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Bioreactor Treatment of Process Waters for MACT-Related Contaminants

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Final Report to the Georgia Consortium

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Executive Summary

The feasibility of using microorganisms immobilized in polymer gels to treat methanol in condensate streams was studied Laboratory trials showed that *Methylosinus trichosporium* OB3b immobilized in calcium alginate was able to degrade methanol in weak condensate across a large concentration range at temperatures up to 43°C. However, components in liquor carryover, probably hydrogen sulfide, were toxic to the organism. Two field trials were conducted at Georgia-Pacific's Cedar Springs facility. A problem with gel instability was overcome through reformulation of the gel. Reactive components in condensate imposed a chemical oxygen demand on the system, and this was overcome through pre-oxygenation. Overall, the immobilized organisms were able to successfully treat the stream.

The principal advantage of the system is a small footprint, which should translate to a relatively low construction cost. However, it will require a peroxide generator to remove hydrogen sulfide from the feed, an oxygenation unit, and some chilling of the feed stream. The immobilized organisms will need to be replaced periodically. Further piloting is needed to establish costs and commercialization possibilities.

INTRODUCTION

Anticipated MACT regulations (1) will regulate methanol in evaporator condensate. There are four technologies potentially available to remediate methanol: hardpiping to secondary treatment, steam stripping, anaerobic (2-6), and aerobic biodegradation. Aerobic degradation is conventionally achieved with an activated sludge system. However, these tend to have large footprints, and we proposed to study the feasibility of using polymer encapsulated microorganisms (7-9) which held out the promise of coupling high efficiency with a small footprint. In this technology, an organism tailored to the constituent of interest is immobilized in a polymer bead which greatly slows down growth of the organism. Thus, nutrient requirements are small. Also, since the organisms are not contaminated by other populations, pure cultures can be used, which leads to high efficiency. In this study, we demonstrate the feasibility of the technology and identify the factors that will need to be overcome for its application. Organisms were first screened and selected in laboratory work, and operating conditions defined. Two pilots were then run at the Georgia-Pacific Cedar Springs mill.

LABORATORY STUDIES

Selection of microorganisms

Three microorganisms were screened: two mixed cultures from the effluent treatment systems at Georgia-Pacific's Brunswick and Cedar Springs mills, and a methanol degrading pure culture, *Methylosinus trichosporium* OB3b (9,10), purchased from the American Type Culture Collection. The pure culture proved to be roughly two orders of magnitude more efficient in degrading methanol in condensate, and it was used in the remainder of our studies.

Culture and Encapsulation Procedures

A bacterial growth medium formulation suitable for *Methylosinus trichosporium* OB3b was obtained from the literature and modified according to recommendations made by Park, Hanna, et. al. (10). Batch cultures of *M. trichosporium* OB3b growing in this medium were assayed for ATP (adenosine triphosphate) (11). Since these results indicated a drop in ATP content after 6 days, the cells were subcultured once every 6 days.

Prior to encapsulation, cells were harvested from the growth medium by centrifugation and rinsed in a saline solution isotonic to that of the growth medium. Harvested cells were encapsulated as follows. A wet cell pellet (30 g.) was resuspended in the saline solution to a volume of 300 mL which was then added to 200 mL of 5% w/v sodium alginate. After the cells were evenly dispersed throughout the liquid alginate, the mixture was extruded through a pipet into a calcium chloride solution, whereupon gellation into 5mm. beads occurred instantly. The cultures, were initially pink in coloration, but faded to tan during experimentation.

The gel protects the entrapped organisms beads from contamination with other cultures, and restricts growth so that bacterial slime is not produced, and nutrients are not required. The density of the beads is high enough to enable fluidized bed operation, and floc

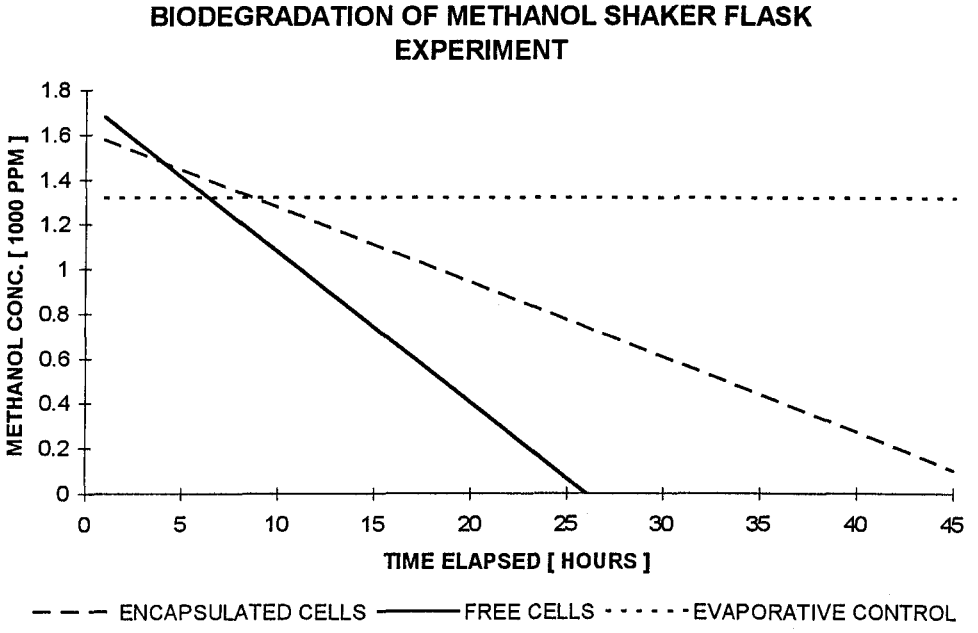


Figure 1: Comparison of biodegradation efficiency of free and encapsulated cells

problems typical of activated sludge are avoided. However, a loss of efficiency is incurred, and this was measured through paired runs with free and encapsulated organisms. Three 250 mL flasks, each containing 50 mL of sterile growth media with an initial methanol concentration of 1,400 ppm, were placed in a shaker incubator. One flask received 5 g. of a free cell suspension. Another received an equal quantity of biomass encapsulated in 3 mm. beads, and the third served as an evaporative control. The contents were analyzed for methanol by gc after 20 and 42 hours, and the results are illustrated in Figure 1. Evaporative loss of methanol was insignificant over the 42 hour test period. The free cells degraded methanol at a rate of 60 ppm MeOH/hr, and no methanol was detected after 42 hours. The encapsulated cells removed methanol at a rate of 33 ppm MeOH/hr. Hence, encapsulation retards the rate by about a factor of two. This is a small price to pay in return for the benefits of encapsulation.

Methanol is oxidized by the process: $CH_3OH + 1.5\ O_2 \rightarrow H_2O + CO_2$. In order to establish that this stoichiometry was obeyed, both methanol and DO drop was measured in a bioreactor. After correction for evaporative loss, 400 ppm of methanol was removed over 7 days which translates to 2.4 g. of methanol in 6L of feed. This requires 3.6 g. of oxygen. An average of about 1.5 ppm O_2 was consumed across the bioreactor, which, at a flow rate of 200 mL/ min., translates to 0.3 mg O_2/min for 200 mL. This equates to about 3 g of oxygen over 7 days, which compares well with the theoretical value of 3.6 g. Since DO is much

easier to measure than methanol, it was used as an index of biodegradation for the remainder of the study.

