 L min^{-1} for 60 min at room temperature with an agitation rate of 100 rpm Samples were withdrawn from the reactor at different times during the ozonation experiment and analyzed for residual ozone concentration, BOD_5, COD, heterotrophic bacteria population, and N-NH_4^+, N-NO_3^-, N-NO_2^-, and P-PO_4^{3-} concentrations. Prior to analysis, the residual ozone in the samples was neutralized with 0.1% (w/v) $Na_2S_2O_3$ solution. The exhaust gas from the reactor was passed through a glass column packed with Carulite® 200 manganese dioxide/copper oxide catalyst (Carus Corp., Peru, IL) to destroy any remaining ozone before being released into the atmosphere.

Microbial Cultivation Experiments on Ozonated Wastewater

The ozonated test wastewaters were then tested as potential growth media for a consortium of oleaginous microorganisms (OM) developed at MSU-RFCL. The consortium contains 12 yeast strains and 1 bacteria that have well-documented lipid-accumulating abilities at certain media conditions. Eight hundred milliliters of ozonated wastewater contained in 2-L shake flasks with (a) no glucose supplement and amended with (b) 1 g L^{-1} or (c)10 g L^{-1} glucose were inoculated with 30 mL of the OM culture and incubated at 27°C with shaking at 110 rpm Each treatment was done in triplicates. Samples of the cultures were obtained at daily intervals and were measured for optical density at a wavelength of 600 nm using a Genesys 20 Spectrophotometer (Thermo Scientific, Waltham, MA). The level of microbial growth in the ozonated wastewater cultures was then correlated with the optical density measurements. For comparison, cultivation runs involving raw and autoclaved (121°C, 240 kPa, 15 min) primary effluent wastewater were also conducted using the same conditions.

RESULTS AND DISCUSSION

Disinfection Kinetics

Figure 1 shows the average inactivation trend of the indigenous heterotrophic bacteria in the municipal wastewater primary effluent using ozone. The data were presented as typical semi-log plots of the fraction of surviving microorganisms, N/N_0 as a function of the transferred ozone dose (TOD); where N_0 and N are the number of viable microorganisms initially and at contact time t, respectively. The TOD values are obtained by calculating the difference between the ozone dose supplied and the amount of ozone leaving the reactor at different contact time intervals and represent the amount of ozone utilized

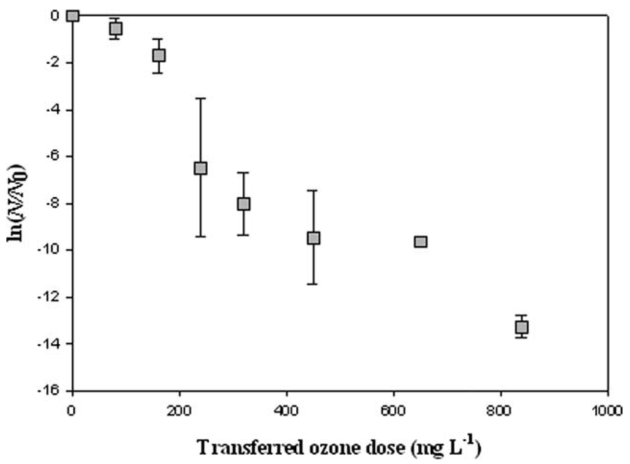

Figure 1. Survival ratios of heterotrophic bacteria in primary-treated wastewater (in semi-log scale) as a function of transferred ozone dose.

for disinfection and oxidation. The plot shows that the general inactivation trend follows a multiphasic curve consisting of an initial shoulder followed by a short exponential drop and a tailing-off pattern towards the end of the run. The initial shoulder which represents a low initial disinfection rate that subsequently increased may be attributed to several factors such as delays in ozone diffusion and/or multiple oxidation targets such as the pre-existing oxidizable material in the wastewater. On the other hand, the tail may be caused by aggregation of microbial cells, cellular debris, or suspended solids that may produce a shielding effect from the effects of ozone, or microbial subpopulations with varying resistances to ozone inactivation [18]. The latter is expected since the heterotrophic bacterial population in the wastewater is thought to consist of different bacterial strains. This same pattern has been found in previous studies involving the disinfection of heterotrophic bacteria from water where a major portion of the heterotrophic bacteria was rapidly inactivated but there always remains an ozone-resistant subpopulation, hence the tailing-off pattern [18]. In another study, the existence of the tail was explained as a result of other competing reactions in the wastewater matrix with ozone which are favored over microbial inactivation at that extent of the ozonation process [19]. Despite this, the remaining ozone-resistant microbial population represents on average less than 0.0002% of the initial heterotrophic bacteria counts.

