Course on Industrial Biotechnology Professor Debabrata Das Department of Biotechnology Indian Institute of Technology Kharagpur Lecture 57 Module 12 Anaerobic Effluent Treatment Process: Bioethanation Process

Welcome back to my course Industrial Biotechnology in the last class I tried to point out that how the biological treatment treatment process is wastewater treatment processes plays important role in the industry I told you whatever organic waste discharged by the different chemical and biochemical industry, more than more than 70 percent of that is treated by biological means and we have two processes with us one is aerobic wastewater treatment process another is anaerobic wastewater treatment process.

In the last two lectures I tried to cover the activated sludge process and also will dealt with the wastewater characteristics and I I made a comparative study that how the carbon and energy balance that we have in the both aerobic and anaerobic wastewater treatment processes.

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Now one thing what I discussed in the last class I want to little bit clarify that FM ratio that is food microorganism ratio when you design the activated sludge process is like this that F by M is like and this can be written as I told you food is substrate that is F0 into x0 and divide by V into x, now F is the flow rate volumetric flow rate you can see this is the volumetric flow

rate and the unit is volume per unit time and the x0 is the concentration of the sorry concentration of substrate this is concentrate mass per unit volume so volume volume will cancel so it will mass per unit time.

And here this is the volume of the reactor and this is the concentration this is mass per unit volume, so if you this will cancel so what we will get this is mass mass mass will cancel so the unit will be time inverse. Now here here here I told you this is equal to S0 V by F0 into x now V by x0 is the hydraulic retention time so it is theta into x this is how in the last class I did not clarify that so I hope this should be clarified so this will be this S0 by x into S.

Now today my topic is anaerobic treatment processes now before I begin let me tell you the the advantages of this process now I told you that whenever we go for installation of any chemical and biochemical industry that we shall have to show that that kind of effluent that is generating by the industry that should be properly treated because we have no right to damage the environment, so so central pollution control board they put some kind of rules and regulation just to safeguard our environment they have disposable standard and that every industry should follow.

Now when when any industry go for that in the early occasions that they found the 10 per cent of the total expenditure of the industry goes for the waste water treatment process but but in a true sense the activated sludge process does not give any any monetary feedback from the process because most of the money that you spent that you cannot get much return from that but due to the due to the development of this anaerobic digestion process now it is it is possible the sum of the money that you spent for the wastewater treatment process you can get back.

I can give a typical example the IFB agro industry that is located close to Calcutta that they are using the anaerobic digestion process for treating their distillery effluent and they observe their 50 per cent of their distillery distillation plant energy required for the distillation plant can be can be shared from the this methane and methane that produce from the anaerobic digester. So it is a great (())(05:03)and not only that I shall you that different western country and also also they are largely using this anaerobic digestion process and used for different purpose I can give the example that if you go I have seen in Sweden, I have seen in Germany they are running the busses with the help of biogas and also in India also now we started using biogas for running the busses.

So it has great different uses that we have so and not only that if you look at the history of biomethanation process what you call biogas generation process, it is very old it is it is invented by American scientist or western scientist and since our the economic conditions of our country as compared to the western countries is not is little bit poor as compared to western country so it is largely applied because this this technology in the both in India and China because this has this has both small scale as well as large scale applicability and India we have millions of biogas plant that is located in the villages and also China they have the millions of biogas plants in the villages.

So you know that they they take the advantage of the degradation of this raw materials organic materials and generate the methane and carbon-di-oxide and that is used for cooking purpose or you know some other purpose and digested material they can be used as a biofertilizer I told you this biofertilizer is very good because this increase the water retention property of the soil this is how the fertility of the soil increases.

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Now let me start this this is if you look at methane that is gas that is produced through this anaerobic digestion process is a colourless odour less gas it does not have any kind of odour and primary component of the natural gas because if you because I can I can I can tell you here gonna share with you some information that you know couple of years back that in Delhi, we have lot of environmental pollution problem due to use of petrol and diesel and because lot of particulate matter lot of you know the fog has occurred so government of India they implement the CNG compress natural gas for running the vehicles and by using this this fog problem has been reduced drastically.

