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Constraints of Bio-augmentation in Improving Performances of Biological Treatment Process

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미생물 활성 촉진에 의한 생물학적 처리효율 평가에 있어서의 제한성에 관한 연구

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Abstract

생물학적 처리공정내 미생물은 물리화학적, 생태학적으로 처리공정의 운전조건과 유입하·폐수 특성에 따라 존재량 및 반응조내 존재하는 우점종이 달라진다. 특히, 폐수의 생물학적 처리시 유입수내 존재하는 독성 및 저해물질은 생물반응조내 존재하는 미생물의 양을 감소시키거나 미생물의 종류를 단순화시키거나 환경조건 및 폐수 특성변화에 능동적으로 대처할 수 있는 능력을 감소시킨다. 따라서 이러한 문제점을 극복하기 위한 방안으로 생물능력향상(bio-augmentation)이 필요하며, 여러 가지 방법으로 생물능력향상을 이룰 수 있다. 본 연구에서는 생물능력향상을 위하여 기존의 효소활성이 강한 미생물균을 투입함으로써 처리효율향상에 미치는 영향 및 제한성을 고찰하였다. 처리효율 향상을 위해서는 생존성, 폐수에 대한 순응기간 및 미생물의 체류가 매우 중요하였으며, 특히 투입된 미생물균이 처리효율을 향상시키기 위해서는 일정기간 이상의 순응기간이 필요하였다. 한편, 토착미생물균인 UB 1의 경우 기존 생물능력향상 미생물균과 거의 동일한 처리효율을 나타내고 있으며, 사전에 순응기간을 거친 UB 2의 경우도 기존 생물능력향상 미생물균과 비슷한 처리효율을 보였다. 따라서 본 결과로 볼 때 생물능력향상 미생물균 투입에 의한 처리효율의 향상은 크지 않으며, 하·폐수의 생물학적 처리공정에 적용하는데는 다소 제한성이 있는 것으로 판단되었다.

I. INTRODUCTION

In an operating bio-reactor used for waste treatment, an indigenous bacterial population arises which is unique from the standpoint of species diversity. This population parameters. In bio-reactors treating a variable mixture of toxic or inhibitory wastes, bacterial diversity is reduced by the increased selective pressure on the population due to the presence of selected waste components. Reducing the types of bacteria present, can diminish the genetic pool of the reactor population, and decrease the ability of the population to respond to changes in the environment

Table 1. Compilation of current supplementation reports

Test Setting	Waste Type	Bio-augmentation Effective		Reference
		Yes	No	
Field	Pharmaceutical	x		(1)
Field	Petrochemical	x		(2)
Field	Refinery	x		(3)
Field	Refinery	x		(4)
Field	Municipal	x		(5)
Field	Dairy	x		(6)
Laboratory	Dairy		x	(7)
Laboratory	Hazardous		x	(8)
Laboratory	Synthetic		x	(9)
Laboratory	Chlorinated Organics		x	(10)

and/or waste composition[1]. Because of the diminished capacity for adaptation, biological hazardous waste treatment processes are often plagued by upsets and are unable to degrade new compounds entering the waste stream. Bio augmentation is the process of adding non indigenous bacterial supplements to a bio-reactor for the purpose of artificially increasing the bacterial diversity and/or activity of the reactor population. Bio augmentation may increase the biological diversity and activity of the population by: 1)adding bacteria with enzymatic systems which allow degradation of previously non biodegradable organics; or 2)adding bacteria which have higher metabolic rates. Many bacterial supplements are available commercially to augment existing populations of biological hazardous waste treatment processes.

To date, no clear consensus has been reached on the relative merits of bio-augmentation. The purpose of this paper is to discuss and illustrate, with data from experimental studies, some of the constraints bio augmentation in improving biological treatment performance. Numerous presentations, reports, and articles have been delivered and published on the subject of bio augmentation. The reported advantages of bio augmentation are mixed. The merits of bio-augmentation have been promoted most heavily in trade literature and conference proceedings. Relatively few articles on bio augmentation have been published in refereed journals. Generally, those that have been published in the refereed literature, are not supportive of the bio-augmentation concept.

