Sulfur-Oxidizing Bacteria Convert Problematic Waste into Marketable Resource

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ABSTRACT

Flue gas desulfurization (FGD) air pollution control technology for coal combustion gases commonly uses aqueous slurries of pulverized limestone or lime to capture sulfur dioxide (SO_2) as crystalline calcium salts. Under forced oxidation (excess oxygen) conditions, FGD byproduct contains almost entirely (greater than 92%) gypsum ($CaSO_4 \cdot 2H_2O$), a useful and marketable byproduct. In contrast, FGD byproduct in oxygen deficient systems contains a high percentage of hannebachite ($CaSO_3 \cdot 0.5H_2O$) to yield a material with no commercial value, that dewaters poorly, and that is typically accumulated on site or landfilled off site. Hannebachite can be chemically converted to gypsum using large quantities of acids or strong oxidizers which could be dangerous and expensive. However, we demonstrated a novel application of microbial physiology using a natural consortium of sulfur-oxidizing bacteria (SOB) that achieved the same conversion of hannebachite-enriched FGD byproduct into commercially valuable gypsum without using reactive chemicals. Physiological studies on the bacteria revealed that they were aerobic, used carbon dioxide rather than organic carbon as a food source (chemolithotrophic), required a steady supply of ammonia, preferred a near neutral pH, and converted hannebachite to gypsum at rates approaching five percent per day in a 20 to 40 percent FGD solids slurry. The process converted FGD waste with less than 50% gypsum into a commercially viable resource containing 96% gypsum with 40 to 100 micron crystals and less than 2% residual hannebachite, making the product a marketable resource produced at low cost, under mild conditions, and without dangerous and expensive reagents.

Introduction

Approximately 560 coal-fired electricity generators operate in the USA[1]. Byproducts of fossil fuel combustion present in the flue gas include sulfur dioxide gas (SO_2) which is both a contributor to acid rain and an indirect greenhouse gas[2]. Discharge levels of SO_2 into the atmosphere have been governed by the Clean Air Act Amendments since November 1990 and regulated by the United States Environmental Protection Agency due to the reaction of SO_2 with water vapor in the atmosphere to form sulfuric acid[3]. Flue gas desulfurization (FGD) is a well-established air treatment technology for coal and oil combustion gases that commonly uses lime or pulverized limestone aqueous slurries to precipitate sulfur dioxide (SO_2) as crystalline calcium salts[4]. The resultant salts, hannebachite ($CaSO_3 \bullet 0.5H_2O$) or gypsum ($CaSO_4 \bullet 2H_2O$), precipitate in different ratios depending on the availability of oxygen during the process. Under forced oxidation (excess oxygen) conditions, FGD byproduct contains almost entirely (>92%) gypsum ($CaSO_4 \cdot 2H_2O$), a useful and marketable commodity. In contrast, FGD byproduct formed in oxygen deficient oxidation systems contains a high percentage of hannebachite ($CaSO_3 \cdot 0.5H_2O$) to yield a material with no commercial value, poor dewatering characteristics, and that is typically disposed in landfills. One of the highest value uses of gypsum is for manufacturing wallboard which consumed 7.4 million tons in 2013[5]. Other uses for gypsum include admixture in cement where it acts as a set retarder[6], agricultural soil amendments to diminish soil acidity and make nutrients more bioavailable[7], and fire resistant coating[8].

Reclamation and conversion of FGD byproduct containing excessive $CaSO_3 \bullet 0.5H_2O$ to $CaSO_4 \bullet 2H_2O$ is chemically possible, yet costly. The conversion processes include the addition of H_2O_2[9] or acid, preferably H_2SO_4[10]. Hydrogen peroxide oxidizes sulfite whereas sulfuric acid can be used to acidify the pH which allows auto-oxidation. The lower the pH, the faster the auto-oxidation[11]. Both chemical reactions involve the addition of substantial amounts of oxidant or acid. An alternative to these chemical reactions exploits the biogeochemical oxidation of reduced sulfur (sulfite) to sulfate. Bacteria characterized as sulfur-oxidizing include a wide variety of genera, display broad habitat diversity, and may be heterotrophic, mixotrophic, chemolithotrophic, or photoautotrophic[12]. Sulfur-oxidizing bacteria (SOB) fulfill an important role in the conversion of reduced sulfur (sulfide) and partially oxidized forms of sulfur (e.g., elemental sulfur, thiosulfate, and sulfite) into sulfate. This work describes a novel, patent pending application of microbial physiology where a natural consortium of sulfur-oxidizing bacteria (SOB) was used to convert hannebachite-enriched FGD byproduct into a commercially valuable, gypsum-enriched product (US Patent Assignment 503373611).