So it has it is a this is used from the natural gas so it is a primary component methane is the primary component of the natural gas chemical formulae is CH4.

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Now methane is a chief component of the natural gas is produced in nature by bacterial decay of vegetation and animal waste because I shall show you how the degradation takes place and this process is called anaerobic digestion.

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Now anaerobic digestion is a series of biological process in which the microorganism breakdown the biodegradable organic matter in absence of oxygen. One of the end products of the biogas is biogas which is combusted to generate electricity and heat, or can be processed into renewable natural gas and and transportation fuel as I mentioned before. So this is the organic waste then the organic precipitation we we preparation then we pass through the anaerobic digestion process we get the biogas so then it can be used heat and electricity generation we can use and digested material can be used as a organic fertilizer and this water you can recycle back to the system.

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Now this is this is the process largely applicable throughout the world and if you look the steps of that degradation process that is involved in this anaerobic digestion process is is like this hydrolysis first the organic material carbohydrate present in the form of starch, cellulose and and then we have proteins we have fats all are bigger molecules. They hydrolyse I told you hydrolysis take place when any water molecule involved in this reaction it produce sugar, amino acid and fatty acid and it passes through the acidogenesis where through which they produce volatile fatty acids and this volatile fatty acids again converted to acetic acid and hydrogen and carbon-di-oxide and through the methanogens it converted to ethane and carbon-di-oxide.

So here I want to point out that two groups of micro flora that take part in this system, this this hydrolysis and the acid production is usually takes place by acidogens and methane production taking place by the methanogens. So two group of microflora because they are totally different from each other they are not same.

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So acidogens they convert organic waste to volatile fatty acid and methanogens they convert this to methane and carbon-di-oxide. Now if you look at the characteristics of the acidogens characteristics of the methanogens is totally different so they I can tell you the you have seen here the substrate required for acidogens is organic waste which comprises of different polymeric compounds, different bigger molecules like you have cellulose, semi cellulose, starch like this also proteins, fats like this that are hydrolyse I I showed you here and the finally it produce volatile fatty acid like acetic acid, butyric acid, propionic acid like this and this this acid they utilise by the methanogens to convert it to methane and carbon-di-oxide.

So the the characteristics two is different with respect to substrate used not only that with respect to pH that pH this acidogens work as a acidic pH, maybe maybe 5 to 6 is better for this and in case of here it is it is 7\$5 (())(11:50) alkaline pH is better for the growth of methanogens. Now if you look at the growth of the acidogens is quite faster this is quite faster and this is slower so this is slower so so you know that that I I showed you before that when we did the reactor analysis then I I showed you that that how the dilution rate that you know that affects that you know what you call x into The, x into D is nothing but Dx by Dt rate of growth of the cells it is like this.

So this is for the slow growing organism and this is for the fast growing organism, so you can see this is I told you this is the D washout, and this is the D washout this is 1 washout. If you look at that you know that that faster required more the D washout value is much higher as compared to slow so if we maintain the diluteness here so we can easily washout the methanogens from the culture so you can do it very easily.

So so this is we can produce the acidogenic culture separately and methanogenic culture methanogens we can develop by taking the mix culture and put the selective substrate we put only the volatile fatty acid as substrate in the reactor so that only methanogens can grow because methanogens can only convert the volatile fatty acid to methane and so we can segregate the culture like this.

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So that raw materials used for this particular process is animal dung that is like cow dung lot of animal dungs we have then poultry waste I I discussed that how one one particular family in some city that how he able to run his poultry plant from farm without by taking care the public opposition because anaerobic digestion remove the that all the bad smell that is generated by this, particular waste because this is a close chamber this is the anaerobic digestion means it does not require any kind of oxygen so no contact with the air so no gas and digested material does not have any smell, so it will totally free from the odour.

Then plant waste like husk, grass and weeds et cetera, human excreta, industrial waste, saw dust, wastes from the food processing industry and the domestic waste like vegetable peel and waste food materials so these are the different raw materials can be used.

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And if you pictorially it can be explained like this we have energy crops manure, industrial waste and different restaurant waste and wastewater when passed through the anaerobic digester, it looks like this and it can be used as a renewable fuel by digested material can be used as a biofertilizer which increases the growth of the plants very high we can we can use as a for the generation of electricity.