Several previous studies using ozone as a water/wastewater disinfectant have noted the difficulty and complexity of developing kinetic models that accurately describe inactivation kinetics using ozone. Models based on reaction mechanisms are particularly susceptible to error due to the complexity of multicomponent matrices like wastewater, unstable residual ozone due to ozone autodecomposition and/or competing reactions with other organic and inorganic compounds in the treatment matrix, and rapid inactivation rates [14, 20–22]. For this reasons, the use of empirical models based on kinetic rate laws are more widely used. In this study, two exploratory models were used based on the following direct reaction of ozone with microbial cells:

$$O_3 + N \longrightarrow \text{products} \tag{1}$$

where N represents the number of viable heterotrophic bacterial cells in colony-forming units (CFU) per mL of wastewater. The first model expresses the rate of microbial inactivation as

$$-\frac{dN}{dt} = k_N N C_{O_3} \tag{2}$$

where k_N is the disinfection rate constant and C_{O_3} is the dissolved ozone concentration. Since the average initial pH of the wastewater samples were close to neutral (see Table 1) and was not significantly affected by ozonation (pH range 6.78–6.97), it was assumed that hydroxide ion concentrations were low so that ozone oxidation caused by the free radical mechanism was negligible [23]. It was also assumed that O_3 is present in large excess with respect to the initial number of viable microorganisms; therefore Eq. 1 is simplified into a pseudo-first-order rate equation or more commonly known as Chick's rate law [18]

$$-\frac{dN}{dt} = k_N^* N \tag{3}$$

where k_N^* is the pseudo-first-order inactivation rate constant equal to $k_N C_{O_3}$. Integration of Eq. 3 leads to the following equation:

$$\ln\frac{N}{N_0} = -k_N^* t \tag{4}$$

The values of the parameters were estimated using the SOLVER optimization package in Microsoft EXCEL®. The goodness-of-fit of the proposed model with the experimental data were measured by calculating the correlation coefficient:

$$R^2 = 1 - \frac{\sum \left(y_i - y_p\right)^2}{\sum \left(y_i - \bar{y}_i\right)^2} \tag{5}$$

where y_i represents an observed value and y_p represents the model predictions.

To determine the apparent order of ozone inactivation, the simple power rate law was proposed as a second model:

$$-\frac{dN}{dt} = k_N N^m \tag{6}$$

The experimental data were then fit into a nondimensionalized integral of Eq. 6 given as

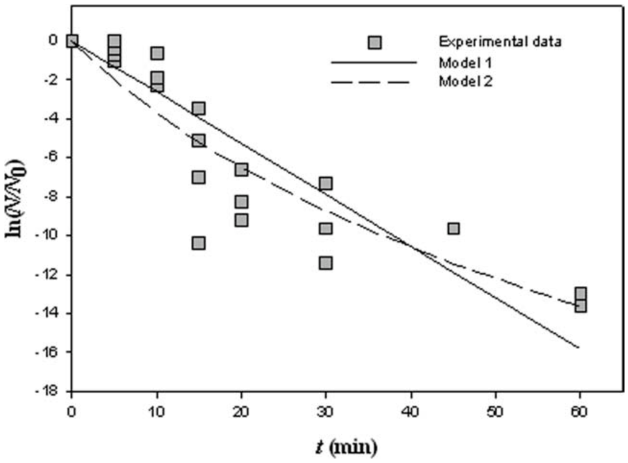

Figure 2. Ozone disinfection kinetic model testing.

