


Biomass Conversion and Biorefinery
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Lecture – 32
Succinic Acid, Propionic Acid, Acetic Acid, Butyric Acid


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Module	Module name	Lecture	Content
11	Organic Commodity Chemicals from Biomass	02	succinic acid, propionic acid, acetic acid, butyric acid



Good morning students. Today's lecture is 2 under module 11. As you know that in this module we are discussing the various organic commodity chemicals that can be produced from biomass and in today's lecture will cover essentially three of them - succinic acid, propionic acid and acetic acid as well as butyric acid. So, let us begin.

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- *Succinic acid*, a four-carbon dicarboxylic acid, is one of the *end products of anaerobic fermentation* and is also an *intermediate of tricarboxylic acid (TCA) cycle*.
 - Various important chemicals, including 1,4-butanediol, tetrahydrofuran, γ -butyrolactone, N-methyl-2-pyrrolidone and adipic acid, can be synthesized from succinic acid.
 - When the U.S. Department of Energy (DOE) identified succinic acid as one of the twelve promising fundamental chemicals to be produced through biotechnology in 2004, it was suggested that bio-based succinic acid will become cost competitive with the petrochemical process if its productivity approaches 2.5 g/L/h.
 - A number of succinic acid producing microorganisms have been isolated or developed, together with the fermentation and recovery systems. *Escherichia coli* and natural succinic acid producers, such as *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, and *Mannheimia succiniciproducens*, have been metabolically engineered to achieve higher yield and productivity.
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First we will discuss about succinic acid. Succinic acid, a four carbon dicarboxylic acid is one of the end products of anaerobic fermentation and is also an intermediate of that TCA cycle that is tricarboxylic acid cycle. Now, various important chemicals including 1, 4-butanediol, tetrahydrofuran, gamma butyrolactone, N-methyl-2-pyrrolidone and adipic acid can be synthesized from succinic acid.

When the US Department of Energy identified succinic acid as one of the 12 promising fundamental chemicals to be produced through biotechnology in 2004, it was suggested that bio-based succinic acid will become competitive with the petrochemical process only if its productivity approaches 2.4 grams per liter per hour. So, this is the target they have faced .

Because some sort of techno-economical evaluation they have carried out and found out that if it is more than 2.5 grams per liter per hour the productivity of the succinic acid from the biological materials then it will become competitive. A number of succinic acid producing microorganisms have been isolated or developed together with the fermentation and recovery systems.

Escherichia coli and natural succinic acid producers such as *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens* have been metabolically engineered to achieve higher yield and productivity.

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- Many available and cost-competitive carbon and nitrogen sources have been investigated for each succinic acid producers for the efficiencies of growth and succinic acid production.
- In addition, various mixed and semipurified carbon and nitrogen sources, including *cane molasses*, *corn steep liquor*, *soybean flour*, *wood hydrolyzate*, *yeast extract*, *polypeptone*, and *crude glycerol*, have been examined for succinic acid production.
- In parallel with the development of the fermentation process of succinic acid production, several different downstream processes for the separation and purification of succinic acid from culture medium have also been investigated and developed. For example, *ultrafiltration* was used widely for the removal of cells and cell debris as an initial separation step.
- Additional steps in the downstream processes, including *precipitation*, *reactive extraction*, and *electrodialysis*, have been developed to selectively separate succinic acid from the fermentation mixture.
- *Ion exchange and crystallization* procedures were adopted to further purify and crystallize succinic acid.

Many available and cost-competitive carbon and nitrogen sources have been investigated for each succinic acid producers for the efficiencies of growth and succinic acid production. In

addition, various mixed and semipurified carbon and nitrogen sources including the cane molasses, corn steep liquor, soybean floor, wood hydrolysate, yeast extract, polypeptone as well as crude glycerol mostly from the biodiesel production industry have been examined for succinic acid production.

In parallel with the development of the fermentation process of succinic acid production, several different downstream processes for the separation and purification of succinic acid from culture medium have also been investigated and developed. For example, ultrafiltration was used widely for the removal of cells and cell debris as an initial separation step.

If you recall even our yesterday's discussion, I was giving a more emphasis that when we produce certain commodity chemicals using the biotechnological routes and even thermochemical routes also, once they are produced, they will be mostly in the aqueous phase. Then you have to carry out the downstream separation part that means that is basically purification.

So, usually 7%, 10%, 15 to 20% will be the initial concentration of the commodity chemicals in the fermentation broth or in the aqueous medium when you talk about thermochemical conversion. So, you need to purify it up to 70%, 80% so that you can use it as a bulk commodity chemical. So, here that is the catch. So, the downstream processing part is supposed to be very costly, still it is very costly.

With the invention of membrane and all these things process cost is coming down slowly and more work is yet to be done and people are doing day and night so much of research on this particular aspect that how to develop low cost as well as sustainably economical downstream processing part for purification of commodity chemicals once they are produced. Membrane has been tried a lot.


