CELLS ON FOAM AND FIBER

R. Clyde, Clyde Engineering, PO Box 740644, New Orleans, LA, 70174

ABSTRACT Cells grow on high area foam and, when a screen is put around the foam, it is made heavier so it can be fluidized. When foam is rotated in a half full RBC (rotary biological contactor), drops are formed and mass transfer of oxygen to drops is much faster. Most fungi and some mammalian cells need oxygen. Corrugated fibers with holes in the valleys also produce drops. White rot fungus needs oxygen and it degrades many chlorine compounds, azo dyes, PAHs (polycyclic aromatic hydrocarbons), and TNT. Old cardboard boxes are readily available and when buried in soil, oxygen is entrapped. In a lake, the boxes expose high area. Celite entrapped in fibers provides even more area. Fibers have high surface area for immobilizing cells and, when the fibers are rotated, fast reactions occur, converting one chemical to another. Sugar has been fermented to alcohol in 10-15 minutes. Ethanol has high octane and does not need lead. Old cars and trucks still use lead, and high levels have been found in the drinking water of several large cities. Bacteria on fibers can remove lead in a few seconds. When an RBC of plain fiber discs is rotated and a light shone in the top, the light hits a thin moving film to degrade chlorine compounds and sterilize water. Titania can be fused to the fiberglass discs. Microbes and light remove sulfur from oil. Calcium magnesium acetate is a non-corrosive road deicer. Salt on roads causes millions of dollars damage to bridges and cars.

KEYWORDS: *Zymomonas*, lead, cesium, oxygen, sponge

SPONGE

In a recent article [1], Kargi removed COD and nitrogen from waste water with wire mesh sponge in a fluidized bed bioreactor. This design is covered in Clyde patent [2]. Wire mesh sponge can also be used in a half-full RBC where drops are formed. Mass transfer of oxygen to drops is much faster than to a flat surface [3].

TOXIC METALS

Lead has been found in the drinking water of several large cities. Millions of people in 100 large cities are drinking water that is over the limit of 10 ppb. It has been found in parking lot runoff so it probably comes from old cars and trucks that use leaded gasoline. In my design of cells on rotating fibers, lead can be removed in a few seconds.

Tyvek® fiber is not expensive and it has small pores and many fibers. When the bacterium *Zymomonas mobilis* was immobilized on a piece of white Tyvek® fiber about two inches long and dipped into a colored solution of lead and six valent chromium, the metals got on the fiber immediately, not only on the part immersed, but the color climbed right up the fiber and got on the fingers of the holder. It was not just the wicking effect, because a control showed nothing. In the control, the same procedure was used, including nutrients for *Zymomonas*, except no *Zymomonas* was used. In another experiment 21 ppb of lead was stirred for 3 seconds with 100 Tyvek® discs 45 mm in diameter, and the 100 ml of lead was reduced to 3 ppb. This experiment was repeated two more times on the same discs with the same result. The popular conception of bacteria is that they are poisonous, but Morais found that *Zymomonas* is therapeutic [4]. Several doctors in the Recife (Brazil) area treated their patients with intestinal disturbances, and they showed great mending with disappearance of their symptoms.

Lead was analyzed with a Hitachi Z-8100 Polarized Zeeman automatic absorption spectrometer with a graphite furnace. Presence of the metals was confirmed with a spectrometer at Tulane University, and photos were made. The *Zymomonas* was NRRL 14023 and the Tyvek® 1085B. It took about 30 hours for the *Zymomonas* to grow on the fiber. Nutrients were 100 g/l glucose, 10 g/l yeast extract, and 1 g/l of three salts, $(NH_4)_2SO_4$, $MgSO_4$, and KH_2PO_4 [5]. If the pH was below 6, half the amount of dibasic phosphate was used because it has a higher pH. Instead of yeast extract, inexpensive corn steep liquor can be used. High area celite can be entrapped in the fibers and yeast grown on the celite as shown by Clyde [5].

A patent by Clyde-Whipple [6] covers removing metals with bacteria on fibers. Example 6 in this patent also mentions *Pseudomonas putida* and *cepacia*. Chapatwala [7] degrades cyanides with *Ps. putida.* Cadmium and trichloroethylene can be removed with these. Example 2 in the patent describes uranium and contamination from old fuel rods.