$$\frac{N}{N_0} = \frac{1}{\left(1 + KN_0^{m-1}t\right)^{1/(m-1)}} \tag{7}$$

where $K = \frac{(m-1)k_N^*}{N_0^{m-1}} = (m-1)k_N$. The resulting model fit with the pooled experimental data is shown in Figure 2 and the calculated disinfection kinetic parameters are summarized in Table 2. As shown in Table 2, the nonlinear regression fit (R^2) = 0.77 of the pooled experimental data with the pseudo-first-order relationship described by Eq. 4 resulted into a calculated reaction rate constant $k_N^* = 4.39 \times 10^{-3}$ s^{-1}. When the power law model was used, the calculated apparent reaction order is close to the first order ($m = 1.09$) while the apparent reaction rate constant obtained is $k_N^* = 7.37 \times 10^{-3}$ s^{-1}, which represents the slope of the curve at $t = 0$. The power model also resulted into a better correlation coefficient ($R^2 = 0.84$) than the pseudo-first order approach.

Effects on BOD_5 and COD

The trends of COD, BOD_5, and BOD_5/COD of the test wastewater as a function of ozone contact time are shown in Figure 3 as a ratio of the values of the said parameters at time t and the initial values. The average COD reduction data from three representative runs show an almost linear reduction trend in the total COD of the wastewater. The maximum COD reduction level reached was approximately 30%. On the other hand, the BOD_5 reduction rates were fastest within the first 20 min of ozonation and reached a maximum reduction of approximately 60%. Beyond this point, the BOD_5 levels remained fairly constant and in some instances increased slightly. This observation was consistent with numerous previous wastewater ozonation studies [6, 10, 11, 13, 24] in which the authors attributed the slight increases in the BOD_5 of the wastewater to the partial oxidation of recalcitrant materials into a biodegradable fraction. This phenomenon has been taken advantage of in other studies, particularly those in which the goals of ozonation were to improve wastewater biodegradability and removal of biorefractory organics such as aromatics and unsaturated compounds to assist in the subsequent biological oxidation using the activated sludge process [25]. Furthermore, the BOD_5/COD ratio, which is generally used as an indication of the biodegradability level of wastewater streams was also shown to follow a trend similar to that of BOD_5. A relatively high BOD_5/COD indicates a more biodegradable wastewater stream and vice versa. Although the BOD_5/COD decreased significantly during the first 20 min of ozonation, it appeared to level off until the end of ozonation run.

Table 2. Parameter estimates for the proposed ozone disinfection kinetic models.

Model	k_N^* (s^{-1})	m	R^2
Pseudo-first order, Eq. 4	4.39×10^{-3}	N/A	0.77
Power law, Eq. 7	7.37×10^{-3}	1.09	0.84

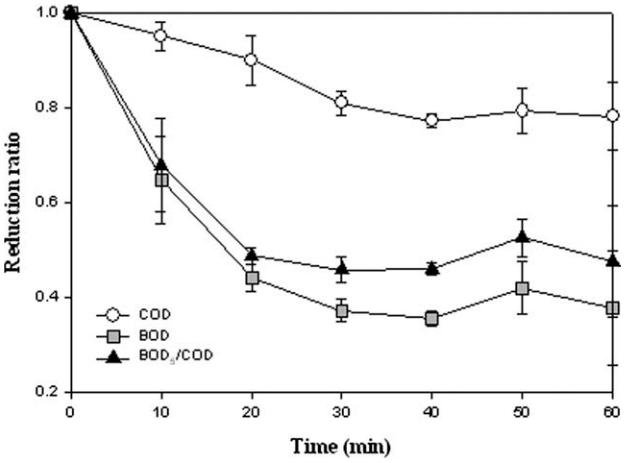

Figure 3. Variation of BOD_5, COD, and BOD_5/COD ratio with ozone contact time.