Apart from membrane there are other processes and we have also discussed that how hybrid processes has also been taken into account and had been successful for certain chemical purification. So additional steps in the downstream processes including precipitation, reactive extraction and electrodialysis have been developed to selectively separate succinic acid from the fermentation mixture. Ion exchange and crystallization procedures were adapted to further purify and crystallize succinic acid.

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Carbon Sources

- Various kinds of carbohydrates are metabolized to succinic acid by diverse succinic acid producers.
- *A. succiniciproducens* is able to catabolize several carbohydrates, including glucose, sucrose, fructose, lactose, soluble starches, and corn syrups (Glassner and Datta, 1992), while *M. succiniciproducens* can utilize sucrose, fructose, lactose, xylose, maltose, mannitol, and arabitol as efficiently as glucose (Lee et al., 2002).
- Moreover, many kinds of carbohydrates, including glucose, sucrose, fructose, lactose, arabinose, galactose, maltose, mannitol, mannose, xylose, soluble starches, and corn syrups, can be used for succinic acid production by *A. succinogenes* (Guettler et al., 1996). Furthermore, more reduced carbon source such as glycerol can be utilized by *A. succiniciproducens* and *M. succiniciproducens*.

Glassner DA, Datta R (1992, Sep 1) Process for the production and purification of succinic acid. US Patent 5141814
Lee PC, Lee SY, Hwang MI, Chang HN (2002) Isolation and characterization of a new succinic acid producing bacterium, *Mandobacterium succiniciproducens* MBEEL31E, from bovine rumen. Appl Microbiol Biotechnol. 58 (3): 693-698.
Guettler MV, Jain MK, Sosa BE (1996, May 20) Process for making succinic acid, microorganisms for use in the process and methods of obtaining the microorganisms. US Patent 5504654.



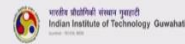
We will talk about some of the carbon sources from where succinic acid can be produced. Various kinds of carbohydrates are metabolized to succinic acid by diverse succinic acid producers. *A. succiniciproducens* is able to catabolize several carbohydrates that include glucose, sucrose, fructose, lactose, soluble starches as well as corn syrups while *M. succiniciproducens* can utilize sucrose, fructose, lactose, xylose, maltose, mannitol and arabitol as efficiently as glucose.

Now moreover, many kinds of carbohydrates including glucose, sucrose, fructose, lactose, arabinose, galactose, maltose, mannitol, mannose, xylose, soluble starches and corn syrups can be used for succinic acid production by *A. succinogenes*. Further more reduced carbon sources such as glycerol can be utilized by *A. succiniciproducens* and *M. succiniciproducens*.

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- In the fed-batch fermentation of *A. succiniciproducens* with glycerol as a carbon source, high succinic acid yield of 1.6 g/g was achieved with low accumulation of by-products (Lee et al., 2000). In the same manner, by using the sorbitol as a carbon source, succinic acid yield was increased compared to the glucose in *E. coli*.
- The reason is that glycerol and sorbitol are more reduced than glucose and provides more reducing power.
- In another study, employing carbohydrates using a transport system other than the phosphotransferase system was demonstrated to increase the succinic acid yield, because more carbon flux could be directed toward oxaloacetate formation at the PEP node (Lin et al., 2005). In addition, co-fermentation of fructose, xylose, or galactose with glucose was studied for *E. coli* and *A. succiniciproducens* (Andersson et al., 2007; Lee et al., 2008).

Lee PC, Lee WG, Kwon S, Lee SY, Chang HN (2000). Batch and continuous cultivation of *Aerobacterium succiniciproducens* for the production of succinic acid from whey. *Appl. Microbiol. Biotechnol.* 54 (1): 23–27.
 Lin H, Bennett GN, Sun KY (2005). Effect of carbon sources differing in oxidation state and transport state on succinate production in metabolically engineered *Escherichia coli*. *J. Ind. Microbiol. Biotechnol.* 32 (1): 87–93.
 Andersson C, Hodge D, Berglund KA, Kwon S (2007). Effect of different carbon sources on the production of succinic acid using metabolically engineered *Escherichia coli*. *Biotechnol. Prog.* 23 (2): 384–388.
 Lee PC, Lee SY, Chang HN (2008). Succinic acid production by *Aerobacterium succiniciproducens* ATCC 39462 growing on galactose, galactose-glucose, and galactose-xylose. *J. Microbiol. Biotechnol.* 11 (11): 1792–1796.



In the fed-batch fermentation of *A. succiniciproducens* with glycerol as the carbon source, high succinic acid yield of 1.6 gram was achieved with the low accumulation byproducts. Now in the same manner by using the sorbitol as a carbon source, succinic acid yield was increased compared to glucose using *E.coli*. The reason is that glycerol and sorbitol are more reduced than glucose and provides more reducing power.