A compilation of findings from published papers on bio augmentation are presented in Table 1. For each paper referenced, the location of the testing, waste type, whether or not treatment performance was enhanced by bio augmentation, and reference are given. Based on the information presented in Table1, enhanced biological treatment performance has been attributed to bio augmentation for those studies conducted under field conditions, while those studies conducted in the laboratory have been generally negative.

In summary, published results from recent research dealing with the effectiveness of bacterial supplementation have been inconclusive. However, on the basis of literature review, a number of criteria which may impact on the potential

success of bio augmentation were identified. These criteria are: 1) the viability of the bacterial supplements should remain high when added to the indigenous reactor population; 2) once supplements are added they should initiate biodegradation of the target compound rapidly, or degrade compounds not degraded by the indigenous population; and 3) added supplements should have the necessary characteristics to maintain their population in the reactor.

II. Materials and Methods

Laboratory studies were undertaken to investigate the factors found important for supplementation success. The approach and methods used for these experiments are delineated briefly in this section.

1. Study Approach

For bio augmentation to be an acceptable option in efforts increase biological treatment performance, bacterial supplements added must remain viable after addition to indigenous population. In this study, a mass balance approach was employed to determine the survival rate of selected supplements after they were added to reactors containing indigenous populations. Enumeration of supplement viability was performed from plate counts which employed strain specific nutrient media. Results from these tests were verified using cellular Adenosine Triphosphate (ATP) analyses and COD removal results.

Tests were conducted to determine the degradation capacity of bacterial supplements. A spread plate growth test procedure was used to analyze and screen all supplements studied. Selected supplements identified as promising from the preliminary growth tests were further rested using batch shaker flask COD removal tests. On the basis of the spread plate screening and the batch shaker flask COD removal tests, further study of supplement acclimation to waste constituents and environmental conditions was warranted. Two conditions were studied: adaptation to waste constituents and temperature.

The culture retention characteristics of supplements were investigated during the study. Retention characteristics such as growth rate, floc size, and numbers of dispersed growth in supplement reactor effluents were quantified.

Table 2. Waste characterization for blended TSDF wastewater used during the experimental studies

Parameter	Concentration[a]
COD	5,100
TDS	12,000
TSS	345
VSS	70
phenol	150
TOX	77.7 as Cl
pH	7.4 pH units
Cu	6.0
Ni	4.1
Cr	0.4
Zn	0.2
Fe	16.0
total bacteria	$4.1*10^7$/㎖
viable bacteria	$2.5*10^7$ CFUs/㎖

[a]All concentrations are in ㎎/ℓ unless otherwise noted.

2. Study Methodology

The purpose of this section is to describe: 1) the nature of the waste studied; 2)the bacterial supplements investigated; and 3) the experimental protocol for the various tests.

2.1 Nature of Waste.

Wastewater for this study was a blend of individual waste streams obtained from a hazar-

Table 3. List of bacterial supplements investigated

Target Compounds Waste	Level
Heavy Metal	A-1
Phenol	B-2, C-2
Chlorinated Organics	B-3, C-3, F-1, F-2, F-3
Fats and Proteins	A-3
Hazardous Wastes	D-2
Industrial Wastes	A-2, D-1
Food Wastes	C-1
Septage	C-4
General Wastes	E-1
Cold Weather	B-1
Study Waste	UB-1
Soluble Starch	UB-2

dous waste treatment, storage, and disposal facility(TSDF). An analysis of the blended wastewater is presented in Table 2. The waste was characterized by moderately high chemical oxygen demand(COD) concentrations(5,400㎎/ℓ), high total dissolved solids(TDS) concentrations (42,000㎎/ℓ), and nearly neutral pH. The phenol concentration of the waste was 150㎎/ℓ. Total organic halogen(TOX) concentration for the waste was 77.7㎎/ℓ as Cl. Metals concentrations in filtered waste(GF/C filters) ranged from 0.2㎎/ℓ for Zn to 16㎎/ℓ for Fe. The blended wastewater had a viable bacteria count of 2.5×10^{7} colony forming units (CFU)/㎖.

