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A UREASE BIOREACTOR FOR WATER RECLAMATION ABOARD MANNED SPACECRAFT

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Abstract. Development of a fixed bed continuous flow bioreactor, utilizing urease immobilized on diatomaceous earth, for decomposition of aqueous urea in a spacecraft closed loop environmental life support system is described. The results of small scale bioreactor experiments investigating the effects of throughput, temperature, pH and conductivity are reported. The design and performance of a full scale bioreactor are also presented.

Key words: bioreactor, urease, immobilization, spacecraft water reclamation.

INTRODUCTION

For long duration manned missions, such as a space station, a manned Mars mission, or a permanent lunar base, closed loop environmental life support sub-systems are required for purification, replenishment, and reuse of air and water. Typical aqueous wastes occurring aboard spacecraft are crew hygiene waters which arise from shower, oral hygiene and hand wash facilities; laundry water, humidity condensates collected from the cabin atmosphere via condensing heat exchangers; and human urine[1]. To a lesser extent, additional wastewaters may originate from experimental animal metabolism, and as the result of chemical, biological and materials science experimentation. Hygiene water, urine and experimental animal metabolic wastes contain significant levels of urea[2]. Current spacecraft water reclamation methodology for treatment of humidity condensate, laundry, and hygiene waters utilizes either ion exchange/sorption technology, known collectively as multifiltration (MF), or reverse osmosis (RO)[3,4]. Urine is processed using these techniques following a preliminary distillation separation by Vapor Compression Distillation (VCD) methods[5].

Urea is a highly water soluble, polar non-ionic contaminant and hence is not effectively removed by sorption onto ion exchange media, granular activated carbons, or organic polymer sorbents. Urea rejection by RO membranes is also quite poor. Distillation of urine using the highly energy efficient VCD technology yields a distillate which is contaminated with urea and organic acids, and which requires further purification[2]. Low temperature aqueous phase heterogeneous catalytic oxidation using molecular oxygen and noble metal catalysts has proven effective at destroying the low concentrations of urea which are present following MF treatment of hygiene water and urine distillates[6]. Electrolysis of high concentrations of urea followed by electrodialytic separation of the

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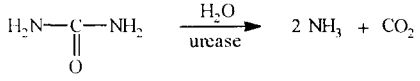
ionic decomposition products has also been demonstrated[7-9]. Despite these successes, more energy efficient and passive means are sought for the removal of polar low molecular weight organics such as urea.

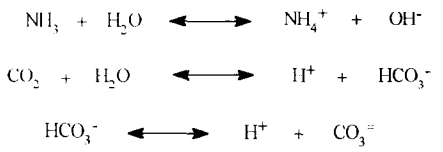
As long ago as 1966, a microencapsulated soluble urease shunt was employed for the hydrolysis of blood-borne urea[10]. Fixed bed reactors containing urease immobilized by adsorption onto alumina have been used for regeneration of urea loaded hemodialysate[11]. The enzyme, immobilized on polymethacrylate macroporous cation exchange resin using 1-cyclohexyl-3-(2-morpholino-ethyl)carbodiimide metho-p-toluene sulfonate, has also been demonstrated for this purpose[11]. Several workers have investigated the use of immobilized urease for the treatment of urinous wastewaters. Davidson and co-workers treated a synthetic urine consisting of sodium chloride and urea with a combined reverse osmosis, urease bioreactor and ion exchange system[12]. The urease in this study was immobilized on collagen. Husted[13] investigated the utility of immobilized urease in conjunction with ion exchange sorption of the ammonium ion as a means of preventing urea toxicity during a 30-day Orbiting Frog Otolith (OFO) flight experiment in which the urea source was frog urine. This work evaluated urease immobilized with acrylamide gel on nylon netting, glass wool, and nitrocellulose, prepared following the method of Guilbault and Montalvo[14] with modifications. The immobilized urease was challenged with aqueous urea concentrations ranging between 20-70 mg-L^{-1}. The test results indicated low hydrolysis efficiency for the immobilized enzyme and hence the use of free soluble urease was recommended for the flight experiment.

