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반응촉진 유기물 첨가에 의한 혐기성 분해율의 향상에 관한 연구

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Enhancement of Anaerobic Degradation by Organic Stimulants Addition

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Abstract

혐기성대상과정중 메탄생성균(methanogenic bacteria)에 의한 메탄생성시 주요 기질인 아세트산(acetic acid)을 분해할 경우에 여러 가지 복합기질중 아미노산 첨가에 의한 분해속도증가에 미치는 영향과 투입된 아미노산이 미생물에 의하여 생체량으로 합성되는 정도를 고찰하였다. 실험결과 메탄생성균은 glycine, serine, threonine, aspartic acid, trytophan 등의 혐기성미생물의 생체량합성에 필요한 물질을 투입할 경우에 아세트산의 분해속도가 증가하였으며, 여러 가지 아미노산을 혼합하여 주입한 결과 분해속도가 17%향상되었다. 한편, 메탄생성균의 lysing에 의하여 생성된 유기물은 메탄이나 이산화탄소의 최종산물로 전환되기보다는 새로운 메탄생성균의 생체량을 형성하는데 직접 이용되었으며, 아세트산의 분해속도를 52% 증가시켰다. 단순기질(sole substrate)과 복합기질(complex substrate)의 분해는 미생물의 생체량합성에 필요한 여러 가지 중간대사산물간의 상호자극효과에 의하여 복합기질이 용이한 것으로 나타났으며, 유입기질내 활성이 강한 슬러지의 농도는 혐기성처리에 매우 중요한 부분을 차지하였다.

Ⅰ. Introduction

Anaerobic digestion is usually thought of as being a multi-step process in which complex organics are degraded to short-chain fatty acids(SCFA) by facultative organicsm and then degraded primarily to carbon dioxide and methane by methangenic bacteria as shown in Fig. 1. The methange fermentation step has been observed to be the slowest or rate-limiting step in this sequence[1] and thus kinetic values describing this step would be useful in digester design. Subsequently, enrichment cultures of anaerobic organisms have been subjected to pure feeds of SCFA, and their carbon removal rates and their energy utilization efficiency or organisms yield determined[2,3,4]

While such studies have provided valueable information which can be incorporated into the design of these bacterial mediated treatment

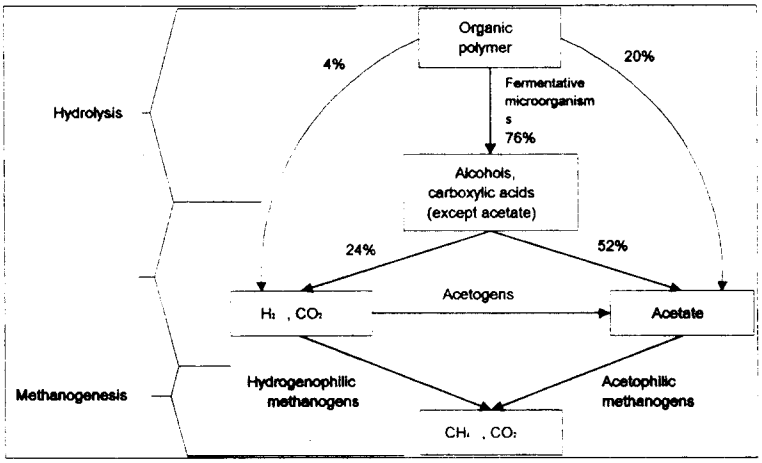

Fig. 1. Anaerobic decomposition of organic matter.

units[5], certain field units have been shown to be more efficient in removing wastes than any of the laboratory units receiving pure volatile acids feeds. It appears that under certain undefined conditions, the anaerobic bacteria can degrade organics at rates faster than that observed in laboratory units which are fed a sole carbon source. For example, Torpey[6] was able to succesfully digest concentrated primary and activated sludge at detention times are as low as 2.6days. Lawrence and McCarty[2] found that detention times of 3day or greater would be necessary to prevent washout of methanogenic bacteria from occuring when the sole carbon source was a single volatile acid. Attempts to stimulate the biological activity of anaerobic digestion facilities have centered around providing some unknown growth factor, usually a heavy metal or vitamins. Others have advocated pre-packaged enzymes, assuming that these will have the same effect as maintaining a high active organism population. Some success has been achieved with these attempts, chiefly the delineation of required heavy metals by Speece and McCary[7]. Other attempts have been provided erratics results, at times providing surges in gas production but generally failing to generate a consistently higher rate. One method of stimulation which has been successful although it has not found widespread use in the field has been to return a portion of the dry digested sludge material. McCarty and Vath[8] found acetic and butyric acid degradation rated could be enhanced by the addition of dried supernatant solids. The stimulatory matter in these solids was apparently of organic origin since addition of the ash of this material caused no increase in gas production.