In another study employing carbohydrates using a transport system other than the phosphotransferase system was demonstrated to increase the succinic acid yield because more carbon flux could be directed towards the oxaloacetate formation at the PEP node. In addition, co fermentation of fructose, xylose or galactose with glucose was studied for *E. coli* as well as *A. succiniciproducens* by various researchers.

Some of the references are given. If you are interested to learn more, you can please browse through these particular references, these are classical works.

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- Instead of employing purified carbohydrates as carbon sources, some cost-effective feedstocks such as *wood hydrolysates, cane molasses, cheese whey, and straw hydrolysates* have also been used for bio-based succinic acid production.
- In 2000 and 2003, *Lee et al.* investigated an anaerobic fermentation process for succinic acid production from whey and wood hydrolysates by *A. succiniciproducens*.
- In the batch fermentation with 20 g/L of non-treated whey and 7 g/L of glucose, the yield and productivity of succinic acid of 0.95 g/g and 0.46 g/L/h, respectively, were obtained.
- When wood hydrolysate (equivalent to 27 g/L glucose) was used as a carbon source, the succinic acid yield of 0.88 g/g was obtained.
- In the case of *M. succiniciproducens*, whey and wood hydrolysates-based medium were utilized as economical carbon sources to produce succinic acid (*Kim et al., 2004; Lee et al., 2003a*).

Lee PC, Lee WG, Kwon S, Lee SY, Chang JN (2003). Batch and continuous cultivation of *Asanobacterales succiniciproducens* for the production of succinic acid from whey. *Appl Microbiol Biotechnol*, 54 (1): 21–27.
Lee PC, Lee SY, Hwang SH, Chang JN (2003a). Batch and continuous culture of *Manduca succiniciproducens* MHEL5E for the production of succinic acid from whey and corn steep liquor. *Bioprocess Biotech*, 18 (1): 43–47.



Instead of employing purified carbohydrates as carbon sources, some cost-effective feedstocks such as wood hydrolysates, cane molasses, cheese whey and straw hydrolysates have been used for bio-based succinic acid production. In 2000 and 2003, Lee et al investigated anaerobic fermentation process for succinic acid production from whey and wood hydrolysates by *A. succiniciproducens*.

In the batch fermentation with 20 grams per liter of non-treated whey and 7 grams per liter of glucose, the yield and productivity of succinic acid of 0.95 gram per gram and 0.46 gram per liter per hour respectively were obtained. When wood hydrolysate that is almost equivalent to 27 grams per liter of glucose was used as carbon source, the succinic acid yield of 0.88 grams per gram was obtained.

So, what you can see from this particular slide is that the succinic acid which is being reported is extremely low when you talk about the 2.5 grams per liter per hour productivity. However, these are classical works which has led a new path or you can say opened up new avenues to work on bio-based succinic acid production whereas we are now using genetically engineered strains to increase the yield as well as productivity.

In the case of *M. succiniciproducens*, whey and wood hydrolysates-based medium were utilized as economical carbon sources to produce succinic acid. So very important 2 papers which are given below you can browse through them if you want to learn more. They are excellent work in this particular field.

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- Using corn steep liquor containing whey-based medium, the succinic acid yield of 0.71 g/g and productivity of 1.18 g/L/h were obtained in *batch fermentation*.
- *Continuous fermentation* resulted in a succinic acid yield of 0.69 g/g and productivity of 3.9 g/L/h.
- By using wood hydrolysates based medium, the succinic acid yield of 0.56 g/g and productivity of 1.17 g/L/h were obtained in *batch fermentation*, and 0.55 g/g and 3.19 g/L/h, respectively, were obtained by *continuous fermentation*.
- Various studies utilizing inexpensive carbon sources such as corn fiber hydrolysates, cane molasses, cheese whey, wheat milling by-products, and straw hydrolysates by *A. succinogenes* have been carried out to examine the possibility for cost-effective succinic acid production (Yi et al., 2013).

Yi, J., Cho, S., Han, M.S., Lee, J.W. and Lee, S.Y., 2013. Production of succinic acid from renewable resources. *Bioprocessing Technologies in Bioenergy for Sustainable Production of Fuels, Chemicals, and Polymers*, pp.317-330.



Then using corn steep liquor containing whey-based medium, the succinic acid yield of 0.71 gram per gram and productivity of 1.18 grams per liter per hour was obtained in batch fermentation. Now, this is batch fermentation. Of course, when you talk about batch fermentation and compare with continuous one it is always less, but it has its own advantages.

Now, continuous fermentation resulted in succinic acid yield of 0.69 grams per gram and productivity of 3.9 grams per liter per hour. You have just noticed that the productivity is almost a double, 1.18 to 3.9 - when we go from batch to continuous fermentation mode. Now by using wood hydrolysates based medium, the succinic acid yield 0.56 per gram and a productivity of 1.17 gram per liter per hour were obtained in batch fermentation and 0.55 per gram and 3.19 grams per liter obtained in continuous fermentation.