2.2 Bacterial Supplements Study

Based on the waste characteristics, a total of 16 bacterial supplements were recommended by various manufacturers as being able to improve degradation. Although the stated applications of the supplements(Table 3.) did not always agree with the nature of the waste, the manufacturers maintained that the supplements would be able to increase the biological activity of the reactor treating the study waste. For confidentiality purposes, the commercial sources are not identified by company name.

In addition to the supplements recommended by various manufacturers, two non-commercial supplements were used for comparative purposes. Non-commercial sources included: activated sludge from a batch reactor which had been treating the TSDF wastewater for approximately 6 months, and activated sludge from a parallel batch reactor treating a readily degradable starch synthetic wastewater. These supplements are identified as UB-1 and UB-2, respectively.

2.3 Experimental Protocol.

During the course of the laboratory experiments, various commercial and non-commercial supplements were used to inoculate solid or liquid media. The reconstitution procedures used for the various commercial supplements studied were performed in accordance with manufacturers' recommendation. Before use as inoculums for the solid or liquid media, all supplements studied were washed in a centrifuge at 5,000 rpm for 3 minutes and resuspended in a phosphate buffer solution. This procedure was performed three to

prevent carryover of auxiliary substrates.

"Acclimated populations" of commercial supplements were obtained from 1 liter aerobic, mixed, batch reactors operated with 10 day HRT which modeled the conditions used to develop UB 1 and UB 2. The study waste was fed to the reactors daily for a minimum 30 days prior to withdrawal of inoculums. To minimize potential cross contamination between reactors, all reactors were sealed. During the study, no wasting from the reactors was conducted, other than incidental solids loss in the effluent and by sampling. Average MLVSS concentrations in the range of 3,000 to 4,000㎎/ℓ were typical for the batch reactors.

With the exception of the *Pseudomonad* enumeration, the solid Agar growth media used during the investigations was nutritionally balanced with the study waste serving as the sole carbon and energy source. *Pseudomonads* were enumerated using selective media plate counts. Plates were incubated at room temperature which averaged 22℃.

The batch shaker flask COD removal studies were performed in 250㎖ Erlenmeyer flasks. At start up, 100㎖ of blended waste, 3㎖ of phosphate buffer, 0.3㎖ of 10㎎ NH4 N/㎖ solution, and 50㎖ of prewashed bacterial seed were placed in each flask. To keep the activated sludge in suspension, the flasks were rotated at 200 rpm throughout the test. Sterile cotton stoppers were used to minimize cross contamination between flasks containing the individual study supplements.

To determine the ATP level of bacterial supplements when exposed to the study waste, the cells were washed three times, diluted to 75 ㎖ and mixed with 25㎖ of the study waste. The mixture was oscillated at 200rpm and 2㎖ aliquots were removed periodically for ATP analysis. Cellular ATP content was assessed as per Standard Methods 1002 H, No.6(Adenosine Triphosphate Method of determination of biomass[11].

Oxygen uptake rate was determined using the Warburg constant volume manometric technique. The TSDF blended wastewater was used as the substrate during the oxygen uptake rate studies and comprised 20%, by volume, of the reaction vessel contents. Bacterial inoculums for the tests, obtained from the 1ℓ batch reactors, and freshly reconstituted commercial strains were diluted to a VSS concentration of approximately 1,000㎎/ℓ and made up the remaining volume. To prevent crossover of auxiliary substrates, the inoculums employed for the oxygen uptake rate test were centrifuged at 5,000 rpm for 3 minutes, and resuspended in phosphate buffer. Each inoculum was washed three times.

