[26] It is not surprising, therefore, that the large bulk of activities directed towards improving metabolic processes have focused on changing the genotype of the microorganism, e.g., through genetic engineering. Genotypic alterations often come at significant expense and require substantial time to obtain the sought performance from a microorganism. Typically most genetically engineered microorganisms lack robustness, e.g., are slow growing and are competitively disadvantaged against invasive microorganisms and are subject to losing plasmids during scale up for quantities sufficient to fill commercial-scale bioreactors and during the bioconversion process itself. Additionally, genetically engineered microorganisms may have to be carefully contained so as not to escape to the environment, and disposal of debris from metabolic processes using genetically engineered microorganisms may be treated as hazardous waste.

Summary

[27] The microorganism-containing biocatalysts of this ~~invention~~ disclosure have a large population of the microorganisms irreversibly retained in the interior of the biocatalysts, and the biocatalysts possess a surprisingly stable population of microorganisms, and hence stable bioactivity, and an essential absence of debris generation from metabolic activity of the microorganisms, and the biocatalysts can exhibit these phenomenon over extended periods of time. These phenomena are contrary to conventional expectations are that microorganisms either escape from physically restricted regions or that the physically restricted region becomes clogged or over populated leading to loss of metabolic activity and ultimate death of the population of microorganisms.

[28] The microorganisms in the biocatalysts of this disclosure~~invention~~ exhibit phenotypic alterations that, in combination with an internal, cavity-containing structure of the biocatalyst, provide highly advantageous biocatalysts including, but not limited to, a metabolic shift from growth of the microorganisms and their population to bioconversion activity (anabolic or catabolic); enhanced tolerance to toxins; enhanced ability to enter a substantial state of stasis even for extended periods of time; and enhanced ability to efficiently bioconvert substrate. These phenotypic changes significantly add to the fact that the microorganisms effecting the bioconversion are retained in the interior of the

biocatalyst to provide advantageous metabolic processes, especially metabolic processes where the biocatalysts of this disclosure~~invention~~ provide desirable bioconversion activity over extended periods of time, preferably at least about 3, preferably at least about 6, and frequently in excess of 12 or 24, months, and sometimes as much as 5 years or more.

[29] While not wishing to be limited to theory, it is believed that the ability of the biocatalysts of this disclosure~~invention~~ to possess the stable population of microorganisms in its interior is due to phenotypic changes to the microorganisms that occur during making the biocatalysts. These phenotype changes are believed to be due to the confluence of three primary factors. First is the use of a high concentration of microorganisms to make the biocatalyst, e.g., at least about 60, preferably at least about 100, grams of cells per liter, such that communication can occur among the cells at the time of formation of the biocatalyst. All references herein to the mass of cells are to the mass of wet cells. The high concentration of cells is also preferred as upon the phenotypic change occurring, little, if any, net growth in the population of the microorganisms occurs in the biocatalyst. In some instances, the net growth in population of microorganisms can be up to three or four fold until the steady-state population occurs. However, in most instances, the population of microorganisms at steady-state is plus or minus about 50, frequently plus or minus about 30, percent of the concentration of the cells initially used in preparing the biocatalyst.

[30] The second major factor is that the biocatalyst when formed contains microorganisms in a plurality of interconnected major cavities of between about 5 and 100 microns in the smallest dimension. Preferably on a volumetric basis, at least about 20, and preferably at least about 50, percent of the interior structure of the biocatalyst (excluding the microorganisms) is composed of major cavities in this range. Although larger major cavities may be present, preferably less than about 25 percent of the interior of the solid structure is composed of these larger major cavities. Preferably the interconnected cavities in the biocatalyst are quiescent. It is believed that the high preponderance of the interconnected major cavities have a smallest dimension of between about 5 and 100 microns enhances the ability of the microorganisms located in the cavities to communicate such that the microorganisms, as a community, undergo phenotypic alteration.