Again you see that from 1.17 to almost double 3.19 gram per liter per hour the productivity has enhanced when we went for continuous fermentation using the wood hydrolysates. So, various studies utilizing the inexpensive carbon sources such as corn fiber hydrolysates, cane molasses, cheese whey, wheat milling byproducts and straw hydrolysates by *A. succinogenes* have been carried out to examine the possibility for cost-effective succinic acid production.

You will see that there are many, I could not report here also, just those which are very important results we have listed here. So, much of work you will see from succinic acid production using two very different approaches. First different types of low cost biomass.

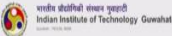
Either it is low cost or it is almost no cost, just like waste only you have to segregate and then little preprocessing and then you can use it.

Second using different types of strains. Mixed strains have also been reported. Then genetically engineered strains have been reported. So, these are the two ways on which most of the succinic acid production research has been carried out.

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- *Propionic acid* ($\text{CH}_3\text{CH}_2\text{COOH}$) is a colorless, naturally occurring carboxylic acid with a slightly unpleasant odor. It is soluble in water, organic solvents and alcohols.
- As a specialty chemical, propionic acid is mainly used as *preservatives* in feed and grain (52%) and bakery and dairy products (18%), and in herbicides (16%) and cellulose acetate propionate (10%) (Kirschner, 2009).
- *Sodium propionate* can also be used in treating dermatoses, wound infection, and conjunctivitis. In addition, esters of propionate are used in artificial fruit flavors (e.g., citronellyl and geranyl propionate) and plasticizers (e.g., glycerol tripropionate and phenyl propionate).
- Propionate esters are also good substitutes for volatile solvents such as xylenes and certain ketones classified as hazardous air pollutants.
- In the past 10 years, the U.S. market for propionic acid continued to grow at an annual rate of ~2.3% with a sale price reaching \$0.92–0.97 per pound in 2009.

Kirschner M 2009. Chemical profile: Propionic acid. Available at: <http://www.ica.com/Articles/2009-03/30/30171/chemical-profile-propionic-acid.html> (accessed February 01, 2013).



Now let us talk about propionic acid. Propionic acid is a colorless naturally occurring carboxylic acid with a slightly unpleasant odor. It is soluble in water, organic solvents as well as alcohols. As a speciality chemical, propionic acid is mainly used as a preservative in food and grains about almost 52% of what is being produced worldwide, bakery and dairy products 18% and in herbicide 16%, cellulose acetate propionate, another classical commodity chemical 10%. Now, sodium propionate can also be used in treating dermatoses, wound infection and conjunctivitis. In addition, esters of propionate are used in artificial fruit flavors as for example citronellyl and geranyl propionate and of course in plasticizers as for example glycerol tripropionate and phenyl propionate.

Propionate esters are also good substitutes for volatile solvents such as xylenes and certain ketones classified as hazardous air pollutants. In the past 10 years, the US market for propionic acid continue to grow at an annual rate of 2.3% with a sale price reaching 0.92 to 0.97 dollar per pound in 2009.

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- Current industrial production of propionic acid is *mainly through petrochemical processes*, from ethylene, CO and steam (*Reppe process*), or ethanol and CO (*Larson process*) in the presence of catalysts.
- Due to the non-renewable feedstocks derived from the increasingly expensive crude oils and environmental concerns of the petrochemical processes, there is great interest in producing **bio-based propionic acid via fermentation** by *propionibacteria*, which are widely used in industry for the manufacturing of Swiss cheese and vitamin B12 (*Boyaval and Corre, 1995; Youngsmith et al., 1982*).
- Propionic acid fermentation using renewable biomass such as *agricultural residues and industrial wastes* as feedstocks is sustainable and environmentally friendly. The bio-based propionic acid is also more appealing to consumers for uses in foods and cosmetics.
- However, conventional propionic acid fermentation is uneconomical due to low product yield (<0.5 g/g), productivity (<1 g/L h), and product concentration (<40 g/L) caused by end-product inhibition.

Boyaval P, Corre C (1995). Production of propionic acid. *Lett* 71: 453-461.
Youngsmith B, Sonomoto K, Taka S, Fukui S (1982). Production of vitamin B12 by immobilized cells of propionic acid bacterium. *Appl. Microbiol. Biotechnol.* 16: 70-74.



Current industrial production our propionic acid is mainly through the petrochemical process. Mostly it is from ethylene, carbon monoxide and steam which is the Reppe process or ethanol and carbon monoxide which is the well-known Larson process. And of course, both are carried out in the presence of catalysts. Now, due to the non-renewable feedstocks derived from the increasingly expensive crude oil and environmental concerns of the petrochemical processes, there is a great interest in producing bio-based propionic acid via fermentation using the *propionibacteria*, which are widely used in industry for the manufacturing of the Swiss cheese and vitamin B₁₂. Propionic acid fermentation using renewable biomass such as agricultural residues and industrial wastes as feedstock is sustainable and environmentally friendly. The bio-based propionic acid is also more appealing to the consumers for uses in foods and cosmetics.