In this paper we report the development and evaluation of a continuous flow fixed bed bioreactor using immobilized urease for the continuous processing of urea containing aqueous wastes[15-17]. The bioreactor has been tested using simulated spacecraft composite waste water containing hygiene waters and urine distillates. The system has also been tested using humidity condensates. The products of urease catalyzed hydrolysis of urea are carbon dioxide, and ammonia,



$$H_2N{-}\underset{\underset{O}{\|}}{C}{-}NH_2 \xrightarrow[\text{urease}]{H_2O} 2\,NH_3 + CO_2 \qquad (1).$$

Speciation of the inorganic reaction by-products is governed by three pH dependent equilibria,



$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^- \qquad (2),$$

$$CO_2 + H_2O \longleftrightarrow H^+ + HCO_3^- \qquad (3),$$

$$HCO_3^- \longleftrightarrow H^+ + CO_3^{=} \qquad (4).$$

The resulting ionic products are conveniently removed from the flowing aqueous stream by the addition of ion exchange media to the bioreactor for sorption of ammonium, carbonate, and bicarbonate ions. Use of hydrogen form strong acid cation exchange media for removal of ammonium ions results in a lowering of pH, producing a shift in the inorganic carbon equilibria toward dissolved CO_2. The resulting carbon dioxide can then be easily removed by the down-stream gas-liquid separator. Because elemental iodine (I_2) is used as a biocide aboard American manned spacecraft[3], the reactor also provides the additional function of de-iodination of the influent to prevent enzyme inactivation, followed by re-iodination of the effluent.

MATERIALS AND METHODS

Two types of jack bean urease (E.C. 3.5.1.5) were used in the present study, type C-3 (Sigma) a high purity crystalline form, and type IX (Sigma) a less pure lyophilized powder. Several immobilization procedures were evaluated including immobilizations by adsorption onto ion exchange resins, and covalent linkage to silanized glass[18]. The best performance was obtained using bioreactors containing urease immobilized on Celite R-648 (Johns-Manville) controlled porosity diatomaceous earth following titanium (IV) oxide activation, and ethylene diamine cross-linking[19,20]. Activities of the immobilized enzymes were generally ≥ 1,000 EU/g (an EU produces 1 μ-mole-min^{-1} of NH_3 at 25°C and pH 7).

Parametric testing was performed using small scale bioreactors consisting of 6 - 7 cm^3 of immobilized enzyme, packed into 1.0 cm diameter borosilicate glass tubing, with glass wool plugs and septa at the inflow and outflow faces. Peristaltic pumps (Barnart 7553-60) were used to establish continuous flow rates in the range between 2.5 - 3.0 cm^3-min^{-1}. Flows were maintained on a continuous 24 hour per day basis, Monday through Friday and shut down over weekends. Empty bed contact times and face velocities for the small scale bioreactors averaged 2.4 minutes, and 3.5 cm-min^{-1} respectively. The small columns were typically challenged with unbuffered aqueous solutions containing concentrations of 60 mg-L^{-1} urea nitrogen. Bioreactor effluents were monitored for pH, specific conductance, NH_3 and urea levels. Urea concentrations were determined spectrophotometrically at 480 nm using the diacetyl monoxime procedure[21]. Aqueous NH_3 was determined spectrophotometrically at 425 nm by Nesslerization[22]. Specific conductances and pH were determined using a conductivity bridge and glass electrode respectively.

RESULTS AND DISCUSSION

At 25°C, small column bioreactors challenged with 60 mg-L^{-1} typically produced effluent urea nitrogen concentrations of less than 0.1 mg-L^{-1} for cumulative flows of 45 - 47 liters per cm^3 of supported enzyme (L-cm^{-3}). For a 6.5 cm^3 bed volume and a flow rate of 2.5 cm^3-min^{-1}, this corresponds to approximately three months of continuous flow. Small column bioreactors were challenged with 60 mg-L^{-1} urea in the presence of a range of HCl concentrations between pH 1.5 and pH 5.5. The immobilized urease beds were found to undergo significant loss of activity for pH < 2.5. Similar challenges using aqueous NaCl solutions with specific conductances ranging between 50 - 860 μS-cm^{-1} resulted in diminished rates of urea hydrolysis for dissolved salt concentrations with specific conductances > 580 μS-cm^{-1}. As shown in Figure 1, challenge of the immobilized enzyme with aqueous elemental iodine concentrations of 0.8 mg-L^{-1} resulted in complete loss of activity after one liter of volumetric throughput per cm^3 of reactor bed volume (L-cm^{-3}). This confirms the necessity for preconditioning of the bioreactor influent to buffer pH and to remove I_2 and excessive levels of dissolved salt upstream of the immobilized enzyme bed.