Laboratory bioreactor performance

The two designs used are illustrated in Figure 2. In the early configuration, the storage tank was aerated, and a sidestream was recycled through the bioreactor. This led to extensive evaporative loss of methanol, which was minimized in the later design by aerating the bioreactor itself. This allowed airflow to be reduced from 2.5 L/min to 0.75 L/min, and the evaporative loss reduced from 20 ppm MeOH/hr at 40°C to about 6 ppm MeOH/hr. Condensate from Cedar Springs was supplemented with methanol to a concentration of 3,600 ppm and used as feed to the bioreactor. The rate of methanol removal from the overall 9L system was a constant 43 ppm MeOH/hr, while the evaporative loss rate from the control was 7ppm MeOH/hr, for a net biodegradation rate of 36 ppm MeOH/hr.

Inhibitory effects of condensate constituents

Evaporator condensate was received from Cedar Springs on 9.15.95. Since this sample had a stronger odor than previous shipments, it was immediately tested by gc. Methanol

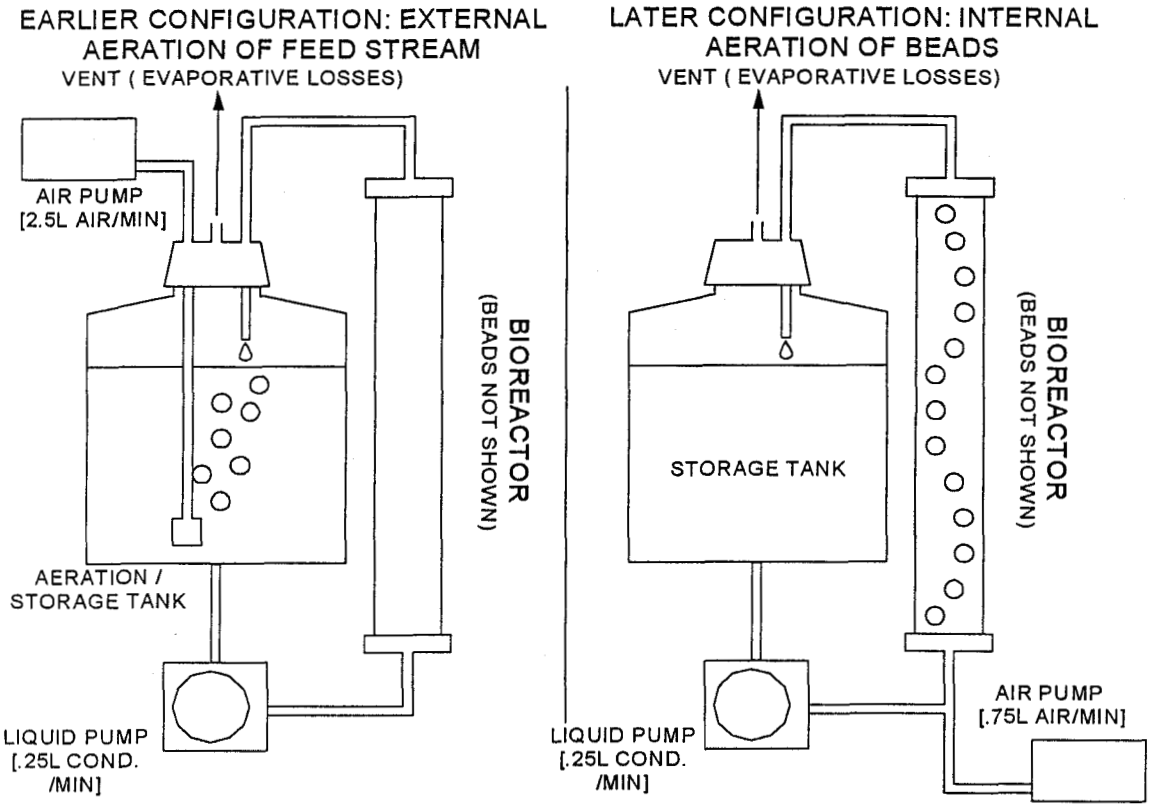


Figure 2: Schematics of two bioreactors

was found at 500 ppm, and two additional small peaks were detected in the gc trace as shown in Figure 3. The elution times of these peaks suggest that they represent compounds containing 3 or 4 carbons. These peaks disappeared when the sample was retested after 3 days.

The beads were transferred to the bioreactor which was maintained at 40°C. The pH of the fresh condensate was amended to 6.5, and its methanol concentration was increased to 4,000 ppm. With the addition of room temperature condensate and beads, the temperature of the system dropped to about 30°C. Within an hour after the introduction of condensate into the bioreactor, the alginate beads swelled, and $CaCl_2$ was added to the system at a concentration of 0.5 g/L, the same concentration used in media formulations. This returned the beads to their initial size, and the system was allowed to stabilize overnight, returning to the target operating temperature of 40°C.

Methanol loss in these experiments was much lower than expected at 6.7 ppm/hr. Since the corresponding evaporative loss was 5.3 ppm/hr, there was little or no methanol biodegradation. Since previous bioreactor experiments had shown degradation rates of up to

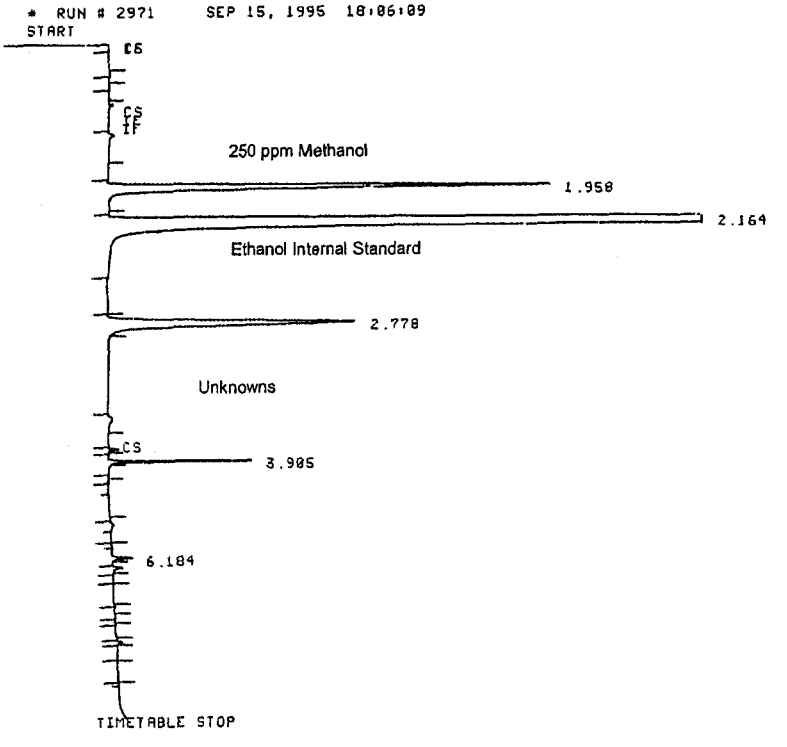


Figure 3: GC trace of fresh Cedar Springs condensate

60 ppm/hr, something in the condensate appeared to be toxic to the bacteria. The obvious possible candidates are the compounds represented by the two additional gc signals. A simple colorometric assay for sulfides utilizing Elmand's reagent qualitatively verified the presence of sulfides in the condensate. Furthermore, the mill reported sulfidity problems at time of sampling.