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Now if you if you look at how the degradation takes place I I told you this is lipid polysaccharides proteins and nucleic acid the lipid will degrade to fatty acid, polysaccharide will degrade to monosaccharide, proteins will degrade to amino acids and nucleic acid will degrade to purines and pyrimidines and simple aromatics, and this again converted to the volatile fatty acids like propionic, butyrate, and and lactic acid and this ethanol and also acetic acid.

But this is higher acid this passes through the acidogenic process and convert the acetate higher higher organic acid like butyrate and propionate when it converted to methane and carbon-di-oxide first they produce the acetate what you call acetogenesis and then this acetate is converted to methane and carbon-di-oxide and also hydrogen and carbon-di-oxide they combine to produce the methane. This is how (())(16:21) I was talking about this is methanogens and this is the acedogens how they take care the reactions.





Now if you if you look at this how the reaction take place this is another way we can explain, this is similar but but different way it can be explained like this and Barker hypothesis for the biomethanation methane formation he has explained like this how its take care in this particular pathway, there is a coenzyme called coenzyme M that very much take take part in the reaction and that is how is carry forward and produce methane here again that this is carboxylation and hydrogenation then again reduction again it is further reduction to methyl groups then it produce methane and this is also can be produced this can be produced with methyl alcohol and acetate like this this is the Bakers hypothesis.

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	Thermodynamics		
	$C_6H_{12}O_6 + 4H2O \rightarrow 2CH_3 H$	COO- + HCO ₃ - + 4H ₂ + 4H+,	$\Delta F^{O} = -49.2 \text{ KCals}$
	$2HCO_3 + 4H_2 + H^+ \rightarrow CH$	3COO- + 4H _{2.}	$\Delta F^{O} = -25.1 \text{ KCals}$
	$C_6H_{12}O_6 \rightarrow 3CH_3COO$ -	Δ	F ⁰ = -74.3 KCals
	$2CO_2 + 4H_2 \rightarrow CH_3 CO_3$	$OH + 2H_2O$,	$\Delta F^{\circ} = -18.7 \text{ KCals}$
	$CO_2 + 4H_2 \rightarrow CH_4 + 2$	H ₂ O,	∆F° = -33.2 KCals
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And this is the thermodynamics behind this process and when when when the glucose is converted acetic acid your free energy changes minus 74\$3 Kilo Cal but when this carbon-dioxide and hydrogen produces acetic acid this is very low and carbon-di-oxide produce methane that is also comparatively low so in this reaction I can always say that that glucose to acetic acid is more favourable as per this reaction is concerned then after that this methane production after this is acetic acid from carbon-di-oxide and hydrogen.

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	$CH_3COO- + H_2O \rightarrow CH_4 + HCO^{-3}$, $\Delta F^\circ = -7.4 \text{ KCals}$	
	$\mathrm{CH_3CH_2COO-} + 2\mathrm{H_2O} \rightarrow \mathrm{CH_3COO^-} + 3\mathrm{H_2} + \mathrm{CO_2}, \qquad \Delta \mathrm{F^\circ} = +19.5 \ \mathrm{KCals}$	
	$\label{eq:CH3CH2CH2COO} CH_3 CH_2 CH_2 COO^* + 2H_2 COO^* + 2H_2 + H^+ \qquad \Delta F^\circ = + \ 9.95 \ \text{KCals}$	
	4 NAD(P)H + 4H ⁺ \rightarrow 4 NAD (P) ⁺ + 4H ₂ , Δ F° = + 18.4 KCals	
	$4H_2 + CO_2 \rightarrow CH_4 + H_2O$, $\Delta F^\circ = -33.2 \text{ KCals}$	
	SUM: $4H^+ + 4NAD(P)H + CO_2 \rightarrow 4NAD(P) + +CH_4 + 2H_2O, \Delta F^\circ = -14.8 \text{ KCals}$	
1		
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Now another another thing very interesting when when we consider acetic acid when it converted to methane the free energy change is minus 11 point 4 Kilo Cal where your propionic acid required plus, say 19\$5 and butyric acid at 9\$95 from this we can easily say

that propionic acid degradation is very much much less favourable as compared to that of acetic acid and butyric acid.