The total COD concentration has usually been adopted as a parameter to indicate the overall concentration of ozone-reacting compounds since it includes both organic and inorganic materials that constitute wastewaters, including microbial cells [11, 26]. Low COD levels usually indicate the presence of low molecular weight carboxylic acids such as acetic, oxalic, and maleic acid, which are considered nontoxic and more susceptible to biological degradation [14]. Hence, the kinetics of COD oxidation was also considered using a pseudo-first-order kinetic model similar to Eq. 3, assuming that ozone is present in excess

$$-\frac{d\mathrm{COD}}{dt} = k_{\mathrm{COD}}^{*}\mathrm{COD} \tag{8}$$

Integrating Eq. 8 leads to the following pseudo-first-order relationship

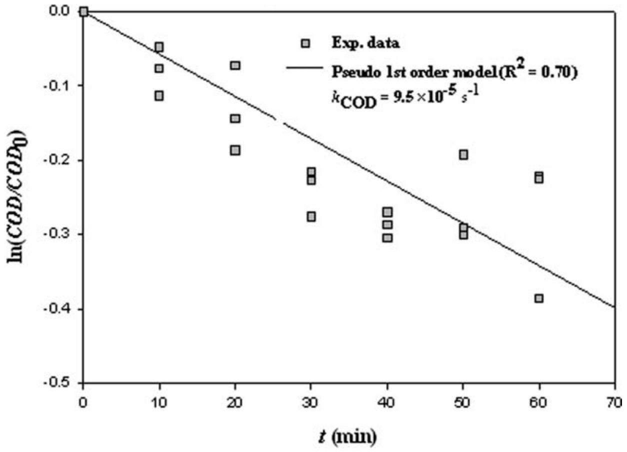

Figure 4. COD oxidation kinetic model testing.

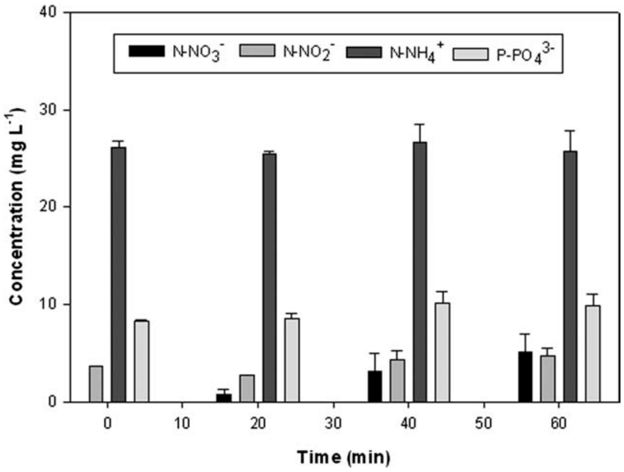

Figure 5. Effect of ozone contact time on the nutrient ion composition of primary-treated wastewater.

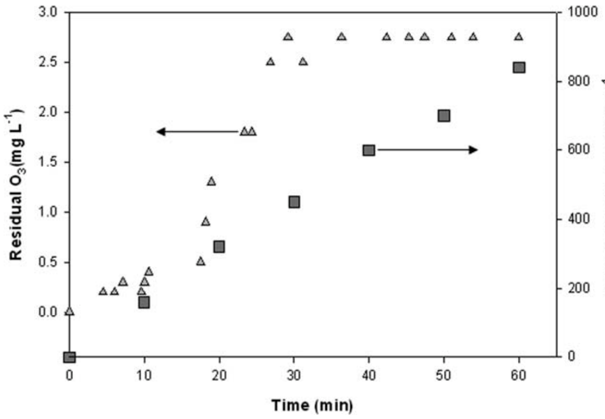

Figure 6. Residual ozone formation and total ozone dose as a function of contact time.

$$\ln\frac{\text{COD}}{\text{COD}_0} = -k^*_{\text{COD}}t \tag{9}$$

where k^*_{COD} is the apparent COD oxidation rate constant. Figure 4 shows that the linear regression fit of the experimental COD reduction data resulted in a satisfactory correlation (R^2 = 0.70) and a calculated rate constant of 9.5 × 10^{-5} s^{-1}, which is much smaller than the disinfection rate constants. Hence, it can be inferred that microbial inactivation kinetics was favored over COD reduction. It may also be considered an artifact of the transformation of recalcitrant COD to BOD, which is also encompassed by the total COD of the wastewater. This may have resulted into an apparent reduction of the rate of total COD oxidation.