A series of shake flask experiments was conducted to measure the methanol degradation of bacterial cultures under different conditions of temperature and feed type. Since sulfides were suspected to be the toxicant in the bioreactor experiment above, a shake flask experiment was carried out to compare methanol loss between standard media, untreated condensate, and condensate treated to remove sulfides. Sulfide was removed through addition of 3% hydrogen peroxide, and degradation of the residual peroxide by MnO_2.

Double strength growth medium (25 mL) was added to three pairs of sterile flasks. A 25 mL sample of water, treated condensate, and untreated condensate, was added to each pair of flasks. One flask in each pair was then inoculated with 1 mL of bacterial culture, and the other was used as an evaporative control. All six flasks were then placed in a shaker incubator at 30°C. Samples were collected from each flask and analyzed for methanol over three days.

For the first two days there was no appreciable change in methanol content since the initial cell density was low. However, on the third day, all the inoculated flasks were cloudy from bacterial growth, and methanol had been completely removed from all three inoculated flasks. This experiment was initiated on 10.5.95, twenty days after the condensate had been received. It would seem that the toxic component of the condensate had disappeared during this time period. Significantly, the two extraneous gc signals had also disappeared, suggesting that a volatile two or three carbon mercaptan was responsible. If these compounds are, indeed, sulfides, then a sulfide probe followed by a peroxide injector will need to be built in ahead of a field bioreactor.

Establishment of the maximum operating temperature

In order to define the maximum operable temperature, double strength media (25 mL) was added to each of four 250 mL flasks at 43°C. Untreated condensate (25 mL) was added to one pair of flasks; the other pair received 25 mL of deionized water. One flask in each pair was inoculated with 5 mL of culture. The flasks were then placed in a shaker incubator at 43°C and samples were tested for methanol over four days. The rate of methanol loss from the evaporative controls was 54 ppm/hr. The inoculated flask containing condensate had a gross methanol loss rate of 178 ppm/hr, or net biodegradation rate of 124 ppm/hr. This rate remained relatively constant throughout the four day period, implying that growth was inhibited, but degradation was not. The inoculated flask containing media showed a gross methanol loss rate of 210 ppm/hr, or a net biodegradation rate of 156 ppm/hr. This rate also remained constant throughout the sampling period. These rates are acceptable and demonstrate that the bioreactor can be operated at 43°C. Analogous experiments conducted

at 55°C showed little or no biodegradation, indicating that 43°C is close to the maximum operable temperature.

Summary of laboratory results

The laboratory work demonstrated that pure strains of *Methylosinus trichosporium* OB3b immobilized in calcium alginate degrade methanol rapidly from evaporator condensate. However, components in condensate (present occasionally or continuously) are toxic to the organisms, and will need to be removed. The maximum operable temperature is about 43°C (109°F), which indicates that the condensate will need to be cooled if the system is commercially deployed.

FIELD STUDIES

In a preliminary field trial, alginate beads containing bacteria was exposed onsite to condensate at Cedar Springs. The beads lost their integrity over a few days, and it was believed that the breakdown was due to calcium loss from the gel, calcium providing the covalent bonds between alginate polymers which creates the gel. Two measurements were taken to replenish the calcium leached out. In the August 1996 trial, solid $CaCO_3$ was placed in the reactor so that calcium slowly dissolved and replenished material lost from the beads. This procedure has been reported to prolong the life of alginate beads (7). In the September/October trial, powdered $CaCO_3$ was included in the bead formulation.

Summary of the August field trial

The biotreatment system illustrated in Figure 4 was located in the evaporator area of the G-P Cedar Springs mill. This system consisted of a series of 9 upright PVC pipes connected in series through which a stream of evaporator condensate passed. Eight of the vertical pipes, referred to as units 1 through 8, comprised four biotreatment pairs, with the ninth unit (not shown) functioning as a heat exchanger. Hot condensate entering the system heat exchanger was cooled by the treated condensate exiting the system in unit 9. Each biotreatment pair consisted of an odd numbered oxygenation unit, followed by an even numbered bioreactor unit.

The condensate stream entered each oxygenator unit at the top and exited from the bottom. A stream of 1:1 oxygen:nitrogen gas was introduced from the base of the oxygenation unit through a medium fritted glass Buchner funnel. The oxygenated condensate stream entered each bioreactor unit at the bottom, and exited from the top. Inserted into bioreactor units 4, 6, and 8 were fine nylon mesh sacks containing either alginate beads. The alginate beads were initially composed of 5% calcium alginate, 5% calcium carbonate, and 10% bacterial biomass by weight. Unit 2 was always kept empty for control purposes.

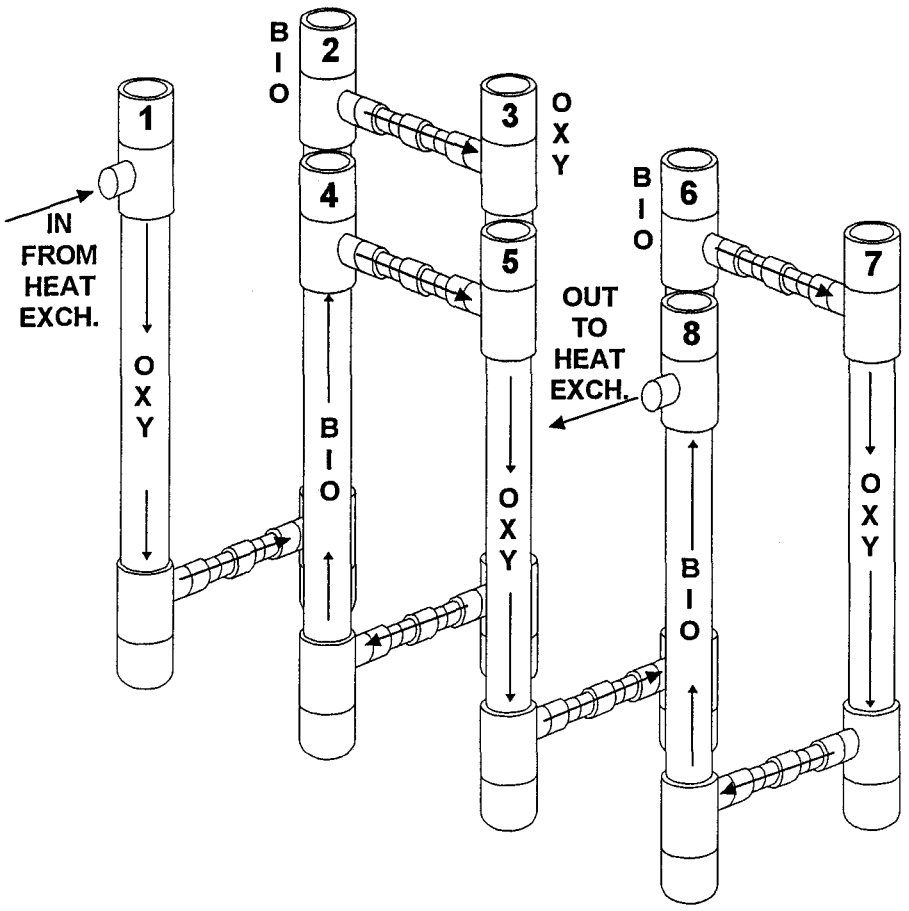


Figure 4: Field bioreactor unit (OXY: oxygenation unit, BIO: biotreatment unit)

Three types of data were collected for this study. DO, temperature, and flow rate was measured several times a day. DO and temperature was measured at the bottom of each oxygenation unit, near its exit, and also above the bacterial bead beds. Additionally, for control purposes, these measurements were collected at 4 inch intervals along the axis in the empty bioreactor unit 2. Altogether, over 1,500 DO and temperature readings were collected over the duration of the field trial.