Bacterial supplement growth rates were determined in batch shaker flasks oscillated at 200rpm at 25℃. Growth was quantified using optical density measured at a wavelength of 540nm. Total and volatile suspended solids, COD, phenol, TOX, pH, and metals measured during the experimental investigation were performed in accordance to procedures outlined in Standard Methods[11].

Ⅲ. EXPERIMENTAL RESULTS

Presented in this section are the results from the laboratory studies conducted during the investigation.

1. Culture Viability

Any culture chosen for bio augmentation must contain a stable population. In this section an example of viability loss and its consequences on degradation performance are illustrated. The viability of three bacterial supplements(*Pseudomonad* bacteria, F 1, F 2, and F-3) was in-

Table 4. Viability of cultures F-1, F-2, and F-3 after addition to indigenous bacterial population

Supplement	CFUs added to reactor	CFUs remaining after two hours.	Percent viable after two Hours
F-1	$7.0*10^{10}$	$2.4*10^{7}$	0.03
F-2	$1.9*10^{10}$	$1.0*10^{8}$	0.50
F-3	$6.5*10^{10}$	$3.6*10^{7}$	0.06

Table 5. Average effluent COD concentrations achieved with supplements F-1, F-2, and F-3 during bio augmentation

Supplement added	Effluent COD(㎎/ℓ)	COD removal(%)
UB-1	1,128	77.4
UB-1	1,120	77.6
F-1	1,068	78.7
F-1	1,059	78.8
F-2	1,185	76.3
F-2	1,142	77.1
F-3	1,138	77.2
F-3	1,173	76.5

vestigated using a mass balance approach. As reported in Table 4, supplements F-1, F-2 and F-3 were developed for enhanced degradation of TOX. The supplements were enumerated immediately upon and two hours after addition to a batch bio reactor containing an indigenous population treating the study waste. To be conservative, the indigenous population was assumed to contain no *Pseudomonads* at the time of addition. The number of viable *Pseudomonads* per unit volume of reactor when added and two hours after addition are presented in Table 5. Two hours after addition to the bio-reactor, less than 0.5% of the added supplemental bacteria were found to be viable.

To confirm the results of the species enumeration technique employed, ATP analyses were used to determine the viability of F-1, F-2, and F-3 when exposed to the waste. ATP is an intermediate energy storage and transfer molecule found in all living cells. Upon cell death, ATP is rapidly lost, making it a suitable parameter for measuring cell viability. As shown in Figure 1, a rapid loss in the cellular ATP pool was observed over the first hour after supplement was exposed to the study waste diluted to 25%. The rapid loss of cellular ATP during the first hour, and the low levels reached by two hours after addition support the viability results obtained using plate counts.

The osmotic stress caused by the high dissolved solids content(42g/ℓ) of the study waste was hypothesized as causing the rapid die off of supplemental bacteria. Consequently, no improvement of effluent quality would be expected from addition of these bacteria to the indigenous population. To further confirm this hypothesis, bacterial supplements F-1, F-2, and F-3 were added daily to a batch reactor containing an indigenous population for approximately 30 days. During that time, effluent COD for parallel control and supplemented reactor was measured and is

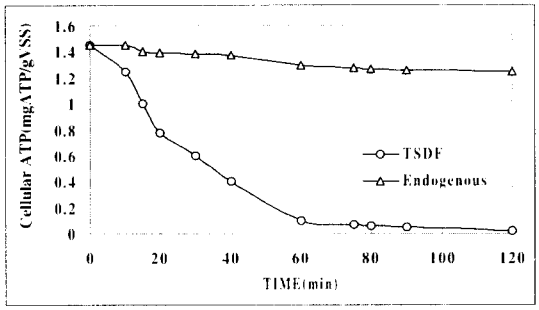

Fig. 1. Cellular ATP level of supplement F-1 after addition to waste.

presented in Table 5. No significant difference between the average effluent COD values was achieved by bio augmentation using the *Pseudomonads* strains F 1, F 2, and F 3.