It appears that many laboratory studies of biological waste treatment processes are conducted using pure substrates so that data analysis can be simplified. This oversimlification may yield incorrect results due to the ability of microorganisms to directly assimilate organic intermediates into the cellular structure therby saving energy. This would suggest that currently used design criteria based on pure substrate removal data may be incorrect and that optimization of energy may result in more efficient operation.

The objective of this study was designed to first delineate the nature of stimulation of acetic acid degradation by the addition of organic materials and to ascertain which organics and what degree of stimulation might be expected, and secondly to determine if the direct uptake and assimilation concept is valid for anaerobic metabolism by methangenic bacteria.

II. Theoretical Background

Sunstrate free energy has frequently been used as a parameter to predict organism yields. An organism degrade organic matter to derive energy for growth. Under a given set of physiological conditions, energy yields should be reasonable constant for an organism degrading a particular substrate, probably varying slightly with the age of the culture. Energy losses have been found to be nearly constant for both mixed and pure cultures of organisms degrading sole substrate[9,10]. In the presence of a mixture of organics, however, it is possible that the energy relationship based on free energy considerations might change due to the direct uptake and assimilation into new cell parts of presynthesized biological intermediates. Helting *et al.*[11] found that mixtures of simple substrates resulted in a higher microorganism mass per unit of energy degraded that for sole substrate. Milholits and Malina[12] while stuying the effects of amino acids on anaerobic digestion of primary sludge noticed that addition of 1.08mM/L of glycine to the sludge produced an increase of about 950 μL of gas in 140minutes. Assuming that all the glycine added was degraded instead of producing stimulatory effect, the amount of increase in gas production should have been only 380 μL. Similary, addition of micromole quantities of L-alanine, L-leusine, DL-methionine, and L-tyrosine increased the gas production rate.

In the growth process an organism manufactures cell parts from low energy nutrients as shown in Fig. 2. If any of the intermediate products can be provided dierectly, an energy savings can be made and yield values and therefore degradation rates should be increased since more organisms will be available to degrade the substrate. Such a phenomenon would provide the basis for stimulation of degradation rates associated with the controlled addition of a variety of organics.

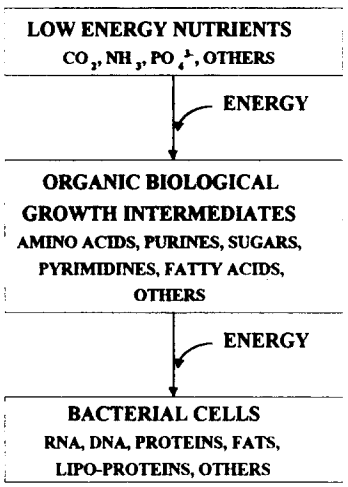

Fig. 2. Synthesis of nutriets in the biological cell

A reasonalbe estimation of those organics capable of providing stimulation can be made. Basically, any cell component should provide stimulation, those requiring the highest energy for manufacture or those most prevalent providing the greatest stimulation. The more complex molecules, RNA, DNA, and whole enzymes probably are too large to pass through cell walls. However, any of the intermediates, amino acid, purines, pyrimidines, and pentoses, or fragments of DNA, RNA, or small proteins may

provode high energy building blocks while passing in the cell. Obviously if a portion of the initial substrate material can be directly used for cell material synthesis instead of undergoing degradation synthesis, the losses associated with these steps will be avoided and yield values and degradation rates will be increased.