Sequence of Events

July 31

Condensate and gas flows were established. The gas mixture of 50% oxygen 50% nitrogen was introduced through fritted glass dispersers in units 1, 3, 5, and 7. Only units 6 and 8 were used for bacteria; the others were used for additional aeration in order to oxidize condensate components that consumed oxygen, reducing the amount available for bacteria.

August 1-2

Alginate beads containing bacteria and solid $CaCO_3$ were introduced into unit 6. DO data collection began.

August 8-9
Alginate beads from unit 6 were moved to unit 8, and new alginate beads placed in unit 6.

August 10-11
Sometime during this period there was a mill shut-down, and both condensate flow and gas supply to the system were stopped.

August 13
The system was discovered shut-down, and filled with very dark condensate indicative of heavy liquor carryover. The system was drained, flow of both gas and condensate re-established, and the alginate beads were placed back into the system.

August 14-15
DO data collection continued.

Results

During the periods of continuous condensate flow, the alginate beads supplemented with $CaCO_3$ did not swell. Changes in beads structure during normal flow of the system seemed to be limited to fracturing and splitting, as well as a gradual loss of bead mass. Bead color did not change appreciably throughout this study. In the previous preliminary field study, when beads broke down, a mass of intact beads imbedded in partially dissolved alginate was formed, which plugged the system. This type of breakdown did not occur with the $CaCO_3$ supplemented beads.

The greatest amount of swelling and loss of bead mass occurred between August 10th and 13th, when the flow was interrupted, and when the beads were immersed for at least two days in condensate containing a large amount of liquor carryover. Partial degradation of the alginate was observed. Reactor unit #4 was used for a control, without any bacteria. DO measurements were taken along its axis over 4" intervals in order to measure the DO drop due to oxidation of condensate components. During this study, with the exception of system upset on the weekend of August 10 - 11, the condensate stream stayed mainly light colored, indicative of minimal liquor carryover. The DO drop along the 20" axis, the distance between inlet and outlet, averaged 2 ppm.

Alginate Beads

Two batches of alginate beads were used in this study, the first being introduced on August 1, and the second introduced on August 8th. For the first batch of beads, on the first day of introduction, the average DO drop was 5 ppm. The next day the DO drop was as high as 14 ppm. However, when readings continued on August 8, the DO drop averaged 1.4 ppm, lower than the DO drop across the control. However, the DO averages for this batch of beads increased to 4 ppm on the 9th, as well as on the 14th the next week. Whatever the cause for the very low level of oxygen consumption on the 8th, the activity in the beads seemed to recover most of their initial level. The second batch of beads, introduced

on the 8th, had greater DO drops than the initial batch. The averages for this batch on the 8th, 9th, 14th, and 15th, were 6.1, 8.8, 7.1, and 8.5 ppm respectively. While there was at least one extended system flow interruption, it did not appear to have permanently damaged the organisms.

The use of $CaCO_3$ to stabilize the alginate beads seems to be successful under continuous flow, low liquor carryover conditions. Most of the loss of bead integrity occurred during the period where the beads were immersed in high liquor content condensate for at least 2 days. The organisms were effective in their ability to drop DO levels in the $CaCO_3$ fortified beads. While the interruption of both gas and condensate flows probably damaged the bacterial populations somewhat, some recovery from the upset occurred. In order to have some control over stream variability, and to reduce the impact of flow interruption, the next set of field experiments run in September/October 1996 utilized a pump and reservoir to control the flow rate through the system.

Summary of the September/October field trial

During the September 31 to October 24, 1996 trial, the biotreatment units (4, 6, 8) in the reactor were filled with fine nylon mesh sacks containing alginate beads. The alginate beads were initially composed of 5% calcium alginate, 5% calcium carbonate, and 10% bacterial biomass by weight. Following the field trial, samples were returned to IPST for methanol analysis. Finally, the beads containing bacteria exposed to the condensate stream were compared to unexposed beads to compare their relative ability to remove oxygen from a standard methanol solution.

In order to increase the stability of the calcium alginate polymer used for the beads, a mixture of two different alginates was used. In addition to the alginate used previously, a different alginate with a higher guluronic acid content was added in a 1:4 ratio. This allowed for a greater amount of calcium crosslinkages within the gel, increasing its overall strength. Previously, condensate flow through the system was controlled by a needle valve in the supply line which allowed for great variability of flow rate, including flow interruptions. Halfway through this study a peristaltic pump was used to draw from overflow tanks to supply the system with a more constant condensate supply to the system.

<u>Sequence of Events:</u>

September 30

The system was set up.

October 3

DO, temperature, and flow data collection was begun. In the afternoon, alginate beads in nylon mesh bags were placed in units 4 and 6.

Table 1: Results from the August 1996 field trial							
date	time	unit #	unit type	DO (ppm)	aerator to top drop (ppm)	temp °C	flow rate mL/min
8/1	12:36 PM	4	empty	10.2		35.6	214
		4	empty	9.9		35.5	214
		6	filled	8.3	-3.6	35.2	214
		6	filled	5.8	-6.0	35.2	214
8/1	2:06 PM	4	empty	8.8	-2.3	36.7	167
8/1	1:59 PM	6	filled	5.0	-6.1	35.1	167
8/1	3:25 PM	4	empty	7.1		36.8	429
8/1	3:23 PM	6	filled	3.2		36.1	429
8/1	3:36 PM	6	filled	3.3		35.5	286
8/1	3:42 PM	4	empty	6.9		36.5	286
8/1	3:52 PM	6	filled	3.7		35.3	207
8/2	8:20 AM	6	filled	0.0		30.9	300
8/2	8:20 AM	4	empty	11.9		31.5	300
8/8	9:35 AM	8	old	11.6	-1.0	34.4	300
8/8	9:35 AM	6	new	6.2	-5.4	34.1	300
8/8	9:42 AM	4	empty	10.2	-2.3	33.6	300
8/8	10:53 AM	8	old	10.5	-2.1	32.6	273
8/8	10:53 AM	6	new	6.5	-4.6	33.4	273
8/8	10:58 AM	4	empty	10.2	-0.9	33.8	273
8/8	11:01 AM	8	old	10.1	-1.3	32.7	273
8/8	11:01 AM	6	new	6.3	-4.8	33.4	273
8/8	11:08 AM	4	empty	9.7	-1.8	33.9	273
8/8	11:12 AM	8	old	10.6	-1.7	32.7	250
8/8	11:12 AM	6	new	6.5	-5.1	33.5	250
8/8	11:19 AM	4	empty	10.1	-2.3	33.9	250
8/8	1:30 PM	8	old	10.7	-2.5	35.1	250
8/8	1:30 PM	6	new	7.3	-5.2	35.6	250
8/8	1:37 PM	4	empty	10.2	-2.1	36.0	250
8/8	1:41 PM	8	old	11.8	0.3	35.2	250
8/8	1:41 PM	6	new	5.3	-5.6	35.7	250
8/8	1:51 PM	4	empty	10.0	-2.1	36.0	250
8/8	1:52 PM	8	old	11.0	-1.6	35.2	231
8/8	1:52 PM	6	new	5.5	-6.5	35.7	231
8/8	2:00 PM	4	empty	9.6	-2.5	36.1	231
8/8	3:06 PM	8	old	13.4	-0.3	34.8	400
8/8	3:06 PM	6	new	6.0	-7.0	35.1	400
8/8	3:10 PM	4	empty	10.8	-1.8	35.5	400
8/8	3:14 AM	8	old	11.4	-1.1	34.9	429
8/8	3:14 AM	6	new	5.1	-7.9	35.1	429
8/8	3:22 PM	4	empty	11.2	-1.3	35.4	429
8/8	3:22 PM	8	old	11.0	-2.3	35.0	231
8/8	3:22 PM	6	new	5.7	-9.0	35.0	231
8/8	3:22 PM	4	empty	12.0	-1.6	35.5	231
8/9	8:06 AM	8	old	10.8	-7.8	29.3	200