[31] The third major factor resides in the polymeric material component of the biocatalyst being hydrated and hydrophilic. It is believed that the microorganisms located in the interior of the biocatalyst as it is being made, especially those in the major cavities and smaller cavities, sense the hydrophilicity of the surface and this sensing of the environment also contributes to the phenotype change. The polymeric material is highly hydrated but yet contains sufficient hydrophobicity that the polymeric material in the biocatalyst is not dissolved or dispersed in water under the anticipated conditions of use. The hydrophilicity and the hydrophobicity of the polymer are such that the microorganisms become substantially irreversibly retained in the interior of the biocatalyst. As the retention of the microorganisms in the biocatalyst is due to a sensing by the microorganisms and their response, this irreversibly retention can be described as a metabolic retention. The biocatalysts can be characterized by having a Hydration Expansion Volume (HEV) of at least about 1,000, preferably at least about 5,000, and most often at least about 10,000. The Hydration Expansion Volume is indicative of the hydrophilicity of the polymeric material, and the higher the HEV, the greater is the hydrophilicity of the polymeric material.

[31a] In one aspect, there is provided a biocatalyst comprising:

a solid structure of hydrated hydrophilic polymer defining an interior structure having a plurality of interconnected major cavities having a smallest dimension of between about 5 and 100 microns and an HEV of at least about 1000 and

a population of microorganisms substantially irreversibly retained in the interior structure, said population of microorganisms being in a concentration of at least about 60 grams per liter based upon the volume defined by the exterior of the solid structure when fully hydrated,

wherein the microorganisms maintain a their population substantially stable.

[32] Accordingly, in ~~its broad aspects~~one example, the biocatalyst composition of this disclosure~~invention~~ comprises:

a solid structure of hydrated hydrophilic polymer defining an interior structure having a plurality of interconnected major cavities having a smallest dimension of between about 5 and 100 microns and an HEV of at least about 1000, preferably at least about 5000, and

a population of microorganisms substantially irreversibly retained in the interior structure, said microorganisms being in a concentration of at least about 60 grams per liter based upon the volume defined by the exterior of the solid structure when fully hydrated,

wherein the microorganisms maintain a their population substantially stable.

[33] Preferably the hydrophilic polymer also forms a skin on the exterior of the biocatalyst composition. Although the microorganisms have been found to become substantially irreversibly retained in the interior of the biocatalyst, generally the case is that the

microorganisms are not significantly, if at all, in direct contact with the polymer although they can be in contact through fibrils, e.g., of extracellular polymeric substance, or strands of polymer. It is believed that the high hydrophilicity of the polymer reduces the ability of the microorganisms to adhere to the exterior surfaces of the biocatalyst under conditions of physical stress such as the flow of fluid over the exterior of the biocatalyst.

[33a] In one aspect, there is provided a method for making a biocatalyst as described herein, the method comprising:

a. forming a liquid dispersion of solubilized precursor for hydrophilic polymer and microorganisms for said biocatalyst wherein the concentration of microorganisms in the liquid dispersion is at least about 60 grams per liter;

b. subjecting said dispersion to solidification conditions to form a solid structure of the hydrophilic polymer wherein the solid structure has an interior structure having a plurality of interconnected major cavities containing said microorganisms, said major cavities having a smallest dimension of between about 5 and 100 microns and wherein the solid structure has an HEV of at least about 1000 said solidification conditions not unduly adversely affecting the population of said microorganisms; and

c. maintaining the solid structure containing microorganisms under conditions that do not adversely affect the population of said microorganisms in the interior of the solid structure for a time sufficient to enable the microorganisms to undergo a phenotypic alteration to maintain their population substantially stable and to become substantially irreversibly retained in the interior of the solid structure

[34] Another ~~broad aspect~~example of this disclosure~~invention~~ pertains to methods for making biocatalyst compositions comprising:

a. forming a liquid dispersion, preferably an aqueous dispersion, of solubilized precursor for hydrophilic polymer and microorganisms for said biocatalyst wherein the concentration of microorganisms in the liquid dispersion is at least about 60, preferably at least about 100, grams per liter;

b. subjecting said dispersion to solidification conditions to form a solid structure of the hydrophilic polymer wherein the solid structure has an interior structure having a

plurality of interconnected major cavities containing said microorganisms, said major cavities having a smallest dimension of between about 5 and 100 microns and wherein the solid structure has an HEV of at least about 1000, preferably at least about 5000, said solidification conditions not unduly adversely affecting the population of said microorganisms; and

c. maintaining the solid structure containing microorganisms under conditions that do not adversely affect the population of said microorganisms in the interior of the solid structure for a time sufficient to enable the microorganisms to undergo a phenotypic alteration to maintain their population substantially stable and to become substantially irreversibly retained in the interior of the solid structure.