However, conventional propionic acid fermentation is uneconomical due to the low productivity yield. It is very low 0.5 grams per gram and the productivity is also less than 1 gram per liter per hour and product concentration which is less than 40 grams per liter caused by the end-product inhibition.

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Propionic Acid Bacteria

- Many bacteria are capable of producing propionic acid (Table 1). Among them, *Propionibacterium* can produce propionic acid as the major product in the dicarboxylic acid pathway (Figure 1) and is the most widely used genus for propionic acid fermentation.
- Another species of interest is *Clostridium propionicum*, which can ferment lactate, alanine, serine, and threonine to produce propionic and acetic acids via the acrylic acid pathway (Figure 1).
- Some species in the genera of *Bacteriodes*, *Fusobacterium*, *Megasphaera*, *Selenomonas*, and *Veillonella* can also produce propionic acid, but not all of them can use sugar or carbohydrates as carbon source (Playne, 1985).
- So far, propionic acid fermentation research has focused on *Propionibacterium* because of their better fermentation performance and broader substrate spectrum compared to other genera.

Playne MJ (1985) Propionic and butyric acids. In: Moo-Young M, ed. Comprehensive biotechnology. Pergamon Press, New York, p 731-739



So let us talk about some of the propionic acid bacteria which are being used by various researchers even in the commercial scale to produce propionic acid. Many bacteria are capable of producing propionic acid. I am just showing you the table 1. You can see here. Here are some propionic acid producing bacteria and then general characteristics are given, we will come back to it later. Among them, *Propionibacterium* can produce propionic acid as the major product in that dicarboxylic acid pathway.

Figure 1, I will show you again what is this dicarboxylic pathway the first one and then acrylic acid pathway of the *Clostridium* species. Anyway, we will come back to it. And is the most widely used genus for the propionic acid fermentation. Another species of interest is that *Clostridium propionicum* which can ferment lactate, alanine, serine and threonine to produce propionic acid and acetic acids via the acrylic acid pathway.


The second pathway, we will go to that. Some species in the genera of *Bacteriodes*, *Fusobacterium*, *Megasphaera*, *Selenomonas* and *Veillonella* can also produce propionic acid but not all of them can use sugar or carbohydrate as carbon source. So far, propionic acid fermentation research has focused on *Propionibacterium* because of their better fermentation performance and broader substrate spectrum compared to other genera.

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Table 1. Some propionic acid-producing bacteria and their general characteristics

Genera/Species	Grain Stain	Substrate	Products	Opt. pH	Opt. Temp. (°C)
<i>Propionibacterium acidipropionici</i> <i>Propionibacterium freudenreichii</i> <i>Propionibacterium shermanii</i>	G(+)	Glucose, sucrose, lactose, lactate, glycerol	Propionate, acetate, succinate, CO ₂	6.5–7.0	30–32
<i>Clostridium propionicum</i>	G(+)	Glycerol, lactate, alanine	Propionate, succinate, acetate, formate, <i>n</i> -propanol	6.8	30
<i>Bacteriodes fragilis</i> <i>Bacteriodes ruminicola</i>	G(+)	Glucose	Acetate, propionate, lactate, succinate, formate	6.5–7.0	37
<i>Veillonella parvula</i> <i>Veillonella alcalescens</i>	G(-)	Lactate	Propionate, acetate, CO ₂ , H ₂	6.5	30–37
<i>Selenomonas ruminantium</i> <i>Selenomonas sputigena</i>	G(-)	Lactate Glucose	Propionate, lactate, acetate	6.8	30–37
<i>Megasphaera elsdenii</i>	G(-)	Lactate	Acetate, propionate, butyrate	7.0	39
<i>Fusobacterium necrophorum</i>	G(-)	Lactate	Acetate, propionate, butyrate	<7.8	39

Wang et al. Propionic acid fermentation, 2013, 333-336



Yes, this is the table 1. Please have a close look at this table. You can see that, what are the different types of the species of (bacteria) that are being used to produce propionic acid. Whether they are gram positive or gram negative? What are the substrates they could degrade? What are the products? What are the optimum pH and temperature? So we will just go, one or two I will just tell you, rest you can see later on when you go through the lecture.

The first is the *Propionibacterium acidipropionici* and the class of propionibacterium species. So, they are gram positive stains. They can degrade various types of substrate whether it is glucose, sucrose, lactose, lactate, even glycerol also. Now the product is propionate, acetate, succinate and carbon dioxide, of course at different proportions. Optimum pH is 6.5 to 7. Optimum temperature is 30 to 32.

So, if you look at some other one, let us say the last one the *Fusobacterium necrophorum*. It is a gram negative bacteria and it will only degrade lactate. It is a lactate based bacteria which will produce propionic acid. The end products are acetate, propionate and butyrate, again at different proportions, pH has to be maintained at less than 7.5 that is the optimum and temperature is slightly higher it is 39. Now, there are others also, you can go through it later on.