Materials and Methods

Media and Cultures

Reagent grade chemicals for culture medium originated from Thermphos International (Flushing, Netherlands), Thermo FisherScientific (Waltham, MA), and Acros (Geel,

Belgium). Continuous culturing of the sulfur-oxidizing consortium and all physiological experiments on SOB were performed using SOB culture medium with sodium thiosulfate as the reduced sulfur source[13]. The SOB consortium was isolated from the FGD byproduct and was maintained through serial dilution in liquid SOB media weekly for 24 months under constant agitation and aeration. The continuously grown SOB consortium (seed reactor) provided biomass for physiology characterization, next generation sequencing, and FGD byproduct treatment tests. When treating FGD byproduct in reactors, the mixed liquor from the seed reactor was added as 5% [v/v] of the working volume of the FGD treatment reactor. During typical operation, reactors converting hannebachite to gypsum were amended with NH_4Cl (0.1 g/L), $Na_2HPO_4 \cdot 7H_2O$ (0.3 g/L) and $MgCl_2 \cdot 6H_2O$ (0.1 g/L). For all experiments the SOB consortium was diluted to a starting OD_{595} of 0.03 or the equivalent of 2.5 to 4 × 10^5 cells/mL.

Cell Density Measurements

Cell densities were monitored throughout the FGD treatment process using correlated ATP levels measured with the Quench-Gone™ Aqueous Test Kit (LuminUltra®, New Brunswick, Canada), optical density at 595 nanometers (OD_{595}) using a Hach spectrophotometer, Model DR 5000 (Loveland, CO), and physical cell counts by means of disposable Cellometer® counting chambers (Nexcelom Bioscience, Lawrence, MA) viewed with an Omax phase contrast microscope (Omax, Korea) at 1000 × magnification.

Growth Temperature Testing

The optimal growth temperature for the consortium was evaluated as a process control parameter affecting the gypsum formation rate. The SOB consortium, maintained at room temperature, was grown at temperatures ranging between 15±1°C and 45±1°C to identify the temperature that would yield the fastest SOB growth rate in SOB medium. To achieve incubation conditions above or below room temperature (22±1°C) the SOB consortium was either warmed in a Precision 2835 water bath (Thermo Fisher Scientific, Waltham, MA) or cooled in a refrigerated unit (Draghetto, Greenaby, WI), using a magnetic stirrer (VWR®, Arlington Heights, IL) and an immersible magnetic stirrer (Electrothermal, Burlington, NJ), respectively. The temperature was monitored with a thermometer (Thermo Fisher Scientific, Waltham, MA). Cell growth experiments continued with monitoring twice daily until the cells/mL started to decline.

pH Sensitivity

The SOB medium has an unadjusted pH of 7.1. The pH was adjusted by altering the molar ratio of KH_2PO_4 and K_2HPO_4 and where necessary by adding H_2SO_4 or NaOH to yield batches of SOB medium with a pH of 3.5, 5.0, 6.0, 7.0, 8.0 and 9.5. Stirred batch growth experiments conducted at room temperature using the pH adjusted media and mixing in a Phipps & Bird jar tester (PB 700, Richmond, VA) at 85 RPM provided data to evaluate the effect of pH on cell growth. The OD_{595} and the pH of each pH treatment

was measured daily. Medium pH was measured with an Accumet XL15 pH meter with a combination electrode (Thermo Fisher Scientific, Waltham, MA). The pH of each treatment was adjusted with either H_2SO_4 or NaOH during testing to maintain the initial pH of the medium.