Table 1 (cont.): Results from the August 1996 field trial							
8/9	8:06 AM	6	new	5.6	-13.1	29.5	200
8/9	8:06 AM	4	empty	16.7	-0.6	29.6	200
8/9	8:23 AM	8	old	11.5	-3.6	29.5	176
8/9	8:23 AM	6	new	6.0	-12.2	29.5	176
8/9	8:23 AM	4	empty	16.8	-0.3	29.6	176
8/9	9:05 AM	8	old	8.2	-1.2	30.1	600
8/9	9:05 AM	6	new	5.0	-4.8	34.0	600
8/9	9:05 AM	4	empty	9.1	-2.2	37.5	600
8/9	9:23 AM	8	old	6.1	-1.8	32.2	429
8/9	9:23 AM	6	new	2.4	-6.3	35.6	429
8/9	9:23 AM	4	empty	8.6	-2.8	36.8	429
8/9	10:10 AM	8	old	5.0	-4.3	34.5	375
8/9	10:10 AM	6	new	3.1	-8.3	34.5	375
8/9	10:10 AM	4	empty	10.4	-2.3	34.9	375
8/9	10:24 AM	8	old	5.5	-4.1	34.3	300
8/9	10:24 AM	6	new	2.6	-8.1	34.2	300
8/9	10:24 AM	4	empty	10.0	-1.4	35.2	300
8/9	10:37 AM	8	old	6.1	-3.2	34.2	273
8/9	10:37 AM	6	new	2.1	-8.6	34.3	273
8/9	10:37 AM	4	empty	9.9	-1.8	35.4	273
8/9	10:55 AM	8	old	5.0	-5.7	33.8	214
8/9	10:55 AM	6	new	2.2	-8.7	34.5	214
8/9	10:55 AM	4	empty	9.9	-1.7	35.4	214
8/14	8:07 AM	8	old	18.2	-0.7	28.2	176
8/14	8:07 AM	6	new	16.4	-6.0	28.4	176
8/14	8:07 AM	4	empty	16.0	-2.8	28.6	176
8/14	9:50 AM	8	old	7.3	-5.0	33.5	300
8/14	9:50 AM	6	new	8.2	-7.1	35.1	300
8/14	9:50 AM	4	empty	8.2	-2.0	35.0	300
8/14	9:50 AM	8	old	8.1	-4.8	33.8	214
8/14	9:50 AM	6	new	6.0	-9.0	34.6	214
8/14	9:50 AM	4	empty	9.0	-2.3	34.6	214
8/14	11:35 AM	8	old	9.7	-5.1	32.9	214
8/14	11:35 AM	6	new	9.7	-6.5	33.6	214
8/14	11:35 AM	4	empty	11.3	-1.9	34.2	214
8/14	12:55 PM	8	old	10.3	-4.6	33.2	214
8/14	12:55 PM	6	new	9.0	-6.8	33.7	214
8/14	12:55 PM	4	empty	11.6	-1.3	34.1	214
8/14	2:02 PM	8	old	10.5	-5.1	33.6	214
8/14	2:02 PM	6	new	9.3	-7.4	34.0	214
8/14	2:02 PM	4	empty	11.9	-3.7	34.5	214
8/15	11:42 AM	8	pooled	9.4	-1.7	33.4	375
8/15	11:42 AM	6	empty	6.5	-1.7	34.9	375
8/15	11:53 AM	8	pooled	8.6	-2.5	33.9	300
8/15	11:53 AM	6	empty	5.5	-2.0	35.0	300
8/15	2:22 PM	8	pooled	8.6	-2.8	35.0	231

Table 1 (cont.): Results from the August 1996 field trial							
8/15	2:22 PM	6	empty	7.7	-1.6	35.9	231
8/15	2:32 PM	8	pooled	8.7	-3.0	35.2	231
8/15	2:32 PM	6	empty	7.8	-1.1	35.8	231
8/15	12:40 PM	8	pooled	7.2	-3.9	34.4	300
8/15	12:40 PM	6	empty	5.8	-2.2	35.5	300
8/15	12:50 PM	8	pooled	7.4	-3.1	34.7	300
8/15	12:50 PM	6	empty	5.6	-2.0	35.6	300
8/15	7:56 AM	8	old	12.3	-3.7	27.4	150
8/15	7:56 AM	6	new	8.5	-8.5	27.6	150
8/15	7:56 AM	4	empty	13.6	-0.5	27.9	150
8/15	3:23 PM	8	pooled	9.4	-3.0	35.1	231
8/15	3:23 PM	6	empty	8.0	-0.9	35.8	231
8/15	3:33 PM	8	pooled	9.3	-2.3	35.3	250
8/15	3:33 PM	6	empty	8.0	-0.8	35.7	250

October 4
Excess beads were removed from unit 4 and 6 due to swelling,

October 5
Some of the beads removed on the previous day were replaced in units 4 and 6.

October 7
Alginate beads from unit 6 were moved to unit 8 and a nutrient feed was started for that unit only. Beads from 4 were moved to unit 6, and beads removed as excess on October 4 were placed into unit 4.

October 9
Due to mill evaporator shut-down, the beads were removed from system and placed in refrigerated storage.

October 13
Liquid and gas flows to the system were re-established, and beads were placed back into bioreactor units 4, 6, and 8. The system operation continued uninterrupted until October 23.

October 14
Flow control by a pumped supply from overflow feed tanks was established.

October 16
Nutrient feed to bioreactor unit 8 was removed.

October 23
The system was shut down.