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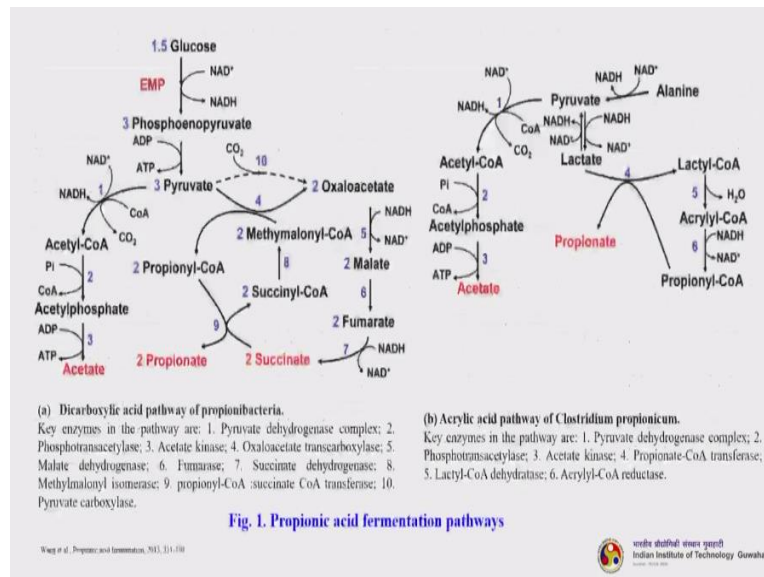


Fig. 1. Propionic acid fermentation pathways

This, I showed you a few minutes before. So, this is the classical propionic acid fermentation pathway, basically 2 different pathways. First is the dicarboxylic acid pathway of the *Propionibacteria*. Second is the acrylic acid pathway of the *Clostridium* species, *Clostridium propionicum*. So, let us try to understand what is happening in that dicarboxylic acid pathway of the *Propionibacteria*.

So, you can see that here the substrate is glucose, it can be something else also. So, glucose is getting converted to 3 phosphoenopyruvate, it is a pyruvate cycle again. Then it is finally degraded to pyruvate. Now, this pyruvate can result in three different products, again using different pathways. Now, when you talk about this particular pathway where the acetyl coenzyme A is getting produced, you remember this we have discussed during fermentation also, a little different right? So, this pathway we have discussed. Now that gives an intermediate product which is called a Acetylphosphate and that finally being degraded to end product which is a acetate. Now, similarly pyruvate can go through a 2 different other pathways. So, one is that oxaloacetate pathway, one is the methymalonyl coenzyme A pathway.

So, if you go through methymalonyl coenzyme pathway it will give you succinyl coenzyme A and finally 2 succinate. And here also if you talk about methynyl coenzyme A, it will go to the propionyl coenzyme A and 2 propionate. Now, 2 methymalonyl coenzyme A is also coming partly from the 2 succinate. Now, this 2 succinate is also getting converted in a backward cycle to 2 succinyl coenzyme A and then methymalonyl coenzyme A.

And if you look at the oxaloacetate pathway, so it gets converted to malate, fumarate. Now, this fumarate is getting converted to succinate. So, this is your dicarboxylic acid pathway of the *Propionibacterium*.

Now, let us talk about the acrylic acid pathway of the *Clostridium* species. Now here you can see that if we start with alanine. So alanine is getting converted to pyruvate. Now pyruvate to acetyl coenzyme A, acetyl coenzyme A to acetylphosphate, acetylphosphate to acetate. Now pyruvate to lactate. Lactate is getting converted to lactyl coenzyme A, then acrylyl coenzyme A, then propionyl coenzyme A and finally propionate. So, these are the 2 major pathways to produce the propionic acid.

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Propionic acid production from renewable feedstocks

- As the raw material cost usually accounts for a major portion of the final product cost in fermentation production of oxychemicals including propionic acid, it is important to use inexpensive or low-cost feedstock for propionic acid fermentation to make it more favorable to compete with the chemical synthesis route.
- There are large amounts of *wastes or by-products generated from food, agricultural, and other industries, including cheese whey from the dairy industry, cane molasses and sugarcane bagasse from the sugar industry, corn fiber and CSL from the corn milling industry, and glycerol from the biodiesel industry,* which may be used as low-cost feedstock for fermentation.
- These wastes containing fermentable sugars or carbon source have low value but high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) contents, posing an environmental threat if not treated properly (*Kushwaha et al., 2012*).

Kushwaha JP, Srivastava VC, Mall JD (2012). An overview of various technologies for the treatment of dairy wastewaters. Crit. Rev. Food. Sci. 11:442-452.

स्वास्थ्य शिक्षण संस्थान
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So, propionic acid production from the renewable feedstocks. We will try to understand what are the biomasses that are being used. As the raw material cost usually accounts for a major portion of the final product cost in fermentation production of the oxychemicals including propionic acid, it is important to use inexpensive or low cost feedstock for a propionic acid fermentation to make it more favorable to compete with the chemical synthesis route.