Ⅲ. Materials and Methods

An enrichment culture of methane bacteria acclimated to sodium acetate was developed from anaerobic digester sludge from municipal wastewater treatment plant. A 6.2L plexiglass laboratory digester was intermittantly fed sodium acetate along with an inorganic nutrient solution on a fill and draw basis for 2 months to insure a complete washout of all but those organism acclimated to acetate. The composition of the inorganic nutrient solution was identical to that used by Lawrence and McCarty[2]. Feeding was conducted on at least a once a week basis and about 1/4 of the contents of the reactor working volume were exchanged. Since methane bacteria are capable only pf degrading a few select SCFA[13], any othe organic substrate would remain undegraded in the presence of the enrichment culture. If direct assimilation and growth stimulation does occur, it would probably not be evidenced by removal of the non-fatty acid organic, but could be most easily seen by monitorung the fatty acid degradation rate or the organism yield. The initial phase of this study was to ascertain the validity of direct assimilation phenomenon and to determine the degree of stimulation by various growth organic intermediates. The second phase of the study consisted of organism yield measurements in the absence and presence of organic stimulatnts. The third phase of the study was to measure the amount of uptake of external organic substrate over an extended period of time.

Batch experiments were perpared by placing 200mL of the enrichment culture into a 500mL erlenmeyer flask containing dry sodium acetate and the particular organic growth intermediates selected for direct assimilation. The flask shown in Fig. 3. was equipped with a gas collector on aone end to pressurize the reactor and an outlet tube and alamp to provide sampling without exposong the contents of the flask to the atmosphere. The flask was filled with to 400mL with tap water and flushed with nitrogen gas. A magnetic stirrer continuous mixed the contents of the flask. The flasks were maintained in a constant temperature incubator at 35℃, which is the general area of digester operation and is the optimal growth temperature for digester methane bacteria. All results in the initial phase were evaluated by comparison to acetate degradation rates in absence of any additional organic substrate. Acetate concentrations were measured as total volatile acids by the gas chromatographic method according to Standard Methods[14]. The concentration of total organic matter was measured as soluble organic carbon. The contribution of acetate to the total organic carbon was calculated from the volatile acid

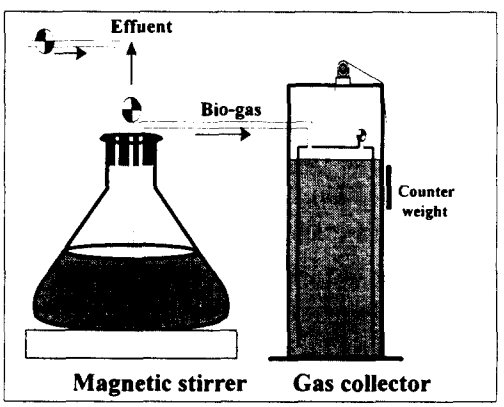

Fig. 3. Experimental set-up of batch reactor.

measurements. In this manner, acetate carbon, total carbon and the carbon concentration of any added growth organics could be determined. A check could then be made to determine if a measurable amount of the additional organics was being removed or degraded.

Growth organics selected for this study consisted of either known biological growth intermediates of a mixture of undefined cellular materials. Amino acids were selected as being capable of direct assimilation because they comprise about 50% of the dry organic solids as protein[15]. Nucleic acids, nucleosides and ribose comprise some of the components of RNA and DNA, so were also considered to be potential stimulants. Components of RNA and DNA used in this study were thyme, uridind, ribose, adenylic acid and quanylic acid. Cellular materials were obtained by lysing both anaerobic and aerobic bacteria. These cellular materials should make up the optimal mixture of assimilable organics since they contain exactly the materials required for organism growth. The following two groups of amino acids were used to investigate direct assimilation.

Group A : glycine, serine, threonine, asparitic acid, and glutamic acid.

Group B : tryptophan, phenylalanine, lysine mo - nohydrchloride, valine, and glutanic acid.