[35] The solidification conditions may, in some instances, include the presence of a cross-linking agent, and the precursor is a solubilized prepolymer. Alternatively, the solidification conditions may comprise a reduction in temperature of the liquid dispersion such that polymer becomes solidified to form the solid structure. Often, the liquid dispersion not encompassed within the solid structure formed in step (b) is separated during or prior to step (c).

[36] ~~Other broad aspects of this invention pertain to m~~Metabolic processes in which the biocatalysts of this disclosure~~invention~~ are subjected to metabolic conditions including the presence of substrate to bioconvert said substrate to bioproduct are provided.

[36a] In one aspect, there is provided a metabolic process comprising subjecting the biocatalyst as described herein to metabolic conditions including the presence of substrate to bioconvert said substrate to bioproduct.

The metabolic processes may be
anabolic or catabolic. In the preferred processes, the microorganisms evidence a metabolic shift as compared to planktonic metabolism under substantially the same metabolic conditions.

[36b] In one aspect, there is provided a processes for reducing the concentration of ultra low contaminants in a water stream comprising:

a. continuously passing said water stream to a bioreactor, said bioreactor being maintained at metabolic conditions including the presence of biocatalyst as described herein comprising microorganisms for bioconverting said ultra low contaminants;

b. contacting said water stream with said biocatalyst for a time sufficient to reduce the concentration of said ultra low contaminants; and

c. withdrawing from said bioreactor a treated water stream having a reduced concentration of said ultra low contaminants.

[36c] In one aspect, there is provided a process for catabolizing dissolved organic carbon and ammonium cation in a wastewater stream comprising:

a. continuously passing said wastewater stream to a bioreactor containing biocatalyst as described herein comprising microorganisms for catabolizing dissolved organic carbon to carbon dioxide and ammonium cation to nitrate anion, preferably an ammonia oxidizing microorganism;

b. contacting in said bioreactor said wastewater stream with said biocatalyst in the presence of oxygen for a time sufficient to provide an oxidized effluent containing less than about 5 milligrams of ammonium cation and having a biochemical oxygen demand (BOD) of less than about 10 milligrams per liter,

wherein substantially no solids pass from the biocatalyst to the oxidized effluent.

[36d] In another aspect, there is provided a process for the biological reduction of soluble phosphate in water comprising:

a. contacting said water in a bioreactor with biocatalyst as described herein comprising phosphate accumulating microorganisms under phosphate accumulating conditions for a time sufficient to reduce the concentration of phosphate in said water and provide a biocatalyst comprising phosphate laden microorganism wherein said phosphate accumulating conditions comprise the presence of polyhydroxyalkanoate within said microorganisms and the presence of aerobic or anoxic conditions in the water:

b. subjecting said biocatalyst comprising phosphate laden microorganism to anaerobic conditions in an aqueous medium sufficient to release phosphate from said microorganisms into said aqueous medium to provide a phosphate rich aqueous medium; and

c. separating said biocatalyst from the phosphate rich aqueous medium for use and step (a).

[36e] In another aspect, there is provided a process for biodegrading ammonium to nitrogen in a feed water containing ammonium and oxygen using an N/D microorganism comprising contacting under metabolic conditions the water with biocatalyst as described herein having said microorganism irreversibly retained therein for a time sufficient to provide a treated water having a concentration of ammonium less than about 50 percent of that in wherein the nitrate concentration of the feed water and a concentration of nitrate ion less than about 1 milligram per liter.

[36f] In another aspect, there is provided a process for the bioconversion of a plurality of substrates using a biocatalyst comprising substantially a single strain of microorganism under metabolic conditions for a time sufficient to bioconvert said substrates to bioproduct, the improvement comprising using a biocatalyst as described herein said microorganism.

[36g] In another aspect, there is provided a process for reducing the concentration of nitrate anion or perchlorate anion or both when present in water comprising contacting the water with a biocatalyst as described herein having therein a strain of microorganism for reducing said anions under metabolic conditions and for a time sufficient to bioconvert such anion.

[36h] In another aspect, there is provided a process for treating water containing at least one soluble compound of metal or semi metal comprising:

a. continuously introducing said water into a reaction zone containing biocatalyst as described herein comprising microorganism for reducing said soluble compound;

b. contacting the water with said biocatalyst for a time sufficient to reduce the concentration of said at least one soluble compound in the water;

c. maintaining said biocatalyst under metabolic conditions sufficient to metabolically reduce the oxidation state of the metal or semi metal to form elemental metal or semi metal or precipitated compound thereof; and

d. withdrawing water having a reduced concentration of said at least one soluble compound from the bioreaction zone.