There are a large amounts of wastes or byproducts generated from food, agricultural and other industries including cheese whey from the dairy industry, cane molasses and sugarcane bagasse from the sugar industry, corn fiber and CSL (corn steep liquor basically) from the corn milling industry and glycerol from the biodiesel industry which may be used as low cost feedstock for fermentation process.

Now, these wastes containing fermentable sugars or carbon sources have low value but high biochemical oxygen demand and chemical oxygen demand, the BOD and COD, posing an environmental threat if they are not treated properly. So it is something you can say that waste to value-added products.

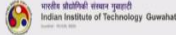
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- Thus, the application of these low-cost and renewable wastes as an alternative feedstock to produce value-added chemicals such as propionic acid has attracted large interests from both industry and academia.
- Some fermentation studies using low-cost biomass feedstock are summarized in Table 2 below.

Substrate	Process	Titer (g/L)	Productivity (g/L-h)	Yield (g/g)	References
Whey permeate	Recycle-batch with FBB	65	0.22-0.68	0.5	Yang et al. (1995)
Cheese whey	Continuous with cells recycled by UF	25	14.3	N/A	Boyaval and Corre (1987)
Cane molasses	Fed-batch with plant FBB*	91.9	0.36	0.46	Feng et al. (2011)
Corn meal	Batch with FBB	40	2.12	0.58	Huang et al. (2002)
Corn cob molasses	Fed-batch	71.8	0.28	N/A	Liu et al. (2012)
Jerusalem artichoke	Fed-batch with FBB	68.5	1.55	0.43	Liang et al. (2012)
Sugarcane bagasse	Fed-batch with FBB	58.8	0.38	0.37	Zhu et al. (2012)
Glycerol/potato juice	Sequential batch with cell recycle	25-50.8	0.29-1.42	-0.6	Dishisha et al. (2012b)
Glycerol	Recycle batch with cells adsorbed on PEI-Poraver	35.2	0.35	0.47	Dishisha et al. (2012a)
	Recycle-batch with FBB	23	0.25	0.58	Zhang and Yang (2009b)
	Fed-batch with FBB	106	0.04	0.56	

*With *Propionibacterium freudenreichii*; all other fermentations were with *Propionibacterium acidipropionici*.
CSL, corn steep liquor; FBB, fibrous-bed bioreactor for immobilized-cell fermentation; N/A, not available.

Wang et al., Propionic acid fermentation, 2013, 333-336



Thus, the application of these low-cost and renewable wastes as an alternative feedstock to produce value-added chemicals such as propionic acid has attracted large interest from both industry as well as academia. Some fermentation studies using low-cost biomass feedstock are summarized in the table 2. So, you can see that the different types of substrates here, processes, the titer, the productivity, the yield and the respective references. Now, this table has been taken from this particular reference, you can browse through later on. So let us just see one or two.

Whey permeate: Recycle-batch with fluidized bed process. So, the titer is 65 grams per liter, productivity is 0.2 to 0.68. It is of course less compared to the theoretical one. Yield is 0.5 grams per gram. Similarly, if you go to corn meal, this is a batch process with again a fluidized bed operation. The titer is 40 grams per liter, however the productivity is higher in this case 2.12 grams per liter per hour and the yield is 0.58 gram per gram. Similarly, if you look at some other things you can see Jerusalem artichoke, a lot of work has been done on this, sugarcane bagasse, glycerol, then glycerol and potato juice and respective productivities as well as the yield.

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- The U.S. dairy industry annually generated more than 80 million tons of whey as a by-product from cheese manufacturing. The main components of cheese whey are whey protein and whey lactose, which makes up about ~75% of whey total solids and are currently in a large surplus with limited uses.
- Propionic acid production from whey lactose and whey permeate by *propionibacteria* has been extensively investigated (Gupta and Srivastava, 2001; Jin and Yang, 1998; Yang et al., 1995).
- In general, high productivity (0.22–0.68 g/L h) and yield (~0.5 g/g lactose) with a high propionic acid concentration of 65 g/L can be obtained from *concentrated whey permeate* (Yang et al., 1995).
- Calcium propionate produced from whey can be used as a *natural preservative* in bakery and dairy products.

Gupta A, Srivastava AK (2001). Continuous propionic acid production from cheese whey using an ultra pure filter. *Biotechnol. Bioprocess Eng.* 4:1-5.
 Jin Z, Yang S-T (1998). Extractive fermentation for enhanced propionic acid production from lactose by *Propionibacterium acidipropionis*. *Biotechnol. Prog.* 14:437-445.
 Yang S-T, Huang Y, Hsu G (1995). A novel recycle batch immobilized cell fermenter for propionate production from whey lactose. *Biotechnol. Bioeng.* 45:379-386.