Cellular organic materials were obtained by the following two methods. Both aerobic organisms (activated sludge) and anaerobic bacteria(enrich culture) were autoclaved for 30minutes, filtered through a glass fiber and ther dried. The dried material was then added to the laboratory batch units for direct assimilation studies. Anarobic bacteria organics were also obtained by bubbling pure oxygen gas thrugh a portion of the enrich- ment culture to cause cell lysis. These materials were then dried and fed to the batch study units. The autoclaving and drying operation emplyed in the first process and the drying in the second would ensure enzyme denaturing and any stimul- atory effects observed would be solely due to direct assimilation of growth intermediates. For the organism yield experiments and the extened period study, a stock solution containing equal weights of valine, threonine, lysine and monohy- drochloride, tryptophan, methionini, phenylalanine and glutamic acid was prepared. Biomass was measured as volatile suspende solids(VSS) as prescribed by Standard Methods[14]. The acetate concentrations were again determined by gas charomatography with FID. The extended period study to evaluate the quantity of amino acid directly assimilated lasted for 6 weeks. Sodium acetate crystals were fed three times during

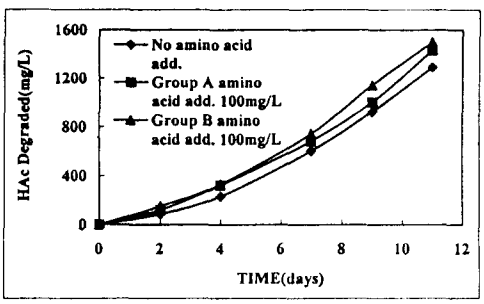

Fig. 4. Accumulation of acetic acid degraded resulting from additions of amino acids.

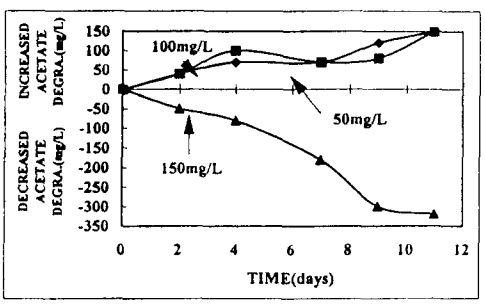

Fig. 5. Effect of varying concentration of Group A amino acids on acetic acid degradation.

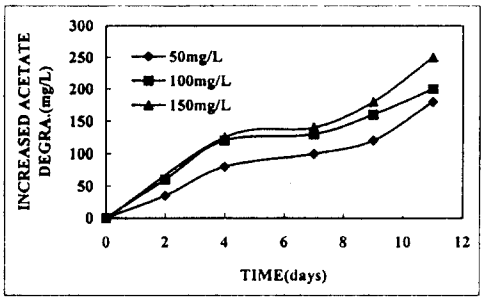

Fig. 6. Effect of varying concentration of Group B amino acids on acetic acid degradation.

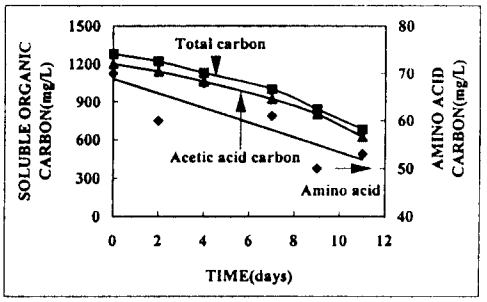

Fig. 7. Variations in soluble organic carbon fractions during degradation.

this period to the batch units to assure that the substrate would not become limiting. The carbon concentrations of amino acids were monitored to evaluated the amino acids uptake.

Ⅳ. Results

The stimulatory effects of amino acids additions are shown in Fig. 4. 5. and 6. Fig. 4. is aplot of accumulated a cetic acid degradation for the amino acids stimulated units and the control. Fig. 4. shows that increased acetic acid degradation occurs in the presence of amino acids for the duration of the experiment.

In Fig. 5. and 6. the effect of concentration of amino acids are shown. For the Group A amino acids, essentially equal stimulation was observed for both 50 and 100mg/L concentration. At the concentration of 150mg/L retardation occurs. These results are in agreement with theindings of Milholtis and Malina[12] who observed that glycine had a stimulatory effect on gas production in laboratlry anaerobic digester units at a concentration of 1.08m moles/L, but at a concentration of 4.0 m moles/L exhibited an inhibitory effect. Since Group A contains glycine, the retardation could have resulted from this amino acid. The Group B amino acids show an increasing rate of acetic acid degradation as the concentration of amino acids increases

From Fig. 7. we could observe that little additional acetic acid removal will be achieved by additions in excess of 150mg/L of the Group B amino acids. In Fig. 7. changes in soluble organic carbon with time are plotted for stimulation by 100mg/L concentration of the Group B amino acids in order to determine the fate the amino acids added. Acetic acid carbon values were calculated from actual acetic acid measurement. The difference between the mea - sured total organic carbon and calculated acetic acid carbon can be assumed to belong to the amino acids. As can be seen from the upper plot in Fig. 7. the amino acid carbon is slowly consumed.