To investigate the effect of influent substrate concentration on bioreactor performance, identical small columns were challenged with influent urea levels of 10,000, 1,000, and 60 mg-L^{-1}. Effluent urea and NH_3 concentrations for these experiments are shown in Figures 2 and 3 respectively. With an influent of 10,000 mg-L^{-1}, the bioreactor was able to hydrolyze 99 percent of the substrate for a cumulative throughput of approximately 5 L-cm^{-3}. At this influent urea concentration, hydrolysis efficiency rapidly dropped to 90 percent by 8 L-cm^{-3}. At 1,000 mg-L^{-1} life was extended to 20 L-cm^{-3} for 99 percent efficiency and to 37 L-cm^{-3} for 90 percent efficiency. This compares to 47 L-cm^{-3} for 99 percent efficiency at an influent concentration of 60 mg-L^{-1} urea nitrogen. In these experiments, anomalously high effluent urea values were obtained at total throughputs of approximately 9 and 37 L-cm^{-3}, for the 60 mg-L^{-1} urea influent concentration. These are attributable to transients following start-up after periods of reactor down time.

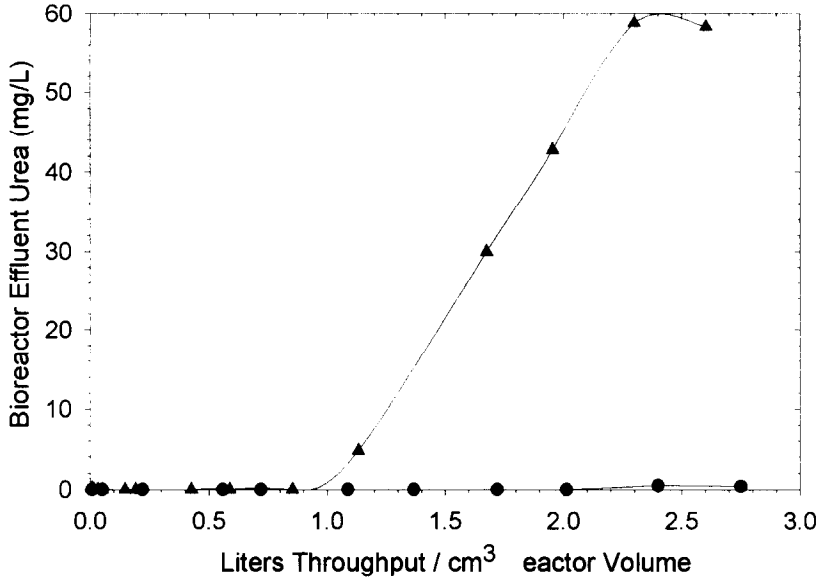


Figure 1. Bioreactor Effluent Urea Levels for 60 mg-L^{-1} Influent: with 0.8 mg-L^{-1} Dissolved Elemental Iodine (triangle), and without Iodine (circle).

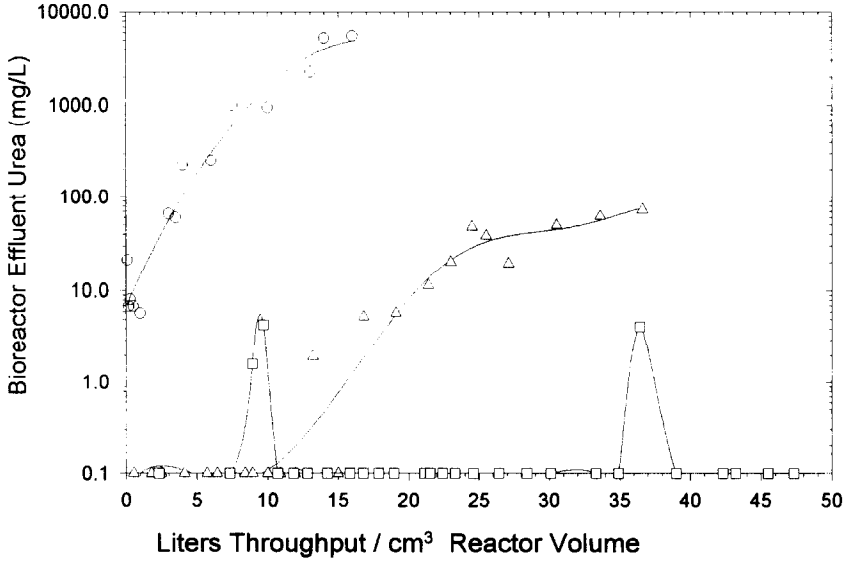


Figure 2. Bioreactor Effluent Urea Levels for 60 mg-L^{-1} (square), 1000 mg-L^{-1} (triangle), and 10000 mg-L^{-1} (circle) Influent Urea Concentrations.