Condensate flow rates

Flow was interrupted and variable during the early part of the study, until a pumped reservoir supply was established on October 14. Instead of flowing directly from the needle valve control to the bioreactor, the condensate was diverted to a pair of 10 L carboys from which a stream was pumped. The overall flow into the carboys was initially set at a rate about twice the flow rate through the pump. When the supply flow rate dropped, there was still enough flow to continue to feed the pump. When the supply flow rate dropped below that of the pump rate, the 20 L reservoir was drawn from. Before installation of this pumped reservoir system, the average flow rate was 302 mL/min with a standard deviation of 128 mL/min. After its installation it was 224 mL/min with a standard deviation of 21 mL/min.

Temperature variations

The overall average condensate stream temperature throughout the system was 30°C with a standard deviation of 5°C. The efficiency of the heat exchanger used to cool the condensate stream was affected by both the ambient air temperature as well as the stream flow rate. With controlled flow rates, the greatest variation in temperature was due to the ambient air temperature, with the lowest values recorded in the morning. The temperaure rose by an average of 1.09°C/hr between 8:00 AM and 5:00 PM. Also, the temperature varied across the system with an average inlet to outlet drop of 0.9°C.

Temperature variations had two effects upon the ability of the bacteria to remove oxygen. First, at lower temperatures the solubility of oxygen increases, resulting in higher initial DO levels. As the temperature increased through the day, the oxygen available to the organisms decreased. Dropping the available DO while maintaining the same outlet DO value made it appear that the efficiency decreased throughout the day. This effect was most noticeable when the biological activity was at its maximum, with outlets at or below 1 ppm.

Variation in chemical composition of the condensate stream

While direct measurements of the chemical composition of the condensate stream in the field were not made, its variability was readily detected. The variability in the chemical oxygen demand of the condensate stream was monitored by the DO measurements in system units 1 and 2. Unit 1 is an oxygenation unit like all other odd numbered units, while unit 2 is an empty unit. At all times when DO data was collected, DO values were taken at the bottom of unit 1, near its exit, and in 4 inch intervals along the 20 inch axis of unit 2. Under conditions of no carryover, the DO reading at the bottom of unit 2 was very close to that at the bottom of 1, and the readings along the axis of 2 were relatively constant. Under conditions of moderate carryover, the DO reading at the bottom of unit 2 were significantly lower than that at the bottom of 1, and there was a continuous drop along the unit 2 axis. Under high carryover conditions, very little dissolved oxygen reached unit 2, and, with all oxygen already consumed, the DO profile along its axis remained constantly below 1 mg/L. Although carryover in the condensate stream consumed oxygen, most of its chemical oxygen demand was met in the first, control biotreatment system. The oxygen consuming effect was

reduced further downstream, and the resulting interference with determining relative biological activity was minimal by the time the condensate stream reached unit 8.

In addition to consuming oxygen, carryover components are probably the chief cause for destruction of both viable bacteria and gel integrity. Biological activity drops outside of an ideal pH range, and caustic carryover from pulping liquors into the unbuffered condensate stream increases its pH well over its normal level of about 8. Alginate is a polysaccharide subject to the same types of degradation reactions from pulping liquors as cellulose. High concentrations of reduced sulfur compounds in carryover are also toxic to our bacteria, and were implicated in the death of encapsulated bacteria in the laboratory study described earlier.

Short-circuiting of the bead bed

The alginate beads used for this field trail were always contained inside the bioreactor units with fine nylon mesh bags, which gave rise to the possibility of short-circuiting. Generally, these barriers were either due to bag size effects or blockage of the nylon mesh. The nylon mesh bags used at the beginning of the study were often cut too long. Later, larger diameter nylon bags of shorter lengths were used, in order to minimize the bypass effect.

As the study progressed, the nylon mesh began to blind, limiting the flow of condensate through the bead mass. These materials were probably a combination of bacterial growth attached to the mesh and alginate breakdown products. The greatest accumulation of solids occurred on the bag containing beads exposed to the nutrient feed stream. This suggests that the fouling was more attributable to bacterial growth. Additionally, biofilms also accumulated on the inside surfaces of all tubes, especially oxygenation units 5 and 7. When the nutrient feed to bioreactor unit 8 was discontinued, the rate at which material accumulation occurred on that unit's nylon mesh bag seemed to decrease. The nylon mesh bags were replaced when fouling was suspected.

DO drop calculations

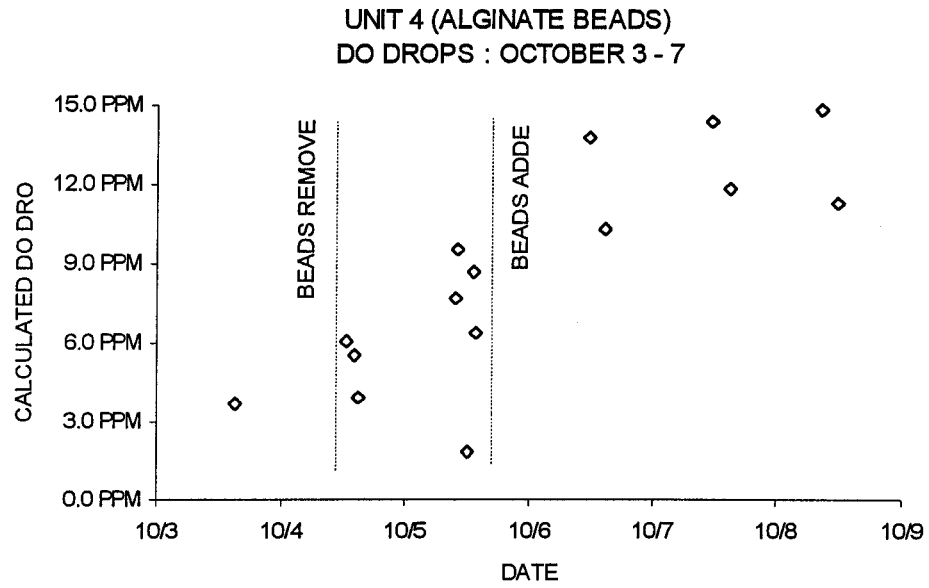
DO measurements were taken at the bottom of the oxygenation unit supplying the beads, and in the liquid over the bead beds. The DO drop across the control bioreactor was attributed to the easily oxidizable compounds in the stream. This effect was strongest upstream, diminishing greatly by the final biotreatment pair. From the control data, a value for this DO drop between oxygenator and bioreactor for each treatment pair was calculated, and was subtracted from the apparent DO drop, to produce calculated DO drops, or ΔDO_{CALC}.

Units 4 and 6 with alginate beads, October 3-7

ΔDO_{CALC} values across the beads were fairly small on October 3, the day beads were installed into units 4 and 6. A ΔDO_{CALC} of just under 3 ppm was recorded for unit 4, and about 1 ppm for unit 6. On the morning of October 4, some of the beads were removed from both of these units due to overnight swelling. The ΔDO_{CALC} after the beads had been

replaced and given time to adjust had risen slightly to about 6 ppm for unit 4, and over 2 ppm for unit 6. On October 5, the ΔDO_{CALC} values for unit 4 had risen, ranging between 6 and 9 ppm, while that for unit 6 ranged from 3 to 7 ppm.

Additional pre-exposed beads were placed back into units 4 and 6 at the end of October 5. The next day there was a significant jump in ΔDO_{CALC} values, with both units reaching up to a drop of 14 ppm, with exiting DO values under 2 ppm. On October 7, unit 4 continued to perform at the previous level, but the ΔDO_{CALC} of unit 6 dropped back to less than 6 ppm.