The effect of thymine, uridine, ribose, adenylic acid, and guanylic acid at a concentration of 50mg/L is shown in Fig. 8. Both thymine, a pyrimidine, and uridind=e, a nucleoside increased the rate of acetic acid degradation over that of the control. Adenylic acid and guanylic acid, both purines inhibited the acetic acid degra dation. The carbon values for thymine and uridine are shown Table 1. These results show that the non-acetate carbon fraction is nearly constant, indicating that increased acetic acid degradation rates are not due to increased

Table 1. Organic carbon fraction during degradation stimulation by thymine and uridine

Substrate	Time(days)	Total soluble organic carbon (mg/L)	Acetic acid carbon (mg/L)	Difference (mg/L)
Acetic acid only	0	545	544	1
	2	445	440	5
	4	355	352	3
	7	180	176	4
Acetic acid + thymine	0	567	544	23
	2	447	425	22
	4	342	320	22
	7	147	128	19
Acetic acid + uridine	0	565	544	21
	2	445	425	20
	4	300	280	20
	7	130	112	18

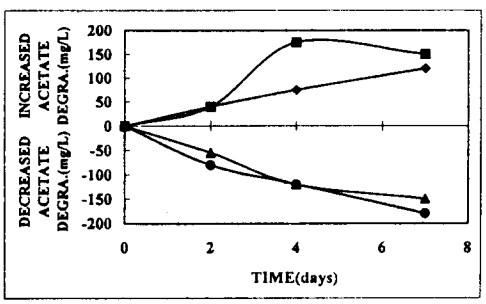

Fig. 8. Effect of nucleic acid components on acetic acid degradation.

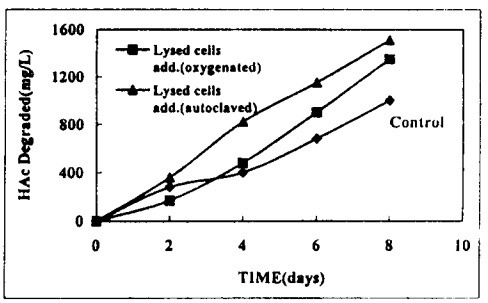

Fig. 9. Stimulation of acetic acid degradation by additions of bacterial lysate.

concentrations of degradable substrate but are the result of stimulation.

In Fig. 9. data obtained for acetate degradation in the presence of biological materials derived from anaerobic bacteria is shown. In 8 days, autoclaved anaerobic bacterial derivatives in - crease the amount of acetic acid degradaded by 52%. This degree of stimulation was the greatest observed during any of the batch experiments. Organics derived by bubbling oxygen gas through the enrichment culture also produced an increase in acetate degradation. A 36% increase in acetate degradation occurred after 8 days.

In Fig. 10. acetic acid degradation and changes in organism biomass in the presence of organicss derived by autocalving the enrichment culture organisms are shown. Organism yields are listed in Table 2. for the enrichment cultures of methangenic bacteria in the presence of and absence of organic stimulants. Amino acids increase the yield from 0.128 to

Table 2. Increase in organism yield in the presence of stimulatory organics

Unit type according to substrate	Time(days)	Acetic acid degraded (mg/L)	Increase in VSS (mg/L)	Yield (mg/mg)
Control 1	3	320	40	0.125
	6	670	90	0.134
	9	1,020	130	0.127
	12	1,560	200	0.128
70mg/L amino acids added	3	360	50	0.139
	6	740	100	0.135
	9	1,280	180	0.140
	12	1,960	280	0.143
Control 2	2	300	35	0.117
	4	740	95	0.128
	6	1,350	175	0.129
Lysed anaerobic cellular matter added(40mgC/L)	2	380	70	0.185
	4	1,060	190	0.179
	6	1,960	355	0.181