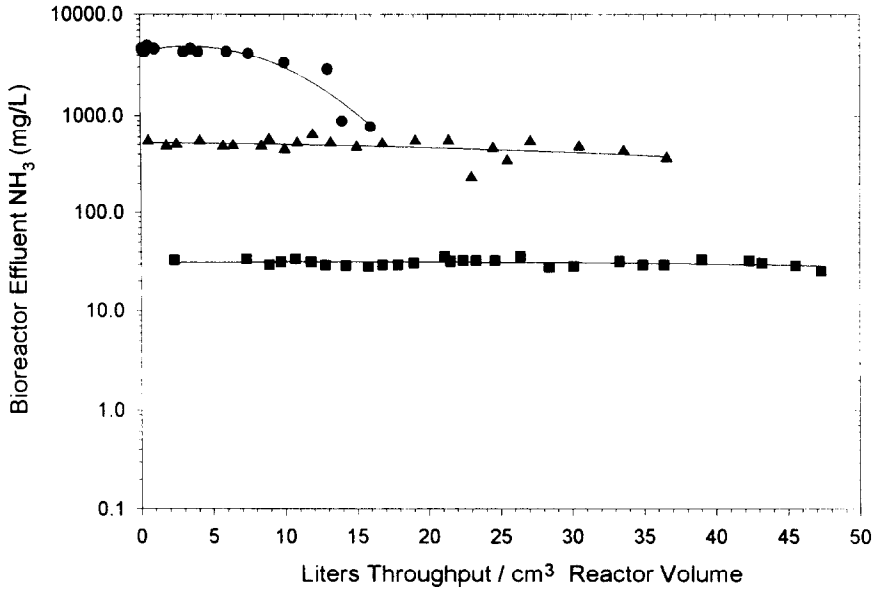


Figure 3. Bioreactor Effluent Ammonia Levels for 60 mg-L^{-1} (square), 1000 mg-L^{-1} (triangle), and 10000 mg-L^{-1} (circle) Influent Urea Concentrations.

The effect of reactor temperature was investigated by challenging three identical small urease bioreactors with 1,000 mg-L^{-1} influent at 25°C, 45°C, and 65°C. The results of these experiments are illustrated in Figure 4. At 65°C the bioreactor rapidly lost activity, showing only 30 percent efficiency after 1 L-cm^{-3} of throughput. At 45 °C the immobilized enzyme bed demonstrated high catalytic activity for the initial 3.8 L-cm^{-3} of throughput, falling to 90 percent efficiency by 5.3 L-cm^{-3}, and rapidly degrading to 60 percent by 6.0 L-cm^{-3}. At 25°C high enzymatic activity was observed for the typical 45 L-cm^{-3}.

Water reclamation systems for regenerative life support in space must produce potable quality water. For this reason microbial contamination is a serious concern. Reclaimed waters are routinely disinfected using a flow-through cartridge of iodinated ion exchange resin, termed the Microbial Check Valve (MCV)[23]. An additional requirement is that sub-systems not introduce any significant additional microbial populations. To this end, all water processor flight hardware must be sterilized prior to installation.

The likelihood of thermal denaturation of enzymatic activity consequent to routine autoclaving, required the development of novel antimicrobial methods for preparation of the urease bioreactors. Two possible sterilization schemes were investigated: deposition of transition metals onto the support and irradiation by gamma rays. Metals tested included platinum, palladium, tin, copper, and silver. Deposition of metallic silver resulted in strong suppression of microbial populations as well as complete inactivation of the biocatalyst. Deposition of the other metals resulted in active immobilized enzyme preparations but showed little effect on microbial contaminants.

Immobilized enzyme columns containing 30 cm^3 were irradiated with 2.8 Mrad from a ^{60}Co gamma ray source and subsequently challenged with 60 mg-L^{-1} urea solutions. No radiation induced enzyme inactivation was indicated. Microbial population densities of < 1 CFU-cm^{-3} on R2A agar were detected within the irradiated bioreactors, compared to a range of 10^1 - 10^4 CFU/cm^{-3} in non-irradiated controls.