Oxygen Requirements

The oxygen requirement of the SOB consortium was assessed by culturing in SOB medium at various dissolved oxygen (DO) concentrations. Nominal 1 L Pyrex® bottles (Corning, NY) were filled with 1.1 L SOB medium and fitted with modified bottle caps to accommodate a twin bubble airlock (Homebrew, Sandusky OH) and ¼ inch inner diameter vinyl tubing (Cole-Parmer, Vernon Hills, IL) through which gas was supplied to control the dissolved oxygen concentration. Compressed N_2 (Airgas, Radnor, PA), breathing air (supplied from the atmosphere using a Top Fin®AIR-4000 diaphragm pump (Petsmart, AZ), or a mixture of both were used to adjust dissolved oxygen. All connections were sealed with silicone and rubber O-rings were used to seal the caps. Cell growth experiments were performed under anoxic conditions (pure N_2), and with dissolved oxygen at 1±0.5 mg/L, 3±0.5 mg/L, and 7.5 mg/L (air saturated). A two-tube gas proportioner (Cole-Parmer, Vernon Hills, IL) blended N_2 and air to provide the lower dissolved oxygen concentrations. The concentration of O_2 was monitored by using a sensION™+ DO6 DL O_2 probe (Hach, Loveland, CO). The pH of all tests was maintained at 7.0±0.5.

FGD Byproduct Treatment

FGD byproduct treatment was performed in a variety of slurry bioreactor configurations. Multiple 500 mL Nalgene bottles containing 250 mL of FGD slurry were incubated at room temperature on a tumbler to provide gently mixing and aeration. This configuration supported initial proof-of-concept testing and preliminary refinement of treatment conditions for consistent gypsum formation.

Three reactor designs were used throughout the testing to maintain the SOB consortium and to evaluate the effect of various control parameters on gypsum formation. A “seed reactor” was maintained to provide SOB biomass for hannebachite oxidation experiments. The SOB consortium was maintained in suspension through continuous circulation of the SOB mineral growth media. Biomass was removed periodically either for use as seed culture for other reactors or to reduce biomass in the reactor. “Stirred slurry reactors” were operated with 20 to 40% solids with airlift mixing. A “CO_2-free slurry reactor”, based on the stirred slurry reactor design, was capable of excluding atmospheric CO_2 to encourage the chemolithotrophic SOB to consume carbonate (CO_3^{-2}).

Harvesting SOB from Reactors

Biomass for identification of SOB in the seed reactor and in slurry reactors (stirred and CO_2-free) exhibiting active hannebachite conversion was collected in 50 ml

polypropylene tubes (Fisher Scientific) by centrifugation at 2100 RPM in a swinging bucket rotor for 10 min in a Bio Lion XC-L5 centrifuge. The supernatant was discarded and the cell pellet was resuspended in residual liquid that remained in the tube. The cell density was verified as greater than 10^9 cells/mL as recommended by the Illumina® MiSeq 16S protocol (Illumina, San Diego, CA). The cells were transferred to a 1.5 ml Eppendorf tube, centrifuged and the cell pellet was stored at – 20°C.
To isolate the biomass in the slurry reactors the FGD solids were allowed to separate from the supernatant prior to centrifuging the supernatant.

16S rDNA analysis

To identify the SOB present in the consortium during FGD treatment, next generation 16S rDNA sequencing (NGS) was performed using the Illumina® MiSeq 16S rRNA Microbial Sequencing kit equipped with primers PCR1_forward and PCR1_reverse to selectively amplify variable regions 3 and 4 of the 16S ribosomal DNA. Following collection of the bacteria, the frozen bacterial cell pellets isolated from the seed reactor and the FGD treatment reactors (stirred and CO_2-free) were shipped on dry ice to Omega Bioservices (Norcross, GA, USA) for DNA extraction and next generation 16S rDNA sequencing using the Illumina® MiSeq platform. Index primers were then attached to the amplicons prior to NGS. Analysis of the sequence data was performed on the Illumina® cloud computing environment Basespace®.

Thermogravimetric Analysis (TGA)
The weight distribution of $CaSO_4 \bullet 2H_2O$ and $CaSO_3 \bullet 0.5H_2O$ in the treatment process was analyzed by step-wise dehydration at two different temperatures[14]. A slurry sample pre-dried at 60°C in a laboratory oven (Quincy Lab, Inc., Model 40 GC) was ground using a mortar and pestle and an aliquot of the sample was weighed into a tared aluminum weighing dish (Fisherbrand) using an Ohaus PA64 balance. The pre-weighed sample was dried sequentially at 180°C and 440°C in a muffle furnace (Ney® Vulcan® D-550) to evaporate the water from the CaSO4•2H2O and CaSO4•0.5H2O, respectively. Each drying step was performed for an hour and the sample allowed to cool in a desiccator prior to weighing. The difference in weight after each drying step was indicative of the mass of chemically-bound water.