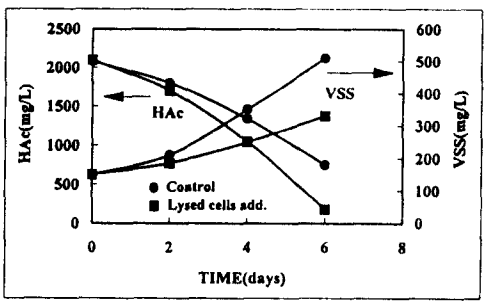

Fig. 10. Effect of bacterial lysate on cell yield and acetate degradation.

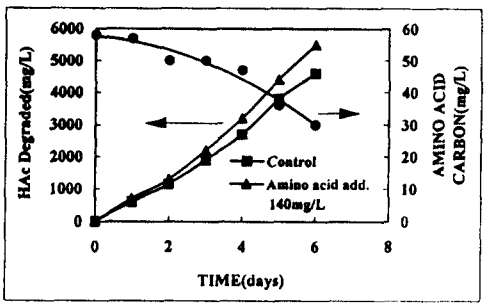

Fig. 11. Uptake of stimulatory organics.

1.35mgVSS/L acetic acid while the cellular organics increase the yields by about 40%.

In Fig. 11. accumulated acetic acid degradation during the extended period study for the control and 20mg/L valine, threonine, lysine, tryptophan, methionine, phenylalanine, and glutamic acid mixture are plotted. Table 2. gives the soluble organic carbon concentrations during the 6 weeks period. As can be seen from the upper plot in Fig. 11. the amino acids carbon decreased from 58mg/L to 29mg/L in 6weeks, indicating that a about 50% of the amino acids have been utilized for direct uptake and assimilation. This corresponds to a direct assimilation of 12.8mg of amino acids per 1,000mg of acetic acid degraded.

V. Disscussions

Acetic acid is degraded more rapidly anaerobically in the presence of organics which are required as cell building blocks. In Fig. 5. 6. and 8. the increasing amounts of acetate degradation in the presence of organic stimulants are plotted with time. The constantly increasing amount of acetate degradation with time in these plots indicates that the effect of stimulatory organics is one of increasing the growth rate of the organism. Since no extra degradation energy sources is provided the effect must be one of direct assimilation of these growth intermediates. The amount of organics used for direct assimilation are apparently small since a carbon loss is slight for most of the batch experiments. From Fig. 5. we could surmize that there is an optimum concentration of Group A amino acids for maximum stimulation. Increasing the concentration above this would inhibit the growth and result in lower degradation rates. This observation is in agreement with the findings of Milholits and Malina[12]. Glysine, one of the ingrements of Group A amino acids is observed to retard growth when present in higher concentrations. Increases in the concentration of Group B amino acids result in increase in acetic acid degradation. It appears that saturation or maximum stimulation would occur at concentration of Group B amino acids in excess of 150mg/L. Stimulation by the nucleic acids components are comparable to that of the amino acid mixture. In 7 days, both thymine and uridine at a concentration of 50mg/L increased the acetic acid degradation by 13% and 17%, respectively. Adenylic acid, and guanylic acid at 50mg/L concentrations showed an inhibitory effect on the organism growth. Since these organics also serve important biochemical functions other than RNA and DNA building blocks, either stimulation or retardation can be explained in several ways. However the anaerobic bacteria are able to successfully grow in the absence of any of the organic growth stimulants so any benificial effect still must be attributed to direct assimilation rather than providing for some essential growth foactor. Natural bilogical stimulants derived by lysing the methanogenic bacteria provide the greatest degree of stimulation. Since autoclaving and drying were used in obtaining these natural biological stimulants, enzyme denaturation would have occurred and therefore the possibility of stimulation by the addition of enzymes is no likely. These natural biological stimulants are probably similar to those found in digester supernatant and would explain the stimulation previously observed resulting from additions of these materials. Other dissolved organics such as vacuum filter liquor or the liquor from sludge heat processes would probably provide an equal degree of bio-stimulation if recycled. In absence of organic growth stimulants, the average microorganism yield coefficient is 0.128mgVSS/mg acetic acid. This is equivalent to a yield coefficiet of 0.137 when expressed in mgVSS/mg COD units.