Effects on Nutrient Ion Concentration

The concentrations of ammonium and phosphate remained fairly constant throughout the ozonation experiment with minor fluctuations while nitrite levels increased slightly as shown in Figure 5. Theoretically, both nitrite and ammonia (which exists in equilibrium with ammonium) are oxidizable by ozone to produce nitrate in the wastewater [27]. Nitrate was not detected initially in the primary effluent but after 20 min of ozonation, it was detected by the IC and increased up to a concentration of 5 mg L^{-1}. These observations were consistent with previous studies that showed ozonation did not significantly reduce the concentration of nitrogen species in the wastewater since it appeared that ozone preferentially oxidizes carbonaceous materials rather than nitrogenous ones [6]. Only very high ozone doses have been shown to significantly oxidize nitrogen compounds into nitrate [28]. It is also possible that the oxidation of microbial cells and other organic suspended solids in the wastewater by ozone could have released these ions in the wastewater matrix to offset those that have been oxidized.

Ozone Consumption and Residual Formation

Reactions between ozone and oxidizable matter in the wastewater matrix are typically fast in addition to ozone autodecomposition so that in simple batch ozonation processes, the ozone supplied is quickly consumed and any residual ozone is usually undetected [22]. To produce a measurable residual ozone concentration in the wastewater matrix, which is usually required in most ozone oxidation and disinfection kinetic models, semi-batch experiments are performed instead. In this study, a compressed, dry-air stream containing 3.0% (w/w) ozone was supplied continuously at approximately 1.0 L min^{-1} into single batches of the test wastewater. This corresponds to an ozone feeding rate of 36 mg L^{-1}·min or a total ozone dose of 2160 mg L^{-1} after 60 min contact time. After taking the difference between the ozone dose supplied and the amount of ozone leaving the reactor at different contact time intervals, the transferred ozone doses (TOD) or the amounts of ozone consumed by the wastewater matrix were obtained. On average, the TODs make up approximately 42% of the total ozone supplied. It should also be noted that compared with previous studies dealing with ozonation

