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Continuous treatment of azo acid dyes by photo-dependent denitrifying sludge

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Abstract: Simultaneous removals of dye and nitrate by photo-dependent denitrifying sludge(PDDS) have been demonstrated in a continuous-flow bench-scale reactor. The best C/N for the degradation of azo dyes by PDDS was 1.5. The specific removal rate of azo dye AB92 decreased with a decrease in hydraulic retention time and increased with a decrease in solids retention time. The degradation rate of TOC decreased with a decrease in hydraulic retention time. AB92, which has nitro and hydroxyl substitutions in non-para positions, was uniquely degraded. During continuous flow treatment experiments using PDDS, complete degradation of azo dyes AB92 and AO20 at influent concentrations of 40 mg/L and 30 mg/L, respectively, was achieved with an HRT of 16.

Keywords: photo-dependent denitrifying sludge (PDDS); continuous treatment; simultaneous removals; azo dye; nitrate

Introduction

Wastewaters from printing and dye industries are typically discharged to domestic collection systems and treated by conventional unit-processes such as activated sludge. While biological treatment is effective in removing biochemical oxygen demand(BOD) and suspended solids(SS), commonly used acid azo dyes tend to be resistant to biodegradation. Color remaining in effluents can thus create a pollution problem in receiving water bodies. In recent years, physico-chemical treatment methods including ozone, activated carbon and cohesion techniques have been considered for removal of dye color. However, due to high treatment costs, the development of more economically favorable methods has been of interest to the dye industry. Furthermore, due to the high urea content of dye wastewaters, the unique requirement for abatement of both dyes and nitrogenous compounds exists.

Recently, it has been reported that some denitrifying sludges can effectively degrade organic compounds that have previously been considered recalcitrant(Nevalaine, 1993; Vannelli, 1990; Evans, 1991; Fries, 1994; Morgan, 1993; Biegert, 1995). In this research, a stable denitrifying sludge including purple nonsulfur bacteria cultured under illumination was used to investigate the simultaneous removals of azo dyes and nitrate in continuous flow treatment. It had previously been discovered in our lab that this photo-dependent denitrifying sludge(PDDS) has the unique capability to remove dye color while utilizing methanol as an organic carbon source for conventional denitrification.

1 Materials and methods

1.1 Photo-dependent denitrifying sludge

The denitrifying sludge used in this research had originally been maintained in translucent plastic containers with exposure to natural light. Under these conditions, the development of a purple tint was inadvertently observed. Upon understanding the unique characteristics of this sludge, it was named photo-dependent denitrifying sludge(PDDS) and further cultivated with methanol as a carbon source for research on treatment of dye wastewaters. Acclimation to dye and nitrate as simultaneous substrates under denitrifying conditions and ongoing maintenance was conducted by fill-and-draw. Though a DNA analysis of the sludge had previously confirmed (with a 99.7% similarity) the presence of the purple nonsulfur bacteria *Rhodobacter sphaerodies*, the AB92 can not be degraded by *Rhodobacter sphaerodies S* which came from Hirosima Electrical Machinery University. This microorganism can reportedly utilize many kinds of organic carbon sources, such as methanol, glucose, DL-malic acid, ethanol and sodium acetate trihydrate. Many details of the bacterium's physiology, however, are yet poorly understood.

1.2 Azo dyes and synthetic wastewater

The congeners of azo acid dyes used in this research consisted of acid blue 92 (AB92), and acid orange 20 (AO20). The molecular structures of these dyes are shown in Fig. 1. The composition of the synthetic wastewater used in this research is shown in Table 1.

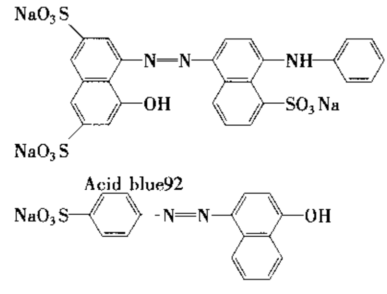


Fig.1 The molecule structure of AB92 and AO20

1.3 Reactor and treatment process

The synthetic wastewater containing dye was fed continuously at a rate of 7.5 L/d to the reactor containing PDDS. The reactor had a total liquid volume of 5.0L with a height (to effluent port) of 50 cm and surface area of 100 cm^2 (Fig. 2). The reactor was operated in complete-mix mode with continuous gentle stirring at 70 r/min and irradiated at 10000 lx with a 100W tungsten lamp over the top. The sides of the reactor were covered with black vinyl sheets. For solid-liquid separation, a 10 cm diameter plastic cylinder was attached to the effluent port (internal to the reactor) with a porous industrial pad attached to the submerged cylinder bottom. Influent and effluent samples were taken for water quality analyses on a daily basis.