When 70mg/L amino acids are present the yield value goes up to 0.139mgVSS acetic acid. In the presence of biological stimulants derived by lysing the methane bacteria, the yield is 0.180mgVSS/mg acetic acid. The higher yield coefficients obtained by addition of growth organics and the increasing differcence in the biomass plotted in Figure 10 suggests that the increased acetic acid degradation achieved in the presence of growth intermediates must be the result of an increase in the biomass and not due to any other phenmenon such as an

increase in enzyme activity. During the extended term study, about 70mg/L amino acids were directly assimilated and increased the acetic acid degradation by 16.6% in 6 weeks. This is equivalent ot an assimilation of 12.8mg of amino acids per 1,000mg of acetic acid degraded. If the organism yield due to uptake of acetic acid is assumed to be 0.128mg cell/mg acetic acid, we can assume 128mg of organisms would be present if we ignore endogenous respiration. Since 12.8mg of the amino acid mixture was assimilated, the direct assimilation would account for about 10% of the biomass accumulated. An additional 740mg/L of acetate removal was achieved by the presence of amino acids and again this large stimulation cannot be explained if we assume biodegradation of 70mg/L of amino acids, instead of direct uptake.

In general, these results indicate that mixed or complex substrates would probably be degraded at faster rates than simple or pure substrates due to the mutual stimulation resulting from direct assi,ilation of biological growth intermediates. Kinetics values for the anaerobic degradation of sole substrates may be considered to be the slowest or limiting case encountered in unhibited digester operations. Sewage sludge degradation rates should be faster than those observed to occur in laboratory studies using pure feeds depending upon the feed concentration of the sludge. Continuous culture theory predicts that effluent substrate concentrations will be independent of influent concentrations when the feed rate is constant and the unit is completely mixed. However, with mixed organic sluges more concentrated sludges may be degraded more rapidly due to the stimulation effect provided by assimilable organics. Dilute feed digester probably will be more subjected to upsets due to the low concentration of stimulants or assimilative organics than units receiving a concentrated feed. Neither detention time nor mass/volume/time units may be exactly appropriate as a digester design parameter due to the stimulation effect which would vary with the feed concentration. Detention times should probably be coupled with sludge concentrations as design parameters. The optimal sludge concentration to attain the maximum rate of degradation is not known, however, it appears that increase in concentration may be beneficial until the ability to mix or the build up of toxic end products like ammonia, hydrogen sulfide becomes limiting(16). In aerobic units receiving pure or nearly pure substrate such as organic industrial wastes, the addition of a variety of organics may add to the stability of these units and increase the substrate degradation rates. The addition of biological materials such as those obtained by autoclaving or oxygenating anaerobic bacteria could be used to increase degradation rates and may aid in digester start-up. Addition of digested slduge to raw feed sludge has been observed to provide increase in volatile solids reduction in digester[17] and such a practice may ne especially beneficial to digester receiving diluted sludge.

Ⅵ. Conclusions

Labortory studies was conducted to inves - tigate the nature of stimulation of acetic acid degradation by the addition of organic materials and to determine the direct uptake and assimilation concept is valid for the anaerobic metabolism by methanogenic bacteria, and follwoing conculsions are derived.

Methanogenic bacteria are able to degrade acetic acid more rapidly in the presence of growht intermediates that are required as

microorganism cellular components. A similar of amino acids at a concentration of 150mg/L increased the acetic acid degradation by 17%. Organic derived by lysing methane bacteria increased the acetic acid degradation by 52 percent. These organics are not degraded but apparently assimilated in the cell and used as new cell builiding blocks. In complex substrate systems, individual waste constituents should be degraded more rapidly than as sole carbon substrate. Therefore, kinetic values obtained for pure substrates may not be applicable to mixed substrate systems. The concentration of substrate sludge may be important in anaerobic digestion facilities. Recycling of aerated biomass may provide a source of natural stimulants which may be used as a means of control to obtain more rapid anaerobic degradation rates.

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