Results

Biomass Measurements

ATP measurements were shown to be reliable for analyzing environmental samples and can be correlated with biomass[17]. To ensure accurate estimation of biomass using ATP, microscopic cell counts and optical density measurements were performed in parallel. The correlation between ATP, microscopic cell counts and optical density proved to be reliable and reproducible allowing ATP levels to be used as a quantitative indicator of biomass (Figure 1).

Growth Characteristics

Understanding the physiology of the SOB consortium would allow for optimized growth condition resulting in the fastest conversion of hannebachite to gypsum. To this end, the optimal temperature, pH, nutrient requirements and oxygen requirements of the SOB consortium was assessed. The SOB consortium proved to be mesophilic as the optimum growth temperature was narrowed to approximately 30°C with little to no growth above 40°C. Growth at 15±1°C was evident but at a rate almost 1.5 times slower than at 30°C. The fastest growth rate was observed to be 0.02 h^{-1} which translated to a doubling time of approximately 34 h. SOB are known to grow across a wide pH range[18]. Therefore, the pH preference of the SOB consortium was evaluated by inoculating the consortium into SOB media ranging in pH from 3.5 to 9.5. Optimal growth was observed at pH 6, and the SOB consortium grew better at pH 7 than pH 5 indicating the neutrophilic character of the culture.

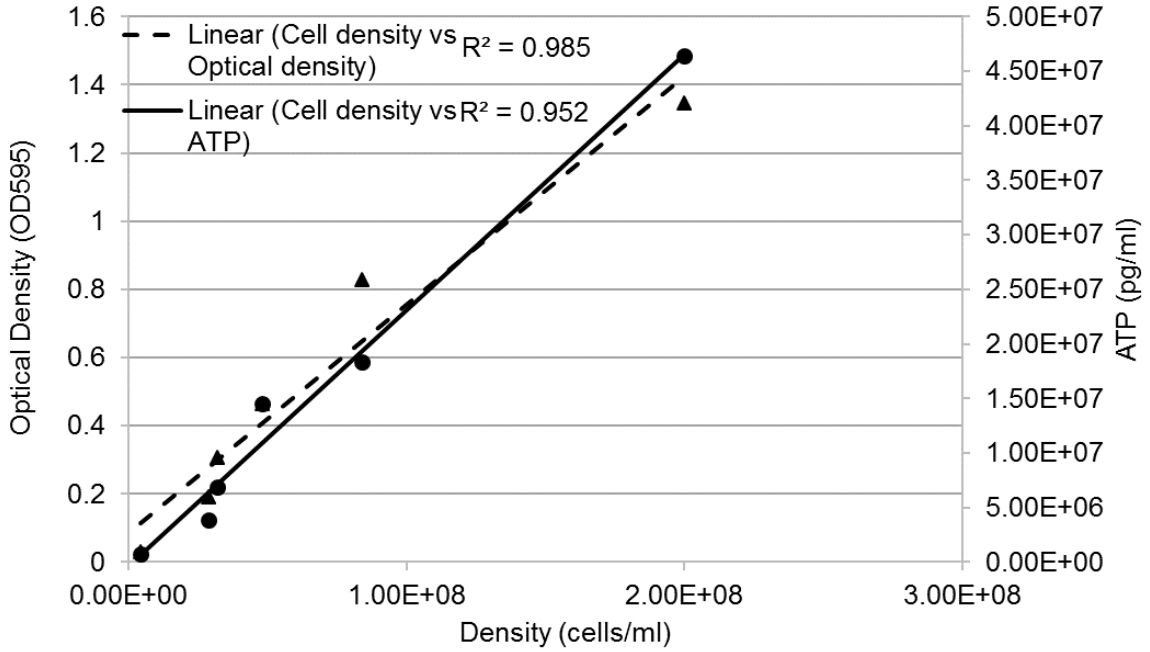

Figure 1. Correlation between cell density, optical density and measured ATP levels during FGD conversion. The correlation indicated that both optical density and measured ATP levels were reliable indicators of biomass.