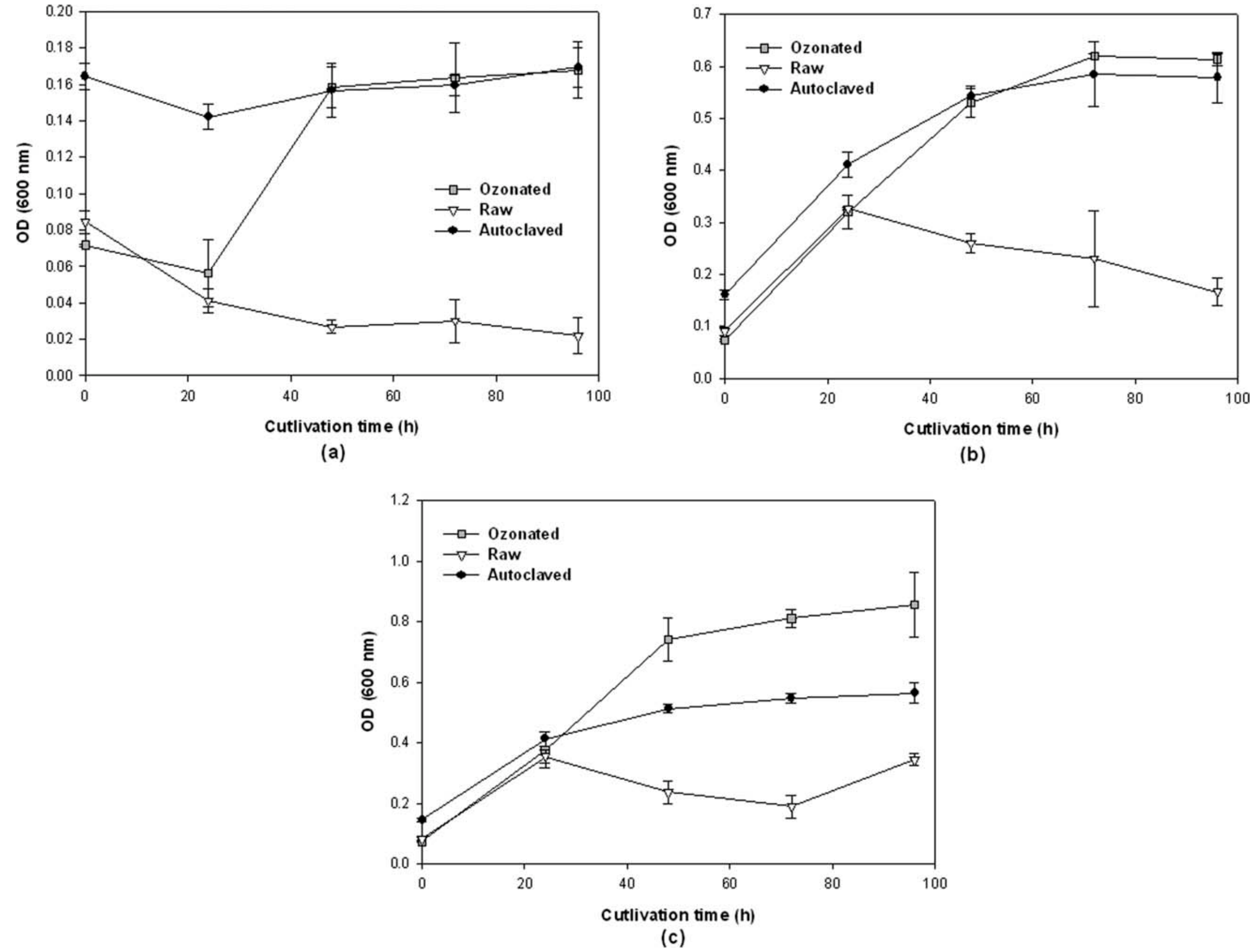

Figure 7. Test cultivation runs using MSU-RFCL oleaginous microorganism consortium on ozonated, raw, and autoclaved primary-treated wastewater. (a) No glucose supplement, (b) 1 g L^{-1}, and (c) 10 q L^{-1} glucose supplement.

of less polluted secondary effluents, the TOD levels used in this study were relatively higher due to higher ozone demands associated with higher COD levels and suspended solids concentration in primary-treated wastewater.

Residual ozone levels (Figure 6) were initially low in the first 20 min of ozonation after which it increased substantially at the 20 to 30 min ozonation period. From 30 min onwards, a constant ozone residual of approximately 2.8 mg L^{-1} was obtained. A constant ozone residual is usually thought of as an indication of complete oxidation of oxidizable materials in the wastewater matrix by ozone. On the other hand, McCarthy and Smith [6] instead define the ozone residual as the 98% air or oxygen that makes up the bulk of the gas mixture that was supplied into the reactor. According to them, the continuous supply of ozone supersaturates the wastewater with oxygen, which originated from the feed gas and from the spontaneous decay of ozone molecules to oxygen.

To verify whether or not the observed ozone residual represents the saturation level, the saturation concentration of ozone at the conditions of the ozonation experiments (20°C and pH 7) was calculated using Henry's law:

$$C^*_{O_3} = \frac{P_{O_3}}{H} \tag{10}$$

where $C^*_{O_3}$ is the saturation concentration of ozone, P_{O_3} is the partial pressure of ozone, and H is the Henry's coefficient, which for ozone was estimated to be 10.3×10^6 Pa dm^3 mol^{-1} at 20°C and pH 7 by Kuosa *et al*. [29]. The calculated ozone saturation concentration (8.5 mg L^{-1}) however, is relatively larger than the observed ozone residual (2.8 mg L^{-1}). It is possible that a kinetic steady state was achieved at 30 min wherein the rate of ozone addition is the same as the rate of ozone consumption or decay.