Table 1 The composition of the synthetic waste water

Composting	Concentration, mg/L
K_2HPO_4	74.4
KH_2PO_4	10.7
NaCl	6.0
KCl	2.8
$CaCl_2$	3.7
$MgSO_4\cdot 7H_2O$	4.1
KNO_3	1000
CH_3OH	555

1.4 Analytical methods

AB92 and AO20 were analyzed by the spectrophotometric method using an optical photometer (U-1100, Hitachi Co.) at a wavelength of 560 nm and 483 nm, respectively. Nitrate was measured according to Japanese industrial standards (JIS, 1986). PDDS concentration was estimated as mixed liquor suspended solids (MLSS). MLSS and effluent suspended solids (SS) were determined by standard methods (APHA, 1995). Total organic carbon (TOC) was measured with a TOC analyzer (TOC 5050A, Shimadzu Co., Kyoto). Dissolved oxygen (DO), oxidation-reduction potential (ORP) and pH were measured by their respective probes.

2 Results and discussion

2.1 Optical irradiation

The influence of optical irradiation on the removal of AB92, TOC and nitrate during continuous flow treatment was investigated. The time course of the removal efficiencies of these constituents under lighted (10000 lx) and darkened conditions are shown in Fig. 3. The removal of AB92 was greatly diminished under darkened versus lighted conditions to approximately 10% versus 60%, respectively. The removal efficiencies of TOC and nitrate, however, were not influenced by the state of illumination. From these results, the unique photo-dependency of this sludge for removal of dye color is demonstrated.

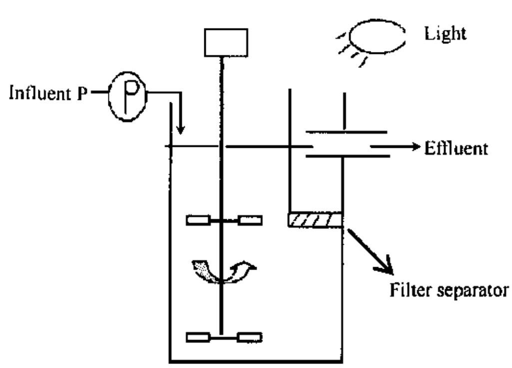


Fig.2 Experimental reactor

2.2 Carbon to nitrogen ratio (C/N)

In the process of denitrification, when the hydrogen was provided by methanol, the chemical equations is as follows:

$$NO_3^- + (5/6)CH_3OH \longrightarrow (1/2)N_2 + (5/6)CO_2 + (7/6)H_2O + OH^- . \quad (1)$$

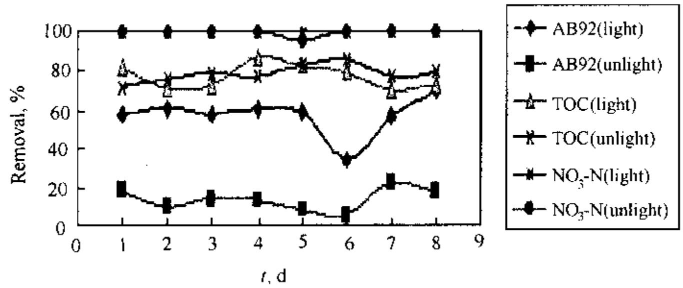


Fig.3 Relationship between AB92 and the optical irradiation in continuous treatment of dye by PDDS (AB92 = 5 mg/L, temp. = 30℃)