Steinberg [8] shows a photo of uranium and *Zymomonas* on fiber. Lovley [9] reduces uranium to an insoluble form with bacteria. Wang [10] reduces chromium in a similar manner. If the bacteria were put on fibers, the process could be run continuously. Macaskie [11] removes uranium with a bacterium. Faison [12] removes strontium with a bacterium. Fujii [13] captures cesium with bacteria. Gas meters are leaking mercury. *Pseudomonas putida* removes mercury and degrades cyanides. Bender and Phillips, at Clark Atlanta University, found that when bacteria were immobilized on floating mats, metals were pulled up from the bottom of the lake. These scientists did not immobilize on rotating fibers, but we have tried so many organisms successfully that we believe the chances are good that other bacteria could be immobilized and the reaction run faster when the fibers are rotated. In another application, electrodes pull metals horizontally in soil and they can be captured with cells on fibers.

ETHANOL

Ethanol is less polluting than regular gasoline, and the EPA has recently suggested we need more ethanol. High rates of mass transfer result when yeast is put on a single fiberglass disc at 90 rpm, say Bringi and Dale [14]. We have found that 30 rpm works well too. With the bacterium *Zymomonas*, sugar can be fermented to ethanol in 15 minutes [15]. Wayman rotated at 40 rpm. We have a 30 cm diameter reactor and we rotate at 22 rpm because the tip speed is greater in the larger diameter. A turbine agitator rotating at 200 rpm uses much more energy. We have found that when you don't rotate you get a bubble here and there, about two per second, but when you rotate, the CO_2 which comes off sometimes blows the cotton right out of the vent. With polyester discs 9.5 cm in diameter, and rotated 40 rpm, Wayman got 96% yield starting with 50 g/l glucose and termed the results "remarkable." Using almost identical equipment except starting with 100 g/l glucose, we got an 80% yield in 10-15 minutes in two runs. Continuous runs have been made for up to two weeks, using the same residence times as in a batch run. Nutrients were the same as those used to take lead out of waste water. Several runs were also made in a smaller unit, 4.5 cm. in diameter. Alcohol was determined by HPLC. Higher concentrations of glucose can be fermented and end product inhibition avoided by stripping off some alcohol and recycling the sugar. Another method to eliminate end product inhibition is to extract

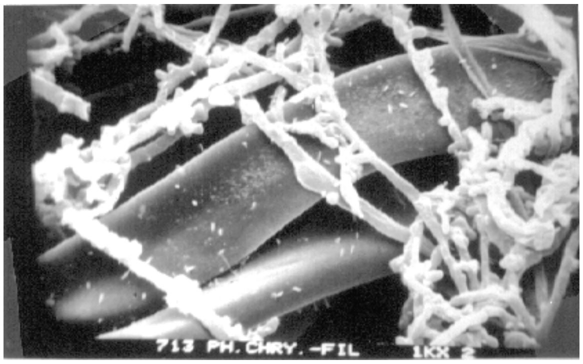

FIGURE 1. *PHANEROCHAETE CHRYSOSPORIUM* ON POLYESTER.

the ethanol with a higher alcohol such as oleyl.

E. coli also grows on fibers. The *Zymomonas* gene can be put into *E. coli* to ferment other sugars. The *E. coli* gene can also be put into *Zymomonas*.

SOIL AND SEDIMENT

Soil has been contaminated in many parts of the world by jet fuel and PAHs. There are over 2,000 contaminated sites from polycyclic aromatic hydrocarbons. White rot fungus grows on fibers (see Figure 1) and degrades these, as well as chlorinated compounds and TNT, but the fungus needs air to grow. A fast way of getting oxygen into cells is described in a recent Clyde patent [16], as shown in Figure 2. Corrugated fibers are rotated in a half full rotary biological contactor. Liquid is carried up into the vapor space and falls through holes in the valleys. Mass transfer of a gas to drops is much faster than to a flat surface [3]. When a liquid is carried up into the vapor space with corrugated fiber or polyurethane foam, drops are formed. With a vertical reactor, oxygen is depleted after passing through the column, but in a long horizontal reactor, oxygen can be added at the end (counter current to liquid) as well as