[36i] In another aspect, there is provided a process to reduce the concentration of 1,4 dioxane in a water stream comprising:

a. continuously passing said water stream to a bioreactor, said bioreactor being maintained at metabolic conditions including aerobic conditions and the presence of biocatalyst as described herein comprising microorganisms for degrading 1,4 dioxane metabolically;

b. contacting said water stream with said biocatalyst for a time sufficient to reduce the concentration of said 1,4 dioxane in the water stream; and

c. withdrawing from said bioreactor a treated water stream having a reduced concentration of 1,4 dioxane.

[36j] In another aspect, there is provided a process for the bioconversion of sugar and optionally carbon dioxide to succinic acid comprising:

a. contacting an aqueous medium with a biocatalyst as described herein comprising succinic acid producing microorganism under metabolic conditions including temperature and the presence of sugar and other nutrients for the microorganism for a time sufficient to produce succinate anion and provide a succinate anion containing aqueous medium;

b. removing at least a portion of said succinate anion containing aqueous medium and said biocatalyst;

c. reusing in step (a) said biocatalyst from which at least a portion of said succinate anion containing aqueous medium has been removed; and

d. recovering succinate anion from said succinate anion containing aqueous medium.

[36k] In another aspect, there is provided a process for the bioconversion of carbon dioxide to bioproducts comprising:

a. maintaining the biocatalyst as described herein comprising a species of Botryococcus in an aqueous medium, said aqueous medium being at metabolic conditions including temperature and the presence of nutrients for the microalgae;

b. contacting the aqueous medium with carbon dioxide for the bioconversion wherein the microalgae secretes bioproduct;

c. irradiating the aqueous medium with light at a frequency and intensity sufficient for the microalgae to photosynthesize carbon dioxide to bioproduct; and

d. removing bioproduct from the aqueous medium.

[36l] In another aspect, there is provided a process for bioconverting substrate contained in a gas phase to bioproduct comprising:

a. continuously contacting the gas phase with biocatalyst as described herein, said contacting being at a temperature suitable for the metabolic bioconversion and for a time sufficient to effect said bioconversion of at least a portion of the substrate to bioproduct, and preferably for a time sufficient to provide a steady state mass transfer to and bioconversion of substrate;

b. cycling at least a portion of the biocatalyst of step (a) to at least one immersion step in an aqueous medium for a time sufficient to substantially fully hydrate the biocatalyst, and preferably wherein the aqueous medium for at least one of said immersion step comprises nutrients for the microorganisms, and said immersion is for a time sufficient to provide nutrients in said biocatalyst;

c. separating the biocatalyst and aqueous medium of the at least one immersion step; and

d. using at least a portion of the separated biocatalyst for step (a).

[36m] In another aspect, there is provided a process for reducing fouling by aquatic macroorganisms comprising contacting water that comes into contact with the aquatic macroorganisms with biocatalyst as described herein comprising microorganisms that are

capable of catabolic conversion of dissolved, metabolizable organic carbon in the water for a time sufficient to provide a treated water having a concentration of metabolizable organic carbon at a level where survival of macroorganisms is inhibited.

[36n] In another aspect, there is provided a process for desalinating water comprising (a) withdrawing a saline water from a water source, said saline water comprising suspended mollusks to provide a withdrawn water stream, and (b) subjecting the withdrawn water stream to desalination conditions to provide a desalinated water stream and a salt containing reject stream, the improvement comprising contacting the withdrawn water stream biocatalyst as described herein comprising microorganisms capable of catabolic conversion of organic carbon in the water for a time sufficient to reduce the concentration of organic carbon to a level where survival of the mollusks is inhibited.

[36o] In another aspect, there is provided an apparatus for the desalination of saline water comprising:

a. at least one raw water inlet conduit adapted to direct saline water into the apparatus;

b. at least one bioreactor in flow communication with at least one raw water inlet conduit and adapted to receive said saline water, said bioreactor comprising biocatalyst as described herein containing microorganisms capable of catabolic conversion of organic carbon in the water;

c. at least one desalination unit; and

d. at least one effluent conduit adapted to direct water from the bioreactor to the desalination unit.