So it has been observed if when you operate the any kind of anaerobic digester for a longer period of time there would be accumulation of propionic acid in the reactor long line as you operate for longer period of time the propionic acid accumulation in the reactor (())(18:49) because the propionic degradation will be very poor.

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Buswell et al. proposed the following equation	
$C_{a}H_{b}C_{c} + (a - \frac{b}{4} - \frac{c}{2})H_{2}O \rightarrow (\frac{a}{2} - \frac{b}{8} + \frac{c}{4})CO_{2} + (\frac{a}{2} + \frac{b}{8} - \frac{c}{4})CH_{4}$	
Klass and Ghosh found out the stoichiometry for kelp as follows	
$\rm C_{2.61}H_{4.63}O_{2.23}{+}0.34H_2O \rightarrow 1.285CO_2{+}1.32CH_4$	
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Now this is the Buswell equation how how the stoichiometry equation how methane can be calculated and Klass and at all their the also did lot of work on this biomethanation process. They use this equation this is the empirical formulae of the kelp, is kind is (())(19:15) and this when it converted to methane and carbon-di-oxide the stoichiometry is like this.

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Now there is a scientist called Sanderson et al reported few disproportionation reactions because this is very interesting that I told you when the anaerobic digestion the acedogens process we get the volatile fatty acid and volatile fatty acid mainly comprises of acetic acid, propionic acid and butyric acid.

Now this is the disproportionation reaction that is that means usually we express the concentration of of concentration of volatile fatty in terms of acetic acid, if you want to express like this 5 moles of acetic acid is equivalent to 2 moles of butyric acid, 7 moles of acetic acid is equivalent to 4 moles of propionic acid this the disproportionate equation developed by Sanderson.

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Now microbiology behind this biomethanation process, the methanogens belong to the archaeobacteria this is kind of special type of (())(20:23). They are distinguish from the eubacteria bacteria by virtue of many contrasting characteristics such as the presence of isoprenoid-rich membrane lipids. They are typical characteristics as compared to and they link with glycerol absence of mauramic acid based on peptidoglycan cell wall and distinct ribosomal RNA.

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So this is this is we have three type of methanogens one is carbon-di-oxide reducing, another methylotrophic, another is aceticlastic this pathway. So carbon-di-oxide reducing carbon-di-oxide is converted to methane, methynotrophic that we also here also is converted carbon-di-oxide convert to methane by oxidizing methyl group containing the substrate such as the methanol, trimethylamine and dimethyl sulphide. Aceticlastic usually convert the acetate to methane this are the different methanogens present one is methanobacteriales then methanolcoccales, then methanomicrobiales, methanosarcinales and methanopyrales, so different types of methanogens they are all obligatory anaerobe.

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Species	Morphology	Substrate	Cell wall composition	
Methanobacterium formicicum thermoautotrophicum	Long rods to filaments	H ₂ , formate H ₂	Pseudomurein	
Methanobrevíbacter ruminantium smithii arboriphilus	Lancet-shaped cocci, short rods	H ₂ , formate H ₂ , formate H ₂	Pseudomurein	
Methanococcus vanniellii voltae thermolithotrophics mazei	Motile, irregular, small cocci pseudosarcina	H ₂ , formate H ₂ , formate H ₂ , formate H ₂ , methanol, methylamines, acetate	Polypeptide subunits	
Methanomicrobium mobile	Motile short rods	H ₂ , formate	Polypeptide subunits	
Methanobacterium cariaci	Motile, irregular, small cocci	H ₂ , formate	Polypeptide	
Methanospirillum hungatei	Motile, regular, curved rods	H ₂ , formate	Polypeptide	
Methanosarcina barkeri	Irregular cocci as single cell methanol packets, pseudoparenchyma	H ₂ , acetate	Heteropoly= saccharide	
Methanothrix soehngenii	Rods to long filaments	Acetate	No muramic acid	
Methanothermus fervidus	Nonmotile	H ₂	Pseudon	
Jource: Das (1985)			0.00	

And this is how they take part in the reaction. This is shown here how they use the different substrate for the production of methane and carbon-di-oxide.