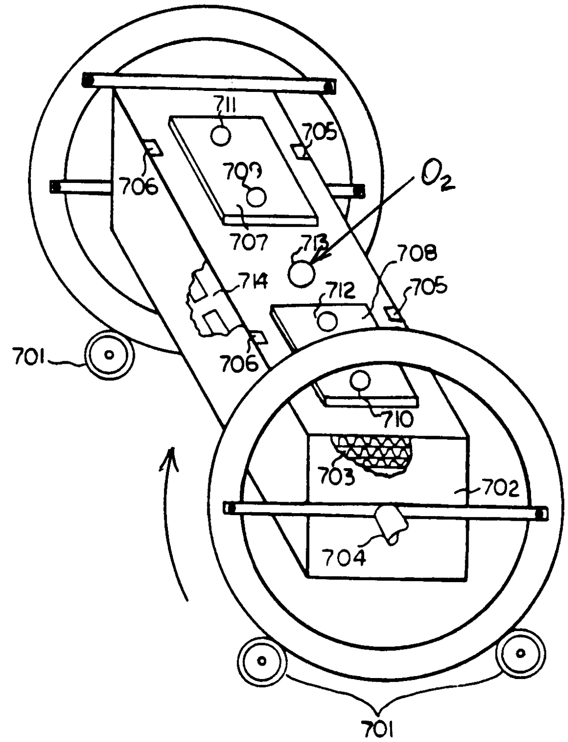

FIGURE 2. FROM PATENT 5,256,570.

in the middle. When a small unit of corrugated fibers was rotated, it took three days to grow white rot fungus, but when holes were put in the valleys, it took a day and a half. The tube was 12 cm long and 4.5 cm in diameter. Holes in the valleys were about 3 mm in diameter and rotation was 6 rpm. The horizontal tube was half full of nutrient. Lamar [17] says *P. laevis* is better than *chrysosporium*.

Some people use pellets or microspheres but it's hard for oxygen to diffuse into the interior of a pellet. At a distance of less than 0.4 mm inside the pellet there is no oxygen. When the corrugations are buried in contaminated soil, air is entrapped for further growth of the fungus. Sawdust was used in Brookhaven, Mississippi, but not very successfully, probably because there was not enough oxygen even though soil was tilled once a week.

Pseudomonads grow on fibers and they solubilize PAHs, say Stringfellow [18], Volkering [19], and recently Paquette [20].

CITRIC ACID

When rotating discs are used with a fungus, there is less energy and no foaming, and the limiting step is oxygen transfer. In an air lift reactor, it takes energy to compress the air, and bursting bubbles damage shear sensitive cells. A yeast can also be used and productivity is increased sharply with an increase in dissolved oxygen, says Sims [21].

AZO DYES

In a recent article, Suominen [22] degrades toxic azo dyes and nine other dyes with white rot fungus on nylon. These dyes are released into the water supply by the textile industry and require energy to remove them by conventional means. Many people in this area (including this author) drink only distilled water but this is a very energy intensive process.

CALCIUM MAGNESIUM ACETATE

Salt on roads causes millions of dollars damage to bridges and cars. People on low salt diets are affected, because the salt gets into drinking water and damages crops. In the future, when electronic devices are implanted in roads, salt will corrode them. CMA is a non-corrosive deicer which can be made with cells on rotating fibers, says Ljungdahl [23]. Cheaper raw materials such as corn steep liquor and cheese whey can be used.

PHOTO REACTOR

When an RBC is run half full and a light shone in the top, the light hits a thin moving film. In other photo reactors, colored or turbid solution blocks the light. Titania can also be fused to fiberglass to produce a faster reaction. Light has been found to degrade some chlorinated compounds, including dioxin. In India, hundreds of children die every day from bad drinking water.

The RBC can be run outside, saving energy by using the sun.

CONCLUSION

Cells on fibers have many applications. Toxic metals can be removed in two seconds. Bender and Phillips found that the attraction was so strong that bacteria on floating fiber mats pulled metals up from the bottom of a lake [24]. Cells grow well on sponges. We have found, and others have confirmed, that when fibers are rotated, fast reactions result. When white rot fungus is immobilized on old cardboard boxes in soil, air is entrapped for growth of the fungus. This fungus degrades many chlorine compounds, azo dyes, TNT, and PAHs. We have grown several *Pseudomonads* on fiber, and they also solubilize PAHs. There are over 2,000 contaminated PAH sites. A better photo reactor is described, where the light hits a thin moving film. Hundreds of children in India die every day from contaminated water. We sold reactors to Professor Wayman (for ethanol) and Ljungdahl (for CMA).