Among SOB, sulfur is either oxidized by anaerobic phototrophs or aerobic lithotrophs[19]. To ascertain the oxygen requirements of the SOB consortium, the bacteria were cultured with dissolved oxygen held constant at less than 0.75 mg/L dissolved oxygen to 7.5 mg/L. No proliferation was observed when the cultures were maintained at 1±0.5 mg/L dissolved oxygen and less. When cultured at 3±0.5 mg/L dissolved oxygen, the bacterial cell growth was similar to that observed under fully aerated conditions 7.5±0.5 mg/L (Figure 2).

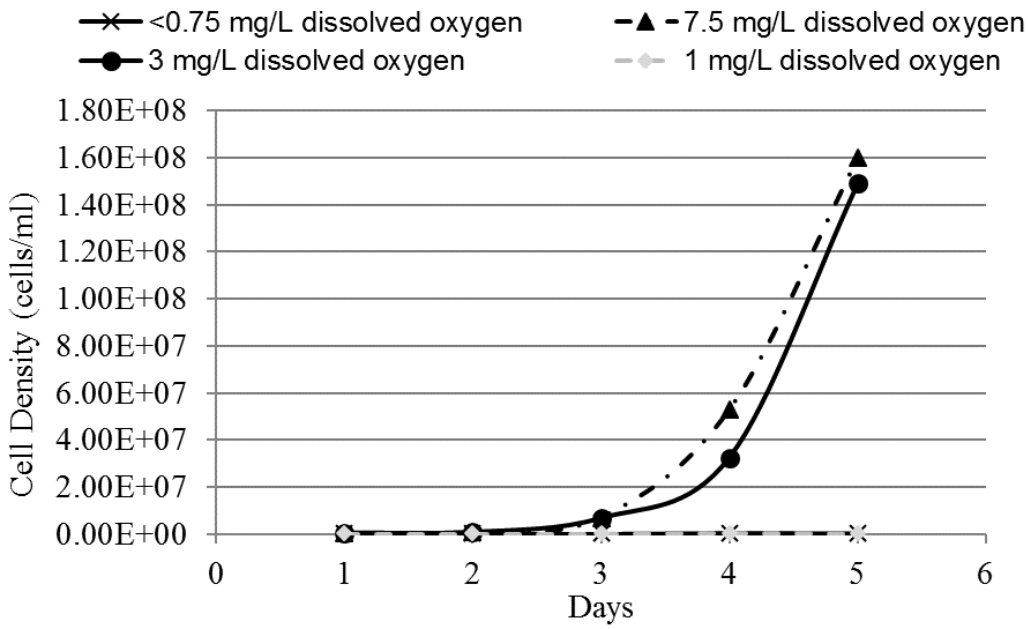

Figure 2. Oxygen requirement of the SOB consortium.

Nutrient consumption by actively growing SOB consortium was evaluated every other day by measuring ammonia and phosphate levels in the culture medium. Phosphate remained constant at approximately 50 mg/L in the culture medium indicating a very low demand for phosphate. The demand for ammonium, however, was high and required frequent additions of NH_4Cl to maintain detectable concentrations. 10^7 cells/mL consumed approximately 11.5 mg of ammonium/L/d.

The conversion of hannebachite to gypsum was assessed using various reactor designs. FGD treatment in the CO_2-free and stirred slurry reactors took between 10 and 21 days to increase the gypsum content from approximately 50% to greater than 80% (Figure 3). This translated to an average conversion rate of 2.1% per day with a maximum observed conversion of 5.5% per day. Acclimated SOB in aerobic slurry bioreactors produced a final product containing up to 96% gypsum, about 2 percent residual hannebachite, and 0.4 percent residual carbonate. This level of gypsum enrichment and overall FGD product purification exceeded the minimum specifications for gypsum used in commercial wallboard production.