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Biochemistry of methanogenesis
The biochemical qualities of methanogenic bacteria are different from other bacteria in the following manner:
 A very restricted range of oxidizable substrates coupled to the biosynthesis of methane
 Synthesis of an unusual range of cell wall components
 Synthesis of biphytanyl glycerol ethers as well as high amounts of squalene
 Synthesis of unusual coenzymes like Co-enzyme M and growth factors
 Synthesis of rRNA that is distantly related to that of typical Bacteria
 Possession of a genome size (DNA) approaching 1/3 that of Escherichia coli
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Then this is the biochemistry behind that biochemistry quality of the methanogenic bacteria different from other bacteria. A very restricted range of oxidizable substrates coupled to the biosynthesis of methane synthesis of an unusual range of the cell wall component. The synthesis of biphytanyl glycerol ethers as well as high amount of squalene. This is the synthesis of unusual coenzyme coenzyme M, I told you this is a typical coenzyme M that present in the methanogens and some growth factors also required.

So so I I was talking about that you know acidogens and methanogens, and since the acidogens require different growth characteristics as compared to methanogens it is advisable that if we can operate this process in two different stages then this will be very easy to control because acidogens they require different environmental conditions as compared to methanogens.

Now if you look at normal anaerobic digester process they have one particular chamber inside both acidogenes and methanogens they they their activity taking place. So rate of their you have to you have to synchronize the process in such a way the rate of acid formation should be equal to rate of methane formation, otherwise the pH of the system will go down and affects the affects the methane production process to a great extent.

This is this is to be taken into account when you operate any kind of anaerobic digester of the biogas plant, because in our in our rural places some of the biogas plant does not work because the villages they they are not have that that how to feed this material to the reactor because I can give it very typical example that you know that I told you that organism they are very sensitive to the environment and particularly temperature plays very important role for the activity of the particular organism.

So during winter or ambient temperature go as low as 20-25 degree centigrade, so that time your actual temperature for the growth of organism is 35 to 37 degree centigrade so activity of the organism will drastically reduce. So then if we if we put the substrate at the same rate as we have in the in the in the hot season when the temperature ambient temperature is 40-45 degree centigrade then the organism will not function in a similar way so you have feed rate you have to reduce to a great extent so that so that you know rate of acid formation and rate of methane formation can be synchronized properly.

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So this is this is not this is easily done by using two stage reactor we pass the substrate in the acidogens and then here the acid formation takes place this acid we take it to the methanogenic reactor to get the methane formation. Now since the rate of reaction of acidogens is much faster as compared to methanogenic reactor size of the methanogenic reactor will be much much higher as compared to that of acidogenic reactor.

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parameters -	Batch system	Two stage system	Batch	Two stage
olatile solids (V.S.)			system	system
duction, %	31	36	35	50
iogas production. ³ .Kg ⁻¹ dry residue	0.102	0.12	0.16	0.231
lethane production, ³ .Kg ^{*1} dry residue	0.061	0.08	0.12	0.174
lethane vield. ³ .Kg ⁺¹ V.S. digested	0.246	0.278	0.468	0.475
nergy recovery as methane,	16.0	20.5	29.56	43.5
crease in NPK value,%	33 🔤	41	34	57
	iogas production. ¹² Kg ³ dry residue fethanę production, ¹³ Kg ⁴ dry residue fethanę vield. ¹³ Kg ⁴ V.S. digested mergy recovery as methane, ¹⁴ trease in NPK value,%	iogas production. 0.102 Ifethane production, 0.061 Ifethane vield. 0.246 mergy recovery as methane, 16.0 io 33	iogas production. 0.102 0.12 fethane production. 0.061 0.08 fethane vield. 0.246 0.278 mergy recovery as methane. 16.0 20.5 ic 33 ¹⁶ 41	iogas production. 0.102 0.12 0.16 fethang production. 0.061 0.08 0.12 fethang videl. 0.061 0.08 0.12 fethang videl. 0.246 0.278 0.468 mergy recovery as methane. 16.0 20.5 29.56 ic 33 ba 41 34

Now this is the kind of analysis because I did I personally done some research work on that I tried to find out that how cow dung is a traditional substrate for the biomethanation process and how this can be comparable with that of mixed residue because we have observed that we we we that that you know in the in the any kind of biochemical industry when it depends on

particular raw materials even that raw materials is cheaper but after some time you will find that the unavailability of this raw materials when the people will find that some material has some importance they will they restrict for for selling that material, or they will increase the price of the material.