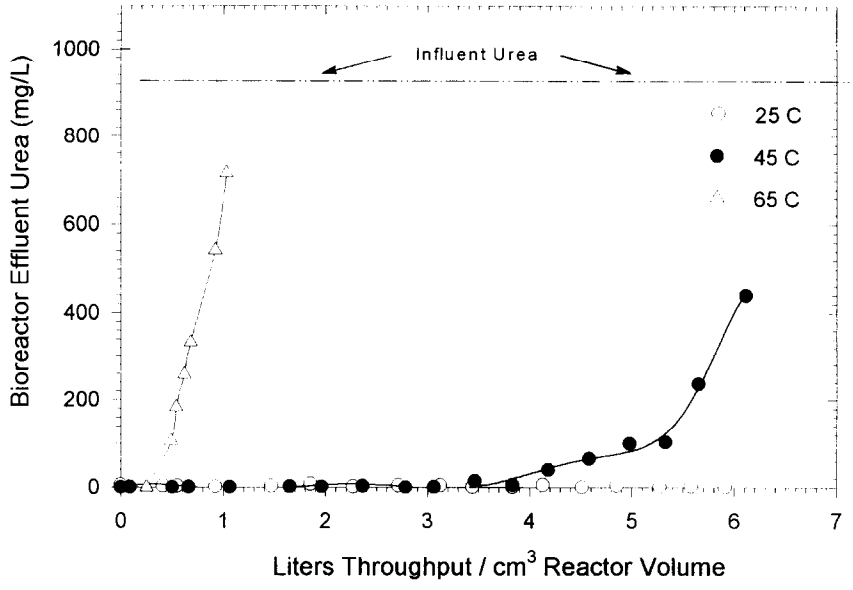


Figure 4. Urease Bioreactor Performance Between 25°C - 65°C for 1000 mg-L^{-1} Urea Influent.

The information gathered as a result of preliminary testing was used to design a full scale breadboard urease bioreactor for delivery to Marshall Space Flight Center for evaluation during Core Module Integrated Facility (CMIF) Phase III water recovery testing of the proposed Space Station *Freedom* water reclamation systems[24]. The then current design for space station water reclamation consisted of individually designed MF processes implemented using layered sequences of carbonaceous, polymeric, and ion exchange sorbents known as Unibeds®*

One unit treated combined hygiene water and urine distillate for production of hygiene water, and a second unit treated humidity condensate for the production of potable water. The urease bioreactors were designed for use downstream of the urine distillate - hygiene water multifiltration train.

Design requirements were for a system capable of continuously processing crew hygiene water at 25 mL-min^{-1}, with a maximum differential pressure of 275.8 kPa (40 psi). The resulting breadboard bioreactor design is illustrated in Figure 5. The system consists of a housing fabricated from a 61 cm length of cylindrical polycarbonate with an inside diameter of 5.08 cm. For a bioreactor of this geometry, minimum bed volumes of 60 cm^3 are required to maintain a satisfactory ratio of length to diameter (L/D) for the minimization of flow channeling. Five beds of media are arranged in layers, separated by polypropylene spacers. From inlet to outlet these are 60 cm^3 of IRN-150, a stoichiometrically equivalent mixture of strong base anion exchange and strong acid cation exchange resins (Rohm & Haas), 200 cm^3 of immobilized urease, 750 cm^3 of IRN-77 strong acid cation exchange resin (Rohm & Haas), an additional 60 cm^3 of IRN-150 resin, followed by 60 cm^3 of MCV® Resin** (Umpqua Research). Quick disconnect fittings are attached at inflow and outflow faces for ease of installation. At

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the design flow rate, this configuration corresponds to a face velocity of 1.23 cm-min.$^{-1}$ and empty bed contact times of 8 minutes and 44 minutes for the urease bed and the complete bioreactor module respectively.

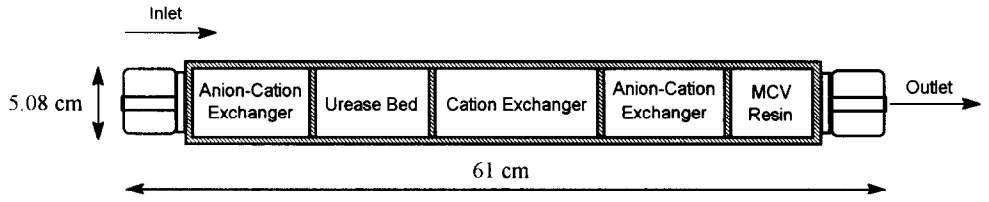

Figure 5. Full Scale Breadboard Urease Bioreactor - Layered Sorbent Module.