Eq. (1) shows that 1.9 mg methanol is required for the denitrification of 1 mg nitrogen-nitrate. Results of experiments conducted to evaluate the influence of C/N on treatment of wastewater containing the azo dye AB92 are shown in Fig. 4. The average removal efficiency of AB92 with a C/N of 2.0(days zero through 5) was 77.5%. While the removal efficiency of color was high, the effluent TOC concentration was quite high. The influent C/N was then reduced to 1.0 (from day 6, Fig. 4) in an effort to reduce the effluent TOC. Under these conditions, however, the removal efficiency of dye color was greatly diminished. The C/N was thus increased to 1.5 (day 13, Fig. 4) and removal of dye color was restored to the approximate efficiency when C/N was 2.0. With an influent C/N of 1.5, the removal efficiency of TOC under steady-state conditions was 80%. A C/N value of 1.5 was thus selected for all further treatment of azo dyes using PDDS

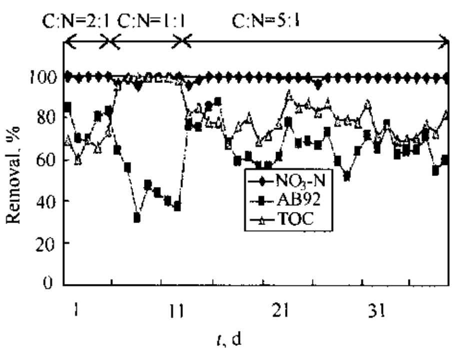


Fig. 4 Relationship between AB92 and C/N in continuous treatment of dye by PDDS(AB92 = 5 mg/L, temp. = 30℃)

2.3 Hydraulic retention time (HRT)

The specific degradation rate of a dye can be defined as

$$R = \frac{\left(\frac{C_i - C_e}{C_m}\right)}{H} \times 24, \quad (2)$$

where R is the specific degradation rate of a dye (mg/g SS/d); C_i is the concentration of dye in the influent(mg/L); C_e is the concentration of dye in the effluent(mg/L); C_m is the concentration of PDDS(mg/L); and H, the HRT (h). The results of continuous flow treatment experiments with azo dye AB92 at various HRT values are shown in Fig.5. Within the range of testing (HRT, 7.5 to 16h), essentially total removal of nitrate was realized. However, with a decrease in HRT, removal efficiencies of AB92 decreased. While a reduction in HRT from 16 to 10h did not have a significant effect on removal of AB92, a further reduction to an HRT of 7.5h resulted in almost complete termination of dye removal. This observation is curious in that removal efficiencies of TOC did not change significantly over the range of HRT values, it may have been due to the different original concentration of methanol and AB92 in the synthetic wastewater. And, nitrate had a high removal efficiencies over the same range of HRT values. The pronounced decrease in removal of dye color at the low HRT may have been due to a reduced efficiency in sludge-dye contact (Fig.6).

2.4 Solids retention time (SRT)

With HRT set at 16h, the influence of SRT on treatment of AB92 and AO20 was studied as shown in Figs.7 and 8, respectively. In experiments with AB92, nearly 100% denitrification was realized at all SRT values greater than 5d. With SRT at 20h, removal of TOC and AB92 were unstable; after increasing SRT of 25h, however, both TOC and AB92 were removed consistently at efficiencies ranging from 60% to

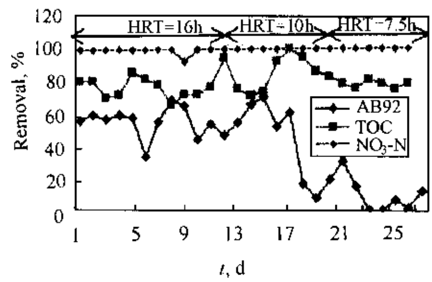


Fig. 5 Relationship between HRT and removal of AB92 in continuous treatment of dye by PDDS(AB92 = 5 mg/L, temp. = 30℃)

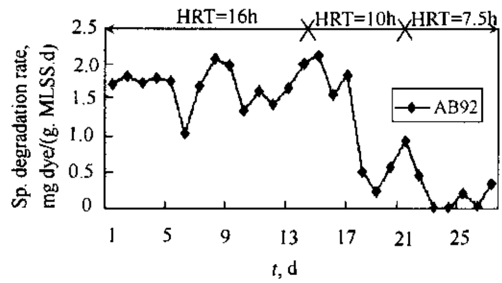


Fig.6 Relationship between the specific removal rate of AB92 and HRT(AB92 = 5 mg/L, temp. = 30℃)