So you have if you have number of different types of raw materials they can be used for that particular process then the cost of the material raw material can be control to a some extent also available of the raw materials that also increases to a great extent. So we use the mix substrate concept, mix biomass we use here, we use cow dung water (())(26:43) and we find we find we find this is must suitable it is not only if you compare with this batch process you will find that methane production here (0\$24) 0\$246 cubic metre per kg volatile solid digested and where two stage process is 0\$475 it is double as compared to that you know that here it is this if you compared this two stage process for cow dung and mixed residue, it is almost double.

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Material balance of biomethanation process	Mixed biomass (A) T.S V.S. C. Cellulose Hemicellulose Crude protein Lignin Minerals V.A. Energy	Inflow 1000g 73% 31% 22.2% 20.8% 14.8% 11.7% 26.7% 0 13.8 MJ	Two stage Biomethanation R.T. 15 days Outflow	Gas Methane Carbon Energy Digested Biomar T.S V.S. C. Cellulose Hemicellulose Crude protein Lignin	231.25 lits 74 lits 112 g 5.916 MJ 58 689 g 53.05 % 26.7 % 19.8 % 6.41% 13.75% 16.8%
Das,D, Ph.D. thesis, IIT Delhi, 1985				Minerals V.A. Energy	39.07% 3 g
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So this is this is the energy analysis of this process we have mix residue here this is the total composition we find during the degradation process lignin content increases here it is 11\$7 here it is 16\$8 because lignin does not take pared in the reaction but cellulose it was 22\$1 here a 19\$8 while hemicellulose is 20\$8 here you have 6\$8. So what I pointed out hemicellulose is a very good raw materials for the biomethanation process which convert the organic material to methane and carbon-di-oxide.

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	Biomethane plants	Biomethane plants feeding the grid	Biogas plants total (incl. LFG, sewage, agricult.)	Agricultural	Biowaste (incl. organic MSW)		ιra
Austria	10	7	503	approx. 300	55	134	14
Croatia			12	9		2	1
France	3	1	269	40	98	60	71
Germany	107	105	9.200	approx. 7.400	100	1.700	
Hungary	1		58	36		14	8
Italy		2	810	498	32	60	220
Netherlands	21	21	235	98	21	75	41
Poland			219	30	2	approx. 200	
Slovakia			57	34	4	10	9
UK	2	2	360	60		100	> 200
Sweden	47	8	229	<u>6</u> 4	23	135	57
Switzerland	17	15	600	140		460	
TOTAL	208	161	12.552	8.659	335	2.950	621

So this is this is some kind of information that we have, how many biogas plant that is this is the year 2013 in the European country how many biogas plant has been has been are in operation total is 208 and Germany playing the the major role 107 and and then Sweden I told you Germany and Sweden there are largely operating their bus with the help of biogas which is environmentally friendly.

So what I what I tried to point out in this particular presentation that you know I told you biomethanation is a process through which we find some kind of revenue of the of the of the of the money that you spent for the for operating that process. So by selling the gas in the form of methane and methane and carbon-di-oxide. And also digested material also we can sell in the form of biofertilizer which is also some selling value.

So with the and this can be easily control with the help of two group of microflora one is acidogens, another is methanogens and if we control this organism properly we can run the plan very very comfortably and we should not have any kind of problem for operation. And we because if we give this information to our villagers who has operating the biogas plant in the rural areas, they can operate the biogas plant very successfully and make use for their for their cooking purpose. It can be used for different purpose I shall show you afterwards then biogas can be has different utilities.

So with this let me stop here and the next class I shall next lecture I shall concentrate on the how this process is largely applied to the bigger scale and also I shall give you the information how this can be comparable with a chemical process and finally I want to discuss

one successful project on anaerobic digestion what you call land fill land fill gas generation process that is in operation in the western country, thank you.