2. Increased Biodegradation Potential

A variety of tests were used to assess the degradation potential of the bacterial supplements. To establish some baseline for comparison, two supplements developed. Supplement UB 1 evolved during approximately 6 months of daily exposure to the study waste constituents, and thus served as an upper limit control for degradation potential of indigenous populations. Supplement UB 2 also evolved over approximately 6 months but was fed a soluble starch synthetic waste with no prior history of exposure to the constituents of the study waste. The initial inoculums for both UB-1 and UB 2 were from the Amherst, New York wastewater treatment facility with receives very little wastewater of industrial origin.

2.1 Spread Plate Screening Procedure.

A spread plate culture procedure was used to assess the degradation potential of various supplemental bacteria as compared to the two indigenous UB supplements. Following inoculation, plates were incubated at room temperature of 22℃ and growth was quantified daily by visual inspection. The results of the screening procedure are presented in Table 6. Only one group of bacterial supplements(B 1, B 2 and B 3) exhibited equal or better degradation capacity than the UB 1 culture. Supplements A 1, A 2, A 3, D 1 and D 2 were similar in ability to the culture UB 2 which as previously stated had no prior exposure to the waste constituents. Supplements C 1, C 2, C 3, C 4, E 1, F 1, F 2 and F 3 showed less ability to degrade the study waste than UB 2. Based on these results, one would hypothesize that few of the recommended supplements would perform better than the indigenous population, and further, a significant percentage of the supplements would require some acclimation period to the waste before initiating degradation.

2.2 Liquid Culture Tests

Simple batch shaker flask COD removal studies were conducted to further examine the degradation properties of the bacterial supplements. As before, supplements UB-1, and UB 2 served as controls. Representative COD removal versus time plots are presented in Figures 2 and 3 for supplements B 2 and C 1. The MLVSS of each reactor was adjusted to approximately 1,000㎎/ℓ at the initiation of the test. As shown in Figures 2 and 3, COD removal by culture B 2 closely parallels that of culture UB 1 while removal by culture C 1 closely follows that of UB 2.

The results of the batch tests confirm the preliminary results of the spread plate screening test and lend further credence to the fact that some of the supplemental supplements suggested by manufacturers require a period of acclimation to the waste to be most effective.

2.3 Oxygen Uptake Tests.

To assess the effect of supplement acclimation,

Table 6. Relative growth of bacterial supplements on solid nutrient media containing TSDF waste as sole carbon and energy source

Supplement	Incubation Time,(days)						
	1	2	3	4	5	6	7
UB 1	+	+++	++++	++++	++++		+++++
UB 2	-	+	+++	+++	+++		+++
A 1		+	+++	+++	++++		++++
A 2	+	+	+++	++++	++++		++++
A 3	-	+	++	+++	+++		+++
B 1	+	++	++++	++++	+++++		+++++
B 2	+	+++	++++	+++++	+++++		+++++
B 3	+	++	+++	++++	++++		+++++
C 1							+
C 2				-	-		+
C 3	+		-				+
C 4	+	+	+	+	+		++
D 1	-	+	+++	+++		++++	++++
D 2	+	++	+++	+++		++++	++++
E 1	-			++	+++		+++
F 1							+
F 2	-			-	-	+	+
F 3			-			-	+

Key to growth indication

Indicator	Description
	no visible growth.
+	very sparse growth.
++	dispersed colonies or very light plaque.
+++	greater than 10 CFUs/㎠ or moderate plaque.
++++	greater than 10 CFUs/㎠ or heavy plaque.
+++++	many colonies or very heavy plaque formation.

both to the substrate and temperature, on the rate of study waste degradation, oxygen uptake rate studies were performed. A pair of typical oxygen uptake curves obtained during this study for a consortium of the B supplements identified as B * are presented in Figure 4. The waste acclimated culture demonstrated a significantly higher oxygen uptake rate(slope of the curve) than the freshly reconstituted culture at 25℃. A summary of the oxygen uptake rates for addi tional waste acclimated and unacclimated supple- ment consortiums are presented in Table 7. In every case, the acclimated consortiums had an increased oxygen uptake rate, indicating an increased rate of biodegradation.