ACKNOWLEDGMENT

We wish to thank George Linthicum, 170 Main St., Bluff City, TN, for fusing titania to fiberglass at 595ºC. We thank Tom Jeffries at Forrest Products Lab for the photo of white rot fungus on fibers and the Biology Department of Tulane University for a slant of the fungus.

REFERENCES

1. F. Kargi and S. Eyiisleyen, Batch biological treatment of synthetic wastewater in a fluidized bed containing mesh sponge particles, Enz. Micro. Tech., 17 (1995) 119-123.

2. R. Clyde, U.S. patent 4,333,893, High area contactor, 1982.

3. Perry's Chemical Engineers Handbook, 6th ed., 1984, pp. 18-38

4. J. Morais, *et al.*, *Zymomonas mobilis* research in the Pernambuco Federal University, J. of Biot., 31 (1993) 75-91.

5. R. Clyde, Methanol dissociation and cells on fibres for ethanol production and pollution control, Fuel Magazine, 70 (1991) 1225-1226.

6. R. Clyde and A. Whipple, U.S. Patent 4,530,763, Method for treating waste fluid with bacteria, 1985.

7. K. Chapatwala, *et al.*, Screening of encapsulated microbial cells for the degradation of inorganic cyanides, J. Ind. Micro., 11 (1993) 69-72.

8. S. Steinberg, In a Ferment, Science News, 124 (1983) 234-235.

9. D. Lovley, *et al.*, Reduction of uranium by Cytochrome c3 of *Desulfovibrio vulgaris*, App. Env. Micro., 59 (1993) 3572-3576c.

10. Y. Wang and H. Shen, Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC 33456, App. Env. Micro., 59 (1993) 3771-3777

11. L. Macaskie, An immobilized cell bioprocess for the removal of heavy metals from aqueous flows, J. Chem. Tech. Biotech., 49 (1990) 357-379. See also Science, 257 (1992) 782-784.

12. B. Faison, *et al.*, Binding of dissolved strontium by *Micrococcus luteus*, App. Env. Micro. (1990) 3649-3656

13. T. Fujii, *et al.*, Kinetic studies on cesium transport in *Rhodococcus erythropolis* CS98 and *Rhodococcus sp.* strain CS402, Biosci. Biotech. Biochem., 59 (1995) 2219-2222.

14. V. Bringi and B. Dale, Experimental and theoretical evidence for convective nutrient transport in an immobilized cell support, Biot. Progress, 6 (1990) 205-209.

15. M. Wayman, *et al.*, Ethanolic fermentation of wood-derived cellulose hydrolysates by *Zymomonas mobilis* in a continuous dynamic immobilized biocatalyst bioreactor, Process Bioch. (1989) 88-91.

16. R. Clyde, U.S. Patent 5,256,570, Bioreactor configured for various permeable cell supports and culture media, 1993.

17. R. Lamar and B. Bogan, Polycyclic aromatic hydrocarbon-degrading capabilities of *Phanerochaete laevis* HHB-1625 and its extracellular ligninolytic enzymes, App. Env. Micro., 62 (1996) 1597-1603.

18. W. Stringfellow and M. Aitken, Competitive metabolism of napthalene, methylnaphthalene, and fluorene by phenanthrene-degrading pseudomonads, App. Env. Micro., 61 (1995) 357-362.

19. F. Volkering, *et al.*, Influence of nonionic surfactants on bioavailability and biodegradation of polycyclic aromatic hydrocarbons, App. Env. Micro., 61 (1995) 1699-1705.

20. G. Paquette, *et al.*, Biosurfactant production by a soil pseudomonas strain growing on polycyclic aromatic hydrocarbons, App. Env. Micro., 62 (1996) 1908-1912.

21. K. Sims, Oxygen uptake and citric acid production by *Candida lipolytica* Y 1095, Biot. and Bioeng., 43 (1994) 131-137.

22. I. Suominen, *et al.*, Decolorization of azo, triphenyl methane, heterocyclic, and polymeric dyes by lignin peroxidase isoenzymes from *Phanerochaete chrysosporium*, App. Env. Micro., 59 (1993) 4010-4016.

23. L. Ljungdahl and J. Wiegel, Chapter 16, In: D. Wise (Ed.), Calcium Magnesium Acetate, Elsevier, p. 412.

24. J. Bender and P. Phillips, Cleansing water with natural slimes, Science News, 147 (1994) 138.