85%. With SRT at the longest times of 50 and 100h, the removal efficiency of AB92 decreased, while removals of nitrate and TOC remained unchanged. Furthermore, when the SRT was shortened after day 100 (Fig. 7), the removal efficiency of AB92 decreased. In treatment studies with AO20, SRT was only varied from 3.3d to 25d over 135 days of study (Fig. 8), during which time nitrate removal was consistently near 100%. With a decrease in SRT (below 12h), removal efficiencies of TOC and AO20 both gradually declined. From these results, it is evident that an SRT can be selected for a continuous treatment system that will allow for effective removals of dye and nitrogen. The correlation between SRT and specific degradation rate of AB92 and AO20 are shown in Figs. 9 and 10, respectively. For both systems, with a decrease in SRT, an increasing trend in specific degradation rate was observed. It appears that with a decrease in the concentration of the PDDS (at lower SRT values), contact efficiency between sludge and the dye substrate was enhanced. In addition, the activity of photosynthesis bacteria may have been stimulated due to greater light penetration at the lower sludge concentrations. Furthermore, it appears that a threshold for the effect for decreasing biomass concentration (MLSS) on specific degradation rate was not passed. In these experiments, it was considered that the influent dye concentrations were very low, especially considering the increase in specific degradation rate with a decrease in SRT (and decrease in MLSS).

2.5 Dye concentration

The influence of dye concentration on treatment of AB92 and AO20 was studied as shown in Figs.11 and 12, respectively. And, concentration was made to change in the range on 5 – 100 mg/L(AB92), 5 – 50 mg/L(AO20), respectively. While a slight decrease in TOC removal also occurred at the highest dye

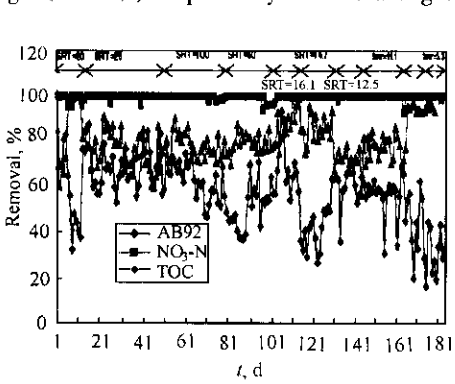


Fig. 7 Relationship between AB92 and SRT in continuous treatment of dye by PDDS(HRT = 16h, temp. = 30℃, AB92 = 5 mg/L)

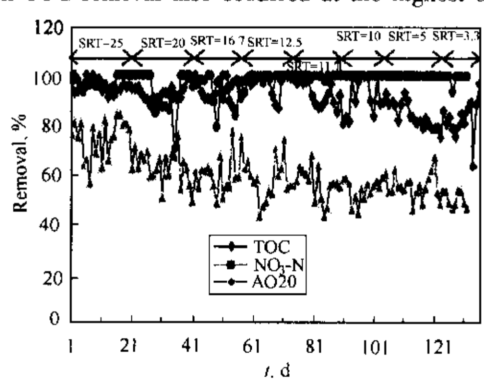


Fig. 8 Relationship between AO20 and SRT in continuous treatment of dye by PDDS(HRT = 16h, temp. = 30℃, AO20 = 5 mg/L)

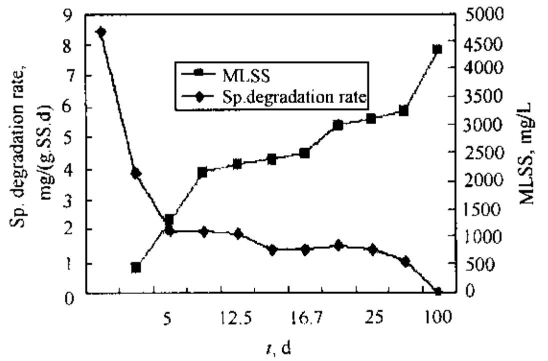


Fig.9 Relationship between the specific removal rate of AB92 and SRT in continuous treatment of dye by PDDS(HRT = 16h, temp. = 30℃, AB92 = 5 mg/L)