Because under normal bio augmentation pro cedures, non waste acclimated supplements will be added to an existing indigenous population, it

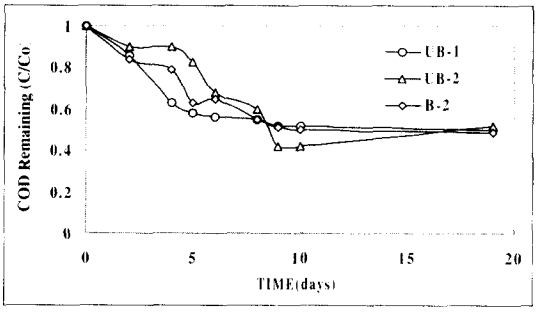

Fig. 2. COD removal by B-2 as compared to UB-1 and UB-2 in batch flask test.

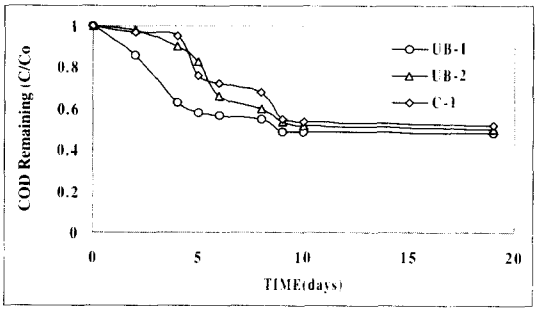

Fig. 3. COD removal by C-1 as compared to UB-1 and UB-2 in batch flask test.

is also instructive to compare the oxygen uptake rates of the non-acclimated consortiums and indigenous population represented by UB-1. In all cases, but especially for the A-* consortium, the indigenous population exhibited a higher oxygen uptake rate. The effect of temperature acclimation on supplement B-1, specifically intended to aid biodegradation under reduced temperature was assessed using oxygen uptake rate data. One sample of B-1 was reconstituted at 2

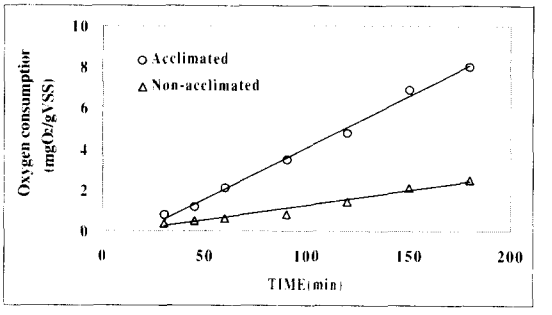

Fig. 4. Comparison of oxygen consumption curves for study waste acclimated and non-acclimated B-# supplement consortium at 25℃.

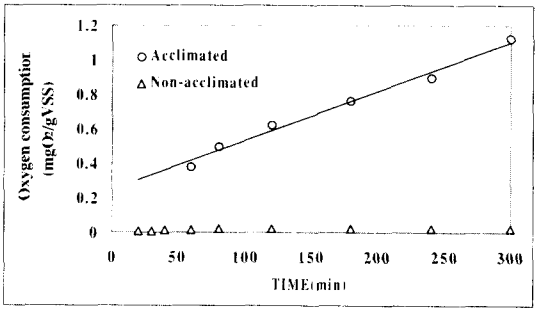

Fig. 5. Comparison of oxygen consumption curves at 10℃ for temperature acclimated and non-temperature acclimated B-1 supplements with no previous exposure to the study waste.