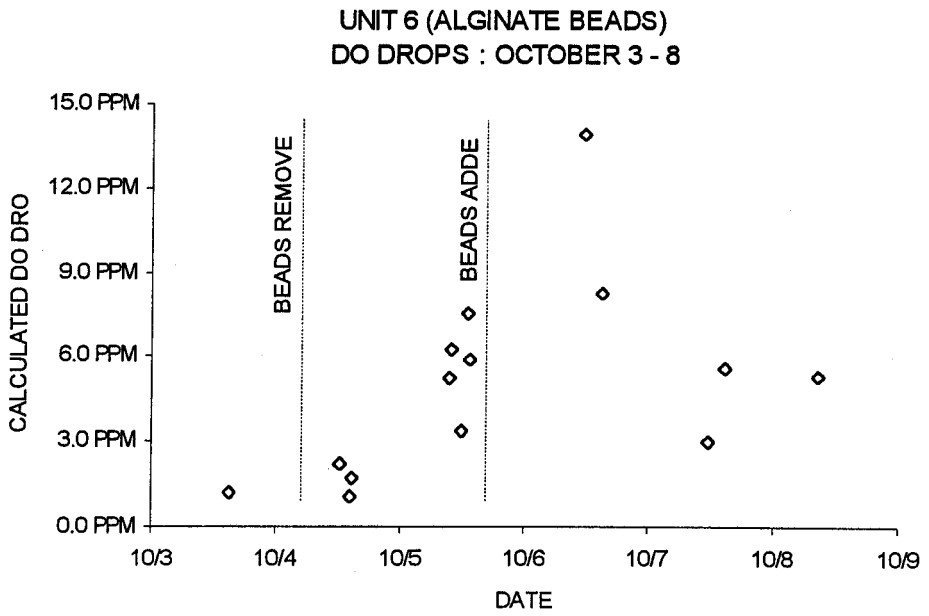


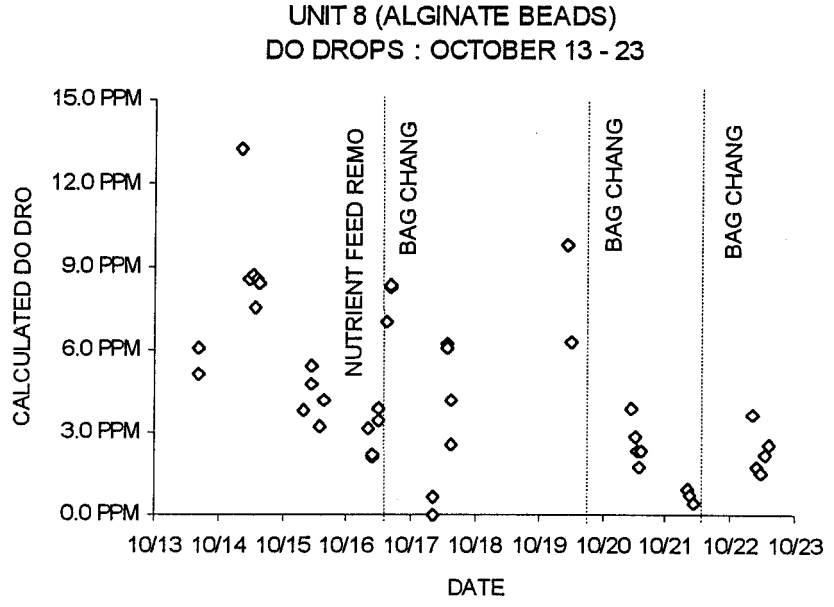
Unit 8 Beads with Nutrient Feed, October 8-16

On October 7, beads from units 4 and 6 were moved downstream to units 6 and 8, with previously exposed beads placed into unit 4. On October 8, a nutrient feed containing 10 g/L $CaCl_2 \cdot 2H_2O$, 2.5 g/L MgCl2 $\cdot$ $6H_2O$, and 10 g/L KNO_3 was turned on at a rate of 1.8 mL/min. The condensate flow rate during the nutrient feed period ranged from 200 mL/min to 333 mL/min, resulting in a dilution rate ranging from 111:1 to 167:1.

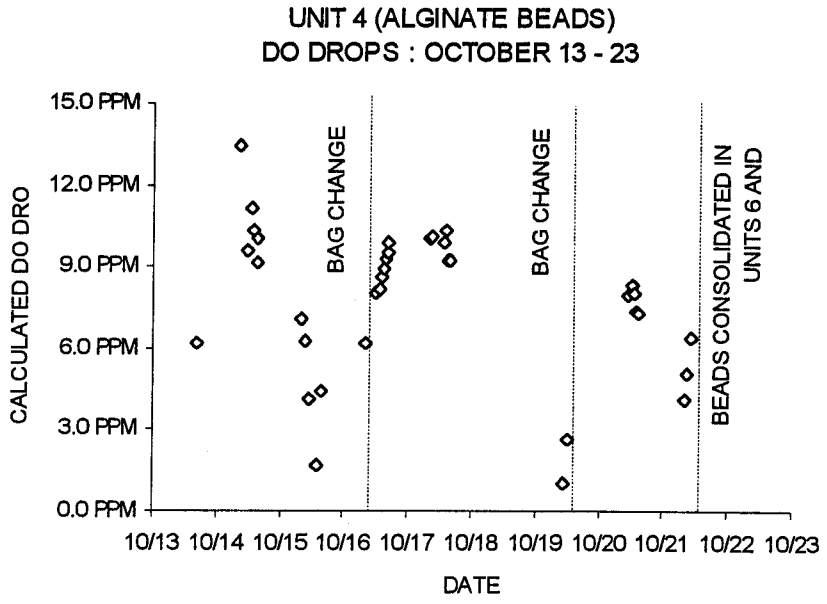
The ΔDO_{CALC} over the beads before were relocated to unit 8 had been about 5.5 ppm. After relocation, but before nutrient addition, the ΔDO_{CALC} dropped below 4 ppm. On October 9, the system was shut down due to evaporator maintenance, and beads from all units placed into refrigeration.

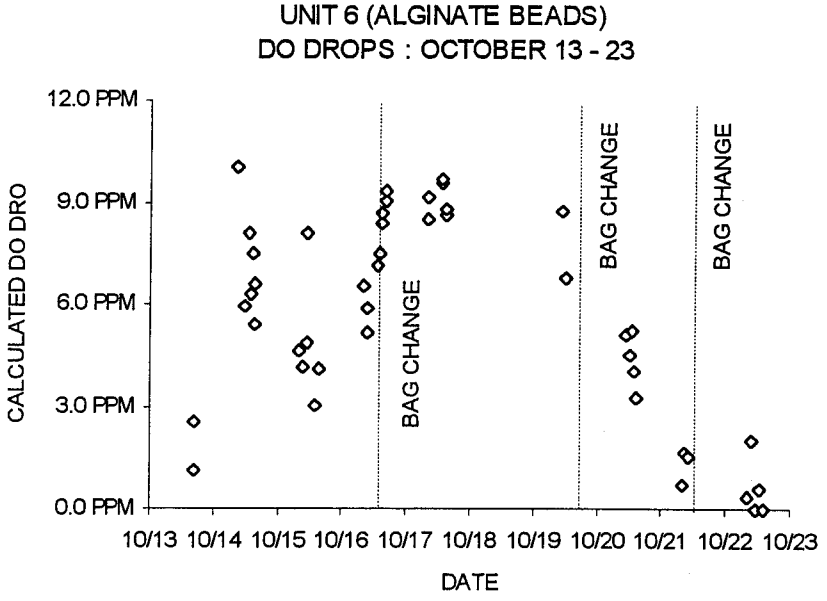
On October 13, beads were returned to all units, and the nutrient feed to unit 8 was re-established. ΔDO_{CALC} for the beads in unit 8 on that day ranged from 5 to 6 ppm. The initial value the next day gave a ΔDO_{CALC} of 13 ppm, the feed DO value being 20 ppm, the exit of 5 ppm, and a calculated drop across the units of 2 ppm. However, this level was not