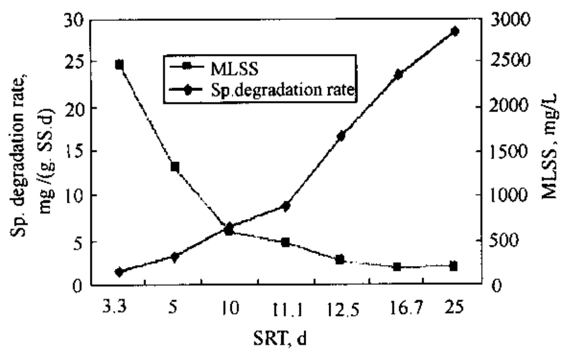


Fig.10 Relationship between the specific removal rate of AO20 and SRT in continuous treatment of dye by PDDS (HRT = 16h, temp. = 30℃, AO20 = 5 mg/L)

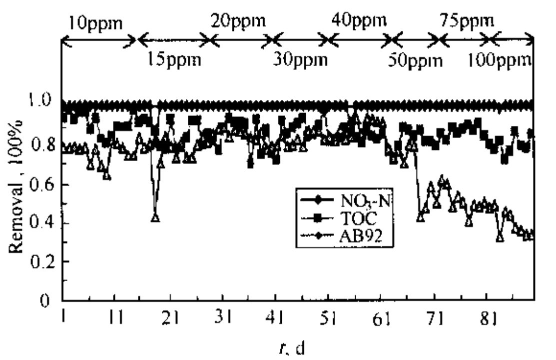


Fig. 11 Relationship between AB92 and concentration in continuous treatment of dye by PDDS(HRT = 16h, temp. = 30℃, SRT = 15d)

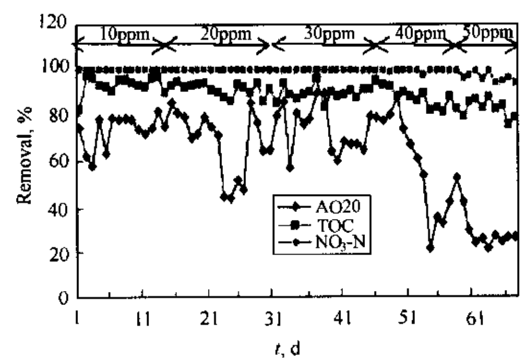


Fig. 12 Relationship between AO20 and concentration in continuous treatment of dye by PDDS(HRT = 16h, temp. = 30℃, SRT = 15d)

concentrations, there was otherwise no significant influence of influent dye concentration on TOC and nitrate removal. Furthermore, nitrate removals were consistently about 100% and TOC, about 90%. Maximum dye removal efficiencies, as well, were realized up to influent dye concentrations of 40 mg/L for AB92 and 30 mg/L for AO20. Considering that dye wastewaters are typically about 20 mg/L, PDDS would be considered an effective option for continuous treatment in a real industrial application. It is reported in the literature that the - N = N - and - OH structures in the para position can be degraded at a higher rates than in the ortho or meta positions (Stucki, 1995; Yatone, 1981; Pasti-Grigsby, 1996). In this research, though, the positions of substitutions for AB92 are not in the para position, the peculiar degradation is shown in Fig. 13. And, it was confirmed in batch experiment too (Furukawa, 1988).

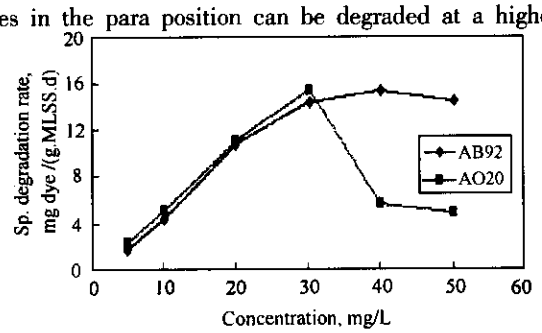
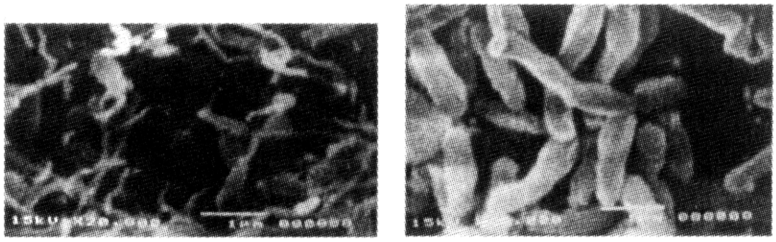