4℃ and reduced in temperature by 2℃/day until reaching the test temperature of 10℃. During temperature ramping, the B-1 was exposed only to the nutrients present in the reconstitution media, and thus was unacclimated to the study waste constituents. The other sample of B-1

Table 7. O_2 uptake rates for study waste acclimated and non acclimated bacterial supplements

Supplement	Rate, mgO_2/g VSS/hr	
	Acclimated	Non-Acclimated
UB-1	1.20	N.A.
A-*	0.41	0.37
B-*	2.98	0.91
D-*	1.17	0.90

Table 8. O_2 uptake rates for non acclimated supplements at 10℃

Supplement	Rate, mgO_2/g VSS/hr
UB-1[a]	0.21
B-1	0.008
B-2	0.003
B-3	0.013
D-1	0.014
D-2	0.001

was reconstituted at 24℃ two hours before testing, and had no prior exposure to the waste constituents or cold temperatures. These latter conditions are representative of expected field application conditions. Uptake rate studies were performed on both supplements at 10℃.

Supplemental bacteria exposed gradually bacteria exposed gradually to reduced temperatures had a much higher oxygen uptake rate than the freshly reconstituted culture as shown by Figure 5. In fact the freshly reconstituted sample of B-1 had no measurable oxygen consumption when exposed to the waste constituents and reduced tempera tures for the first time. As shown in Table 8, this result also was obtained for other supple ments which were known to degrade the waste under acclimated conditions.

3. Population Retention

For bio-augmentation to be an economical alternative, added supplements must have characteristics which allow their special traits to be transferred to bio-reactor. Two mechanisms for this transferal are a physical presence of the added bacterial supplement or a transfer of genetic coding from the non-indigenous to the indigenous strains. For the purposes of this paper, only factors which affect the physical presence of added supplements were investi-gated. Traits deemed important to the population

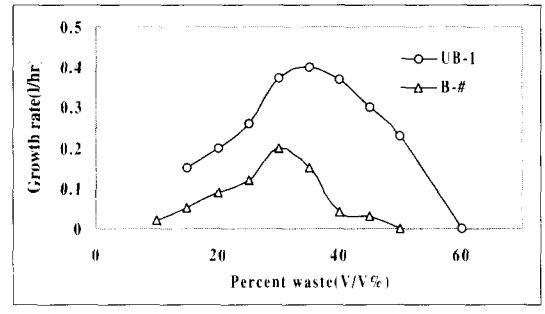

Fig. 6. Growth rates of supplements UB-1 and B-# consortium as a function of percent waste.

retention were supplement growth rate, floc size, and degree of dispersed growth.

3.1 Growth Rate Studies

Growth rate studies were performed with the B-* consortium and supplement UB-1. Presented in Figure 6, is a plot of the specific growth rate of UB-1 as compared to B-* as a function of waste concentration. Both supplements responded in a similar manner to the waste with an increase in specific growth rate until a limiting limit concentration, after which point a toxic response was observed. At high percentages of study waste, no growth was observed. Signi ficant differences observed between the growth characteristics of th two supplements were: 1) the UB-1 population has a higher growth rate than that exhibited by B-* throughout the entire concentration range investigated; and 2) UB-1 was more resistant to the toxic effects of the waste constituents at the higher waste percen tages. Thus, under a competitive growth scenario, the UB-1 population would outcompete the B-* consortium, and repetitive additions of the supplements represented by the B-* consortium would be necessary to maintain B-*.

3.2 Formation of Settleable Flocs.

The ability of the bacteria to form settleable

Table 9. Microscopic observations of bacterial supplements.

Supplement	Ave. diameter(microns)	Dispersed bacteria (per milliliter)
UB 1	100	$3*10^{7}$
A 2	10	$2*10^{9}$
B 1	40	$4*10^{8}$
B 2	20	$9*10^{8}$
D 1	<10	$2*10^{9}$
D 2	20	$7*10^{8}$

aggregates(flocculate) is an important requirement when selecting a culture for bio augmentation. Destabilization of the biofloc is a common occurrence in bio reactors treating industrial and hazardous wastes. The presence of many non-settleable bacteria will cause the effluent to be cloudy and of poor quality. Furthermore, if a supplemental culture does not flocculate, it may wash out of the system, necessitating frequent dosing to maintain the desired reactor population.