sustained. The ΔDO_{CALC} values dropped to about 8.5 ppm for the rest of October 14. On the 15th and 16th, ΔDO_{CALC} values continued to drop, eventually reaching a level of only 2 ppm. At this point the nutrient flow was discontinued, and the beads were removed for inspection. The nylon mesh bag had been mostly blocked at this point, restricting the flow of condensate through the beads. The amount of material blocking the mesh was much greater than similar material found on upstream mesh bags.





Units 4 and 6, October 8-15

On October 8, unit 4 continued operating at the previous day's level with its ΔDO_{CALC} values between 11 and 14 ppm. Unit 6 also maintained its previous ΔDO_{CALC} rate of about 5 ppm. As mentioned earlier, the system was shut down between October 9 and 13. When the system was restarted on October 13, the initial ΔDO_{CALC} values were relatively low for both units, a 6 ppm calculated drop for unit 4, and 2 ppm for unit 6. However, the efficiencies of both units improved the next day, with unit 4 starting out at a better than 13 ppm

ΔDO_{CALC}, and 10 ppm for unit 6. These levels could not be sustained, however. Both units exhibited a steady drop in efficiency through the 15th. At the end of the day on Oct 15, the beads were removed, their nylon mesh bags were replaced, and beads returned to their units.

Units 4, 6, and 8, October 16-22

For the remainder of the study the ability of the beads to remove oxygen from the condensate stream decreased. During this period the nylon mesh bags were replaced on the 19th and 21st. These changes usually resulted in a temporary increase in the ability to remove oxygen, but never to prior efficiencies. On October 21, when bags were replaced, because the mass of beads in the system had dropped, they were consolidated into only 2 bags, which were placed into units 6 and 8. On the 22nd, the ΔDO_{CALC} for unit 6 was less than 1 ppm, and unit 8 averaged 2 ppm. On October 23 with the gas supply exhausted, the remaining beads were removed from the system and refrigerated, and the system was disassembled.

Diatomaceous earth support

Diatomaceous earth has frequently been used to support microorganisms (12,13), since it is porous and of high surface area. In a brief experiment, diatomaceous earth was placed in unit 8, so that any organisms washed out of the beads would colonize the earth. While colonization was successful, it plugged the flow, and the experiments were discontinued.

Methanol determination

Condensate samples were collected for methanol content analysis by GC. These samples were collected five times over a two-day period at 6 different locations throughout the system. The average methanol concentration in the condensate samples was 320 ± 10 ppm.

Viability of the residual beads

The beads removed from the system at the end of the field trial were compared to beads from the same production batch that had never been exposed to condensate in their ability to remove oxygen from a standard methanol solution. The beads were placed in a BOD bottle along with saline and methanol such that the final solution contained 500 ppm of methanol. A DO probe was inserted, and DO readings were recorded periodically, which provided a maximum rate of oxygen consumption. Because the beads exposed to condensate still had residues which consumed oxygen, the oxygen consumption rates for beads with and without methanol were collected, with the difference being attributable to biological oxygen consumption. Differences in the relative biological oxygen consumption rates between exposed and unexposed beads was less than 10%, confirming the viability of the organisms.

Conclusions

The total exposure time of the beads to condensate stream for this field trial was 16 days. The ΔDO_{CALC} over the alginate beads over this period varied greatly, with changes over a bead bed varying as much as 7 ppm within a single day. Replicates within a very short time period often varied as much as 4 ppm. However, alginate beads were often able to reduce DO levels to 1 ppm or less. If a ΔDO_{CALC} of >6 ppm be considered as good, ΔDO_{CALC} of 3-5.9 ppm be called fair, and $\Delta DO_{CALC} < 2.9$ be poor, then good values were seen up through the 13th day.

The measured and calculated losses of the system's ability to remove oxygen from the condensate stream can be thought of in terms of temporary and permanent effects. Temporary effects are due to stream bypass of beads, either from incorrect bag dimensions or fouling of the nylon mesh. Permanent effects were loss of bead mass and cell viability. Both of these were attributable to aggressive components in carryover compounds in the condensate stream. Nutrient addition to the condensate stream probably had more effect upon the growth of bacteria outside of the beads than within the beads. In terms of fouling, this had a negative affect. Additionally, microbial growth within a polymer gel can result in accelerated physical breakup of that gel, another negative effect.

The use of an alginate formulation which combined of calcium carbonate, and two different alginates, based upon relative amounts of glucoronic acid content, provided beads that lasted throughout the trial period. While bead mass did decrease throughout the 16 day period, mass losses did not restrict flow within the bead bed as was observed in earlier studies without $CaCO_3$.

DISCUSSION

The study was a qualified success. We showed that *Methylosinus trichosporium* OB3b encapsulated in calcium alginate beads (supplemented with powdered $CaCO_3$) was able to degrade methanol in evaporator condensate in a field setting, without the addition of supplemental nutrients. Thus, the technical feasibility of the process is established. However, components in carry-over can be toxic to the organisms, and means to remove them from the feed is essential. It is likely that H_2S is the primary toxicant, and it should be removable during brief contact with peroxide. At ambient temperature and in the presence of 100 ppm of H_2O_2, the half live for H_2S removal is 20 minutes at pH 2-7 (14). Short-circuiting is another problem, although this can be engineered around. Our results understate the efficiency of the organisms, since only a fraction of the condensate probably had prolonged contact with the beads. Hence for this system to be viable, a sulfide removal system, and more efficient mixing between the beads and condensate is necessary. The potential advantage of the system is its high efficiency, which translates to a small footprint, and in turn, to relatively low construction costs. However, the beads will need to be replaced periodically, and this will lead to additional cost. Also, oxygen will be needed to provide a sufficiently high DO level. Some chilling of the feed stream will also be required.

From a practical viewpoint, we have three options: hardpiping, steam stripping, and biological treatment. Hardpiping is the least expensive, but the condensate water cannot be recovered, and an additional BOD load will be placed on the treatment system. Steam stripping is expensive. A Paques anaerobic system has been piloted at Boise Cascade (15) with apparent success, although details are proprietary. NCASI is presently running a bioreactor trial at Georgia-Pacific's Leaf River facility. Hence, hardpiping and the anaerobic system (if taken at face value) would appear to be the most viable current options. Our technology is technically feasible but needs more extensive piloting to establish costs, and will not be ready near term.

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