In excess of 500 different bacterial species were detected in the FGD waste. Of these, 26 different SOB species were identified. The SOB present in the waste were also found to be very diverse and included both well studied and poorly understood members. The most dominant SOB species identified belonged to the three genera *Thiomonas*, *Halothiobacillus* and *Thiovirga*. An interesting observation was that both the abundance and the diversity of the SOB varied significantly between the reactors. Despite this observation, gypsum formation occurred at similar rates and completeness in both reactors

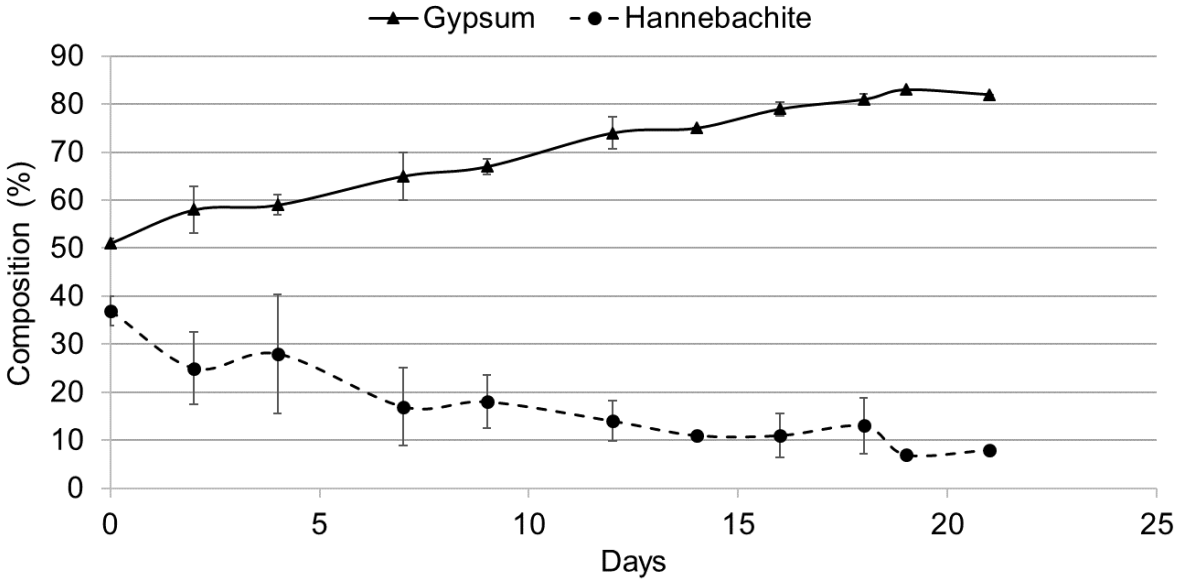

Figure 3. Typical progression of gypsum formation in slurry reactors. Error bars indicates one standard deviation of the mean.

Discussion

The SOB associated with the oxidation of hannebachite were found to be naturally present in the FGD byproduct as part of the bacterial community colonizing the stockpiled byproduct. The successful correlation of ATP, optical density and cell counts resulted in accurate and reliable biomass quantitation and provided an important control parameter for optimizing gypsum formation.
Investigating the oxygen demand of the SOB consortium provided insights into large scale bioreactor designs where oxygen delivery will be an important consideration to ensure gypsum formation will not be impeded for lack of oxygen.

Aeration of industrial water treatment processes is typically expensive, however, the potential to use aeration to both supply DO and suspend the FGD byproduct slurry may help offset the cost of the high oxygen demand of the SOB by eliminating the cost of separate mixing.

The majority of the SOB identified in the reactors, including the three dominant species, were chemolithotrophic. This correlates well with their natural and *in vitro* environments which consist of minerals, nutrients in the form of NH_4 and PO_4, gypsum, hannebachite, calcium carbonate and low concentrations of some metals. In addition, most of the species were Gram negative motile rods that exhibited aerobic, mesophilic, and neutrophilic growth similar to SOB described by others[16, 20-22]. The only other investigation of FGD bacterial communities was performed by Brown and co-workers[23]. Although they did not provide specific detail regarding the species identified, the phyla identified in the FGD slurries they studied correlated with those observed in this study

and included Actinobacteria, Proteobacteria (Alpha-, Beta- and Gamma-), Firmicutes, Cyanobacteria and Bacteroidetes.

This study showed successful oxidation of hannebachite present in FGD byproduct to commercial grade gypsum by the action of a complex mixture of SOB in a variety of reactor configurations. The ability of the SOB to perform at mesophilic temperatures and circumneutral pH suggests the potential for low cost, large scale commercial bio-oxidation processes for sustainable gypsum enrichment in FGD byproduct with high hannebachite content. This novel patent pending (US Patent Assignment 503373611) process proved adaptable and will be feasible in existing open impoundments.

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