To assess the flocculent nature of the commercial supplements, the average floc sizes of the supplement consortiums able to degrade the waste were quantified. Floc sizes of acclimated consortiums obtained from the 1 ℓ batch reactor were determined by microscopic observation. The effluent concentrations of dispersed bacteria from the same 1 ℓ reactors were assessed using a bacterial counting chamber at 1,000× magnification. The average floc sizes and effluent dispersed bacteria per ㎖ of the supplements tested are presented as Table 9. All of the commercial supplements tested exhibited significantly smaller floc particles, and higher effluent dispersed bacterial counts than UB 1 population which served as a baseline for comparison. The inability to flocculate and the accompanying high number of bacteria in the effluent would lead to a high loss of supplemental bacteria from the reactors.

Ⅳ. DISCUSSION OF RESULTS

Based on experimental results of this investigation, the hypothesis that a number of selected factors affect the utility of bio-augmentation was confirmed.

The importance of viability after supplement addition to an indigenous population was demonstrated with bacterial supplements targeted for enhanced degradation of chlorinated organics. Using a mass balance approach the viability of selected supplements added was shown to be minimal two hours after addition to the indigenous population. These results were confirmed using cellular ATP analyses, Obviously, of added supplements are not able to survive under the operating conditions of the reactor, they have little chance in enhancing the performance of the biological process. This expectation was confirmed by comparing the effluent COD values from the supplemented and non supplemented reactors in which there was no significant difference between the supplemented and non-supplemented reactors.

If the supplements remain viable after addition to the indigenous population, a necessary trait of supplemental bacteria is that they should enhance the degradation capacity of the reactor when compared to the existing indigenous population. During the course of the spread plate growth

and batch shaker flask COD removal experiments, none of the bacterial supplements studied performed better than the indigenous population and in many cases did not perform as well, especially during the initial phases of the test. Acclimation of the supplements to the study waste constituents and environment were hypothesized to be important factors in the interpretation of these results.

To test this hypothesis, oxygen uptake studies were conducted with waste and non-waste acclimated and temperature and non-temperature acclimated bacterial supplements. Significant differences were noted between acclimated and non acclimated supplements. Cultures neither acclimated to the waste or depressed temperatures exhibited no measurable oxygen uptake. These results are very important in assessing the feasibility of bio-augmentation because under typical field application conditions, unacclimated bacterial supplements would be added to an indigenous population. Under these conditions, one may conclude that it is likely that little or no positive effect would be through bio-augmentation.

It is also important to note that even when bacterial supplements were acclimated to test conditions they did not perform better than the indigenous population. This result raises further questions about the utility of commercial supplement when compared to an indigenous population.

The importance of population retention factors such as growth rate, floc size, and numbers of dispersed effluent bacteria were investigated. The specific growth rate of the indigenous population(UB-1) was greater than that measured for the supplements. In addition, because the floc size for the supplements was smaller and the number of dispersed bacteria was greater, one may conclude that the non-indigenous supplements would be at a competitive disadvantage over the indigenous population. Therefore, even of the supplements provided enhanced treatment potential, frequent dosages of supplement would be required to maintain the presence of the desired population which would reduce the cost effectiveness of bio augmentation.

V. CONCLUSIONS

A number of factors thought to be important in determining the potential of bacterial supplementation were investigated using a specific waste from a hazardous waste TSDF and bacterial supplements recommended for the wastewater treatment field by the commercial distributors.

Most supplements recommended for the study waste were mot able to initiate degradation of the study waste without a period of acclimation. Parameters such as supplement viability, acclimation, and population retention are important. The population UB 1, which modeled an expected indigenous population, performed as well as any commercial supplement. The population UB-2 which had on prior history to the study waste performed as well as some of the recommended commercial supplements. The feasibility of bio-augmentation is uncertain.

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