Interestingly, the TOD continuously increased with ozone contact time (Figure 6) despite the ozone residual plateau and apparent reduction of the rates of microbial inactivation and BOD_5/COD degradation as shown in their respective plots. This implies that ozone is still being consumed by the wastewater matrix. As mentioned earlier, the pH of the wastewater was virtually unaffected by ozone and remained close to neutral therefore the rate of ozone autodecomposition via the hydroxyl radical is relatively slow with a pseudo first-order rate constant $k < 10^{-4}$ s^{-1} [27]. Although not explicitly quantified, the majority of the TOD might have been utilized for the degradation of refractory compounds comprising the COD albeit at a slower rate, since approximately 75% of initial levels still remained even after 60 min of ozone contact. The ozone supplied could have also reacted with inorganic compounds in the wastewater that were not quantified as well such as H_2S/HS^- and SO_3^{2-}, with rate constants in the range of 10^8–10^{10} $M^{-1}{\cdot}s^{-1}$ at pH 6–8 [27].

Assessment of Ozonated Primary Effluents as Cultivation Media

Figure 7 shows the trend in the growth of the oleaginous microorganism consortium in ozonated primary-treated wastewater compared with raw and autoclaved wastewater with and without glucose supplement. In Figure 7a, it can be seen that there is a two-fold increase in the optical density of the culture using ozonated wastewater whereas in autoclaved wastewater, no significant change was observed. In raw wastewater, a four-fold reduction in the optical density was observed. However, the optical density levels obtained in this setup were too low to make a definitive comparison in the cell growth. This might be due to the low BOD_5 levels in the wastewater both before and after ozonation. Hence, glucose was used to supplement the residual BOD_5 for use as an additional substrate for microbial growth. As shown in Figures 7b (1 g L^{-1} glucose added) and 7c (10 g L^{-1} glucose added), the oleaginous consortium exhibited better growth in ozonated as opposed to raw wastewater, resulting in six- and four-fold increases, respectively. In raw wastewater, an initial increase in OD was observed in 24 h followed by a decrease up to 72 h, after which the OD appeared to increase again. This could indicate competitive growth kinetics between the oleaginous microorganisms and the indigenous microbes in the wastewater. Based on these observations, preozonation of the wastewater resulted in the elimination of competing microorganisms for substrates, giving way for the oleaginous consortium to proliferate more freely in the wastewater. Another possible factor is the presence of more readily biodegradable matter as a result of ozone oxidation of recalcitrance in the wastewater. Moreover, it can also be seen in that the growth of oleaginous microorganisms in ozonated wastewater was comparable with that in autoclave-sterilized wastewater containing 1 g L^{-1} glucose supplement and slightly higher with 10 g L^{-1} glucose. This could be attributed to the fact that ozone decomposition in the wastewater resulted into an increased dissolved oxygen levels which would be favorable for the aerobic growth of the oleaginous microorganisms whereas heat sterilization by means of the autoclave could have released most of the dissolved oxygen in the wastewater.

CONCLUSIONS

This study has demonstrated that ozone is a robust pretreatment process for primary-treated municipal wastewater before its application in fermentative processes for biofuel feedstock generation. After 60 min of semibatch ozonation with 3.0% (w/w) ozone at 1 L min^{-1}, less than 0.0002% of the initial heterotrophic bacteria population remained in a primary-treated wastewater effluent. COD levels were also reduced by 30% while BOD_5 levels remained constant and increased slightly following a 60% reduction within the first 30 min of ozone contact due to the conversion of recalcitrant compounds to biodegradable materials that could be readily utilized by microorganisms as substrates. Ammonium and phosphate concentrations were also unaffected by ozonation; hence, this could reduce the necessity to supplement the wastewater with nutrient salts to induce microbial growth. Cultivation experiments have demonstrated that oleaginous microorganisms could be grown in ozonated wastewater to produce cell density levels comparable to growth in autoclaved-sterilized wastewater, and better than in raw wastewater. Therefore, ozone pretreatment was effective in eliminating microbial competitors for nutrients and substrates without adversely affecting the biodegradability and nutrient content of the wastewater to give way for the cultivation of specialized microorganisms capable of producing large quantities of oil. This process could then potentially generate millions of gallons of renewable and sustainable feedstock for biofuel production using an established infrastructure.

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