[36p] In another aspect, there is provided a process for bioconverting a substrate contained in an aqueous medium to bioproduct under bioconversion conditions including the presence of microorganisms for said bioconversion, wherein an antimicrobial agent is provided to the aqueous medium in an amount sufficient to control contaminating microorganisms, the improvement comprising attenuating the effect of the antimicrobial agent on said microorganisms for said bioconversion by using a biocatalyst as described herein comprising said microorganisms for said bioconversion.

[36q] In another aspect, there is provided a bio detection device comprising a biocatalyst as described herein.

[36r] In another aspect, there is provided an anti fouling coating comprising a biocatalyst as described herein.

[36s] In another aspect, there is provided a biological fuel cell comprising a biocatalyst as described herein.

[36t] In another aspect, there is provided a filter comprising a biocatalyst as described herein.

[36u] In another aspect, there is provided a process for treating wastewater containing organic compound comprising:

a. contacting under thermophilic conditions said wastewater with biocatalyst as described herein comprising thermophilic microorganisms suitable for the bioconversion of organic compounds to methane for a time sufficient to reduce the concentration of organic compound to provide a treated water and a biogas,

b. separating the biogas from the wastewater and

c. separating the treated water from the biocatalyst.

[36v] In another aspect, there is provided a continuous process for the bioconversion of substrate to bioproduct using a microorganism capable of such bioconversion wherein the bioproduct is toxic to the microorganism comprising:

a. continuously supplying substrate and aqueous medium to at least one first bioreactor containing aqueous medium, said at least one first bioreactor having therein biocatalyst as described herein comprising said microorganism;

b. maintaining said at least one first bioreactor under metabolic conditions and continuously withdrawing a first reactor effluent from said at least one first bioreactor at a rate sufficient to maintain steady state conditions and provide a hydraulic residence time sufficient to bioconvert a portion of the substrate, said a first bioreactor effluent containing unconsumed substrate and bioproduct, wherein the bioconversion activity to said bioproduct in said at least one first bioreactor is at a first rate;

c. continuously supplying the withdrawn first bioreactor effluent to at least one subsequent bioreactor containing aqueous medium, said at least one subsequent bioreactor having therein biocatalyst as described herein comprising said microorganism;

d. maintaining said at least one subsequent bioreactor under metabolic conditions and continuously withdrawing a subsequent bioreactor effluent from said at least one subsequent bioreactor at a rate sufficient to maintain steady state conditions and provide a hydraulic residence time sufficient to bioconvert at least a portion of the substrate, said a subsequent bioreactor effluent containing bioproduct, wherein the bioconversion activity to said bioproduct in said at least one subsequent bioreactor is at a second rate which is lower than the first rate;

e. continuously separating a bioproduct rich stream from said withdrawn subsequent bioreactor effluent for product recovery and provide a residual aqueous stream; and

f. continuously recycling at least a portion of the residual aqueous stream to at least one subsequent bioreactor.

[36w] In another aspect, there is provided a process for bioconverting substrate to butanol comprising:

a. contacting an aqueous medium with a biocatalyst as described herein, said biocatalyst comprising microorganisms capable of bioconverting said substrate to butanol, wherein said aqueous medium is maintained under metabolic conditions including the presence of nutrients for said microorganisms and contains said substrate;

b. maintaining the contact between the aqueous medium and biocatalyst for a time sufficient to bioconvert at least a portion of said substrate to butanol; and

c. recovering butanol from said aqueous medium.

Brief Description of the Drawings

[37] Figure 1 is an SEM image of a portion of a cross-section of a biocatalyst in accordance with this invention.

[38] Figure 2 is an SEM image of a portion of a cross-section of another biocatalyst in accordance with this invention.

[39] Figure 3 is a schematic depiction of a photo-bioreactor using biocatalysts in accordance with this invention.

[40] Figure 4 is a schematic depiction of an apparatus suitable for treating municipal wastewater using the biocatalysts of this invention.

[41] Figure 5is a schematic depiction of a bioreactor used for the nitrification of wastewater which also contains a zone for collecting solid debris for hydrolysis in the treatment of wastewater.

[42] Figure 6 is a schematic depiction of an apparatus using five fluidized bioreactor assemblies containing biocatalysts of this invention suitable for removing phosphate anion from water.

[43] Figure 7 is a schematic depiction of a bioreactor assembly contained in the apparatus illustrated in Figure 6.

[44] Figure 8 depicts the sequencing of the modes of operation of each bioreactor assembly contained in the apparatus illustrated in Figure 6.