The primary purpose of the first bed is to remove elemental iodine as the triiodide, pentaiodide, or heptaiodide anion, to prevent inactivation of the enzyme. Secondarily, the mixed bed ion exchanger mitigates the potentially deleterious effects of transient episodes of low pH or high ionic strength in the bioreactor influent. Bed volume was determined by the minimal L/D requirement. Considering the 158 mg-cm^{-3} anion exchange capacity of the resin for I_2, and assuming an average elemental iodine concentration of 2 mg-L^{-1}, in the absence of other ionic perturbations, a bed life of 130 days is expected. In the second bed, urease hydrolyses urea to NH_3 and CO_2. Assuming an influent urea concentration of 60 mg-L^{-1} and a typical effective throughput of 40 L-cm^{-3} for the immobilized enzyme, a bed life of 220 days is projected. The immobilized enzyme bed was deliberately oversized owing to the uncertainty as to the levels of urea to be expected in the bioreactor influent. The ionic reaction by-products are removed in the subsequent two beds, as NH_4^+ cations, and HCO_3^- anions respectively. Considering the cation exchange capacity of 25 mg-cm^{-3} of IRN-77 for NH_4^+ ions, this bed is projected to last only 14 days at a urea influent concentration of 60 mg-L^{-1}. A 7 L bed volume for this resin is required to achieve a 130 day life. The 750 cm^3 bed volume was dictated by the overall size constraint for the bioreactor module. Future urease bioreactors can be designed with expendable or regenerable downstream ion exchange beds. The final two beds are sized at 60 cm^3 based upon the minimal L/D requirement. Additional cation exchange capacity is gained in the mixed anion/cation exchange bed which sorbs ammonium, bicarbonate, and carbonate ions from solution. At the pHs expected, most of the dissolved inorganic carbon will occur as CO_2(aq), hence relatively little bicarbonate and carbonate will be sorbed. The primary purpose of the anion exchange component of this bed is to prevent damage to the immobilized enzyme from I_2 under back flow conditions. The final bed within the bioreactor re-iodinates the effluent water with bacteriostatic levels of elemental iodine.

The full scale integrated bioreactor modules were tested at Marshall Space Flight Center during the Phase III CMIF water recovery test of the space station potable water processor, using real humidity condensates collected in the End Use Equipment Facility (EEF) as influent[24]. Unfortunately, this was not the waste water stream for which the urease bioreactor was designed. The tests were conducted following a redesign of the space station water processor which integrated both hygiene water and humidity condensate streams into a single contaminant stream for purification to produce potable water. No future testing of a hygiene water/urine distillate water processor was envisioned. A full scale test of the 'pre-redesign' potable water processor was in-progress. The

urease bioreactor was integrated into this test. The bioreactor was installed at the outflow of the multifiltration Unibed® train. The results are summarized in Table I. Influent urea concentrations varied between 0.12 - 0.31 mg-L^{-1}. Bioreactor effluent urea levels were at all times less than the 0.10 mg-L^{-1} detection limit over the course of the eleven day test. The unit performed well, but was not subjected to the influent urea levels for which it was intended.

The use of an in-line fixed bed immobilized enzyme bioreactor offers several advantages over other means of water reclamation aboard spacecraft. Stability of the bioreactor is an asset. The catalytic efficiency of urease over a wide range of influent urea concentrations enables the system to function despite upstream process upsets. This is at least in part due to the in-line continuous flow fixed bed configuration which allows system performance to approach the favorable kinetics of a plug flow reactor. In general, immobilization lends stability to the enzyme with respect to loss of activity. Urease inactivation has been shown to proceed preferentially with the free form of the enzyme[25]. The relative simplicity of the passive device is also an advantage. Energy utilization is minimized through passive operation at ambient temperature. The only energy requirement is that necessary to sustain fluid flow. Due to the relatively large particle sizes of both sorbents and enzyme support, the pressure drop across the bioreactor at the design flow rate is 6.2 kPa (0.9 psi), and therefore power consumption is minimal.

Table I. CMIF Phase III Water Recovery Bioreactor Test Results

Test Day	Inlet TOC μg-L^{-1}	Inlet Urea μg-L^{-1}	Outlet Urea μg-L^{-1}
2	7200	200	< 100
3	4900	120	< 100
4	5600	130	< 100
5	5700	170	< 100
6	5700	210	< 100
7	5100	310	< 100
9	4500	140	< 100
11	3860	200	< 100

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