Fig.13 Relationship between the specific removal rate of azo acid dyes and SRT in continuous treatment of dye by PDDS (HRT = 16h, temp. = 30℃, SRT = 15d)

2.6 Bacterial examination(in PDDS)

To further understanding of the unique characteristics of the PDDS, photosynthetic and denitrifying bacteria in the sludge were quantified and taken photographs(Fig.14). It was found out that the photosynthesis bacteria was taken by coryneform fungus of the length 1 μm. And, that PDDS was taken by coryneform fungus and thread-shaped fungus was seen too. Results of MPN assays for AB92 and AO20 are shown in Table 2. From it, the numbers of photosynthetic bacteria were less than that of denitrifies and remained at a constant level in the PDDS can been known.



a. PDDS b. photosynthesis bacteria

Fig.14 The state of PDDS in scanning electron microscope

Table 2 The numbers of photosynthetic and denitrifying bacteria in the continuous treatment of AB92 and AO20 by PDDS

Concentration of AB92, mg/L	Bacteria of photosynthesis, cells/(g·MLSS)	Bacteria of denitrification, cells/(g·MLSS)
5	1.3×10^6	3.9×10^9
10	1.7×10^6	2.4×10^9
20	$1.3 \times x10^6$	3.3×10^9
30	1.7×10^7	2.8×10^9
40	-	1.3×10^9
50	2.1×10^7	2.1×10^{10}
Concentration of AO20, mg/L	Bacteria of photosynthesis, cells/(g·MLSS)	Bacteria of denitrification, cells/(g·MLSS)
5	2.4×10^6	2.8×10^9
10	1.7×10^7	1.0×10^9
20	4.9×10^7	1.7×10^9
30	4.9×10^6	1.2×10^9
40	2.1×10^7	2.7×10^9
50	7.2×10^7	2.2×10^9

3 Conclusions

Continuous flow treatment of azo dyes by PDDS was studied over a two-year period. From this research, the following conclusions can be made: (1) The azo dye AB92 was not degraded under dark conditions. (2) A C/N of 1.5 was shown most effective for degradation of dyes by PDDS under the conditions of testing used here. (3) The specific degradation rate of dye decreased with a decrease in HRT and increased with a decrease in SRT within the range of testing conditions used here. (4) Efficient removal of dye color using PDDS over extended periods of continuous flow treatment was possible. (5) Degradation of AB92 by PDDS was unique in that nitro and hydroxyl substitutions were not in the para position. (6) The numbers of photosynthetic bacteria were considerably less than that of denitrifies and remained at a constant level in the PDDS.

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Editorial Board special recognition:

The Editor-in-Chief of the journal——Dr. Tungsheng Liu is being honored with the 2002 Tyler Prize for his pioneering contributions in recognizing and using terrestrial sediments to understand global environmental change. He has been a trailblazer in developing ways to measure paleoclamatic change over the last 2.5 million years through studies of loess, a windblown dust, that forms thick deposits over much of central China.

Prof. Liu is recognized as the father of paleoenvironmental research on Chinese loess-soil sequences. Liu's research over the last 5 decades has clearly demonstrated that loess provides a complete and accurate continental record of environmental change. The fine-grained dust is now widely considered one of three reliable sources of past environmental information the other two are deep-sea sediments and arctic ice cores. Liu's accumulated work on the loess/paleosol sequences has also led to a deeper knowledge and understanding of the variability through time of the Southeast Asian monsoon system.

Liu's research group was instrumental in discovering the underlying causes of Keshan disease, which affected thousands of people in China and vexed the Chinese medical community for decades. Keshan's is a debilitating disease of the heart muscle resulting in cardiocascular failure. Liu's research group linked the disease to deficiencies of trace elements in local soil and water, a problem now countered by supplements of selenium and other nutrients. These research findings have saved thousands of lives. Further contributions to solving environmental problems in China, resulted from Liu and his colleagues studies on vegetation recovery and environmental geology, which helped government officials in China improve overall environmental quality through urban construction reform.

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The John and Alice Tyler Prize is awarded for environmental science, energy and medicine conferring great benefit upon mankind. The prize is endowed by gifts form the Alice C. Tyler Charitable Trust and the John C. Tyler Trust. The University of Southern California is Administrator of the Tyler Prize. The annual award is \$200,000.