The US dairy industry annually generated more than 80 million tons of whey as a byproduct from the cheese manufacturing process. The main components of cheese whey are whey protein and whey lactose, which makes up to about 75% of the whey total solids and are currently in a large surplus with limited uses. Propionic acid production from whey lactose and whey permeate by *Propionibacteria* has been extensively investigated.

In general, high productivity 0.22 to 0.68 grams per liter per hour and yield a 0.5 grams per gram lactose with a high propionic acid concentration of 65 gram per liter can be obtained from the concentrated whey permeate. Calcium propionate produced from whey can be used as a natural preservative in bakery and dairy products.

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- *Corn fiber and CSL* are two low-value by-products from the corn wet milling industry. They are currently used mainly in animal feed but with a large surplus because of the rapid expansion of the corn ethanol industry in the recent years.
- CSL is rich in amino acids and trace elements (metal ions and vitamins), and is a good nitrogen and nutrients source for industrial fermentation. A high final titer of propionic acid (~ 46 g/L) has been reported by using CSL medium in a continuous *propionibacteria* fermentation (Paik and Glatz, 1994).
- In another study, Huang et al. (2002) reported a high yield (0.6 g/g) and high productivity (2.12 g/L h) of propionic acid in a recycle batch fermentation using hydrolyzed *corn meal* as substrate.
- Molasses is a by-product of the sugar refining industry, and its major component is sucrose, glucose, and fructose. *Propionibacterium* is capable of converting all these three sugars into propionic acid.

Paik H-D, Glatz BA (1994). Propionic acid production by immobilized cells of a propionic culture strain of *Propionibacterium acidipropionis*. *Appl. Microbiol. Biotechnol.* 41:22-27.
 Huang YL, Wu Z, Zhang L, Cheng CM, Yang S-T (2002). Production of carboxylic acids from hydrolyzed corn meal by immobilized cell fermentation in a filter-bed bioreactor. *Bioresour. Technol.* 82:51-59.



Corn fiber and corn steep liquor are two low-value byproducts from the corn wet milling industry. They are currently used mainly for the animal feed purposes but with the large surplus because of the rapid expansion of the corn ethanol industry in recent years, they are being unused. Corn steep liquor is rich in amino acids and trace elements just like some of the metal ions and vitamins and is a good nitrogen and nutrients source for industrial fermentation.

A high final titer of propionic acid, almost 46 grams per liter, has been reported by using corn steep liquor medium in a continuous *Propionibacteria* fermentation. In another study, Huang et al reported a high yield and high productivity almost 2.12 grams per liter per hour of propionic acid in a recycle batch fermentation process using the hydrolyzed corn meal as substrate.

Molasses is a byproduct of the sugar refining industry and its major component is sucrose, glucose and fructose. *Propionibacterium* is capable of converting all these three sugars into propionic acid.

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- A propionic acid fermentation process with non-treated or hydrolyzed molasses as carbon source reached a high propionic acid concentration of ~92 g/L with a yield of 0.46 g/g and productivity of 0.36 g/L h (Feng et al., 2011).
- Sugarcane bagasse is another cheap and abundant by-product of sugar refining, which can be hydrolyzed into glucose and xylose, and then used as carbon source for propionic acid production, achieving a titer of 51 g/L, yield of 0.43 g/g, and productivity of 0.71 g/L h (Zhu et al., 2012).
- Corn cob molasses, a hemicellulose hydrolysate containing glucose, xylose, and arabinose, was used as carbon source for *P. acidipropionici*. The three sugars in the corn cob molasses were simultaneously converted to a propionic acid titer of 71.8 g/L with a productivity of 0.28 g/L h in a fed-batch fermentation with an initial cell density of 14.8 g/L (Liu et al., 2012).

Feng X, Chen F, Xu H, Wu B, Li H, Li S, Cheng P (2011) Green and economical production of propionic acid by *Propionibacterium freudenreichii* CCTCC M03015 in plant fibrous-bed bioreactor. *Bioresour. Technol.* 102:6141-6146.

Zhu L, Wei P, Cao J, Zhu X, Wang Z, Huang L, Xia Z (2012) Improving the productivity of propionic acid with FBR immobilized cells of an adapted acid-tolerant *Propionibacterium acidipropionici*. *Bioresour. Technol.* 112:248-253.

Liu Z, Ma C, Guo C, Xu P (2012) Efficient utilization of hemicellulose hydrolysate for propionic acid production using *Propionibacterium acidipropionici*. *Bioresour. Technol.* 114:711-714.



A propionic acid fermentation process with non-treated or hydrolyzed molasses as carbon source reached a high propionic acid concentration of 92 grams per liter with a yield of 0.46 gram per gram and a productivity of 0.36 gram per liter per hour as reported by Feng et al in 2011. Respective references are given below, if you wish you can browse through them later on.

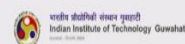
Sugarcane bagasse is another cheap and abundant byproduct of sugar refining, which can be hydrolyzed into glucose and xylose and then used as carbon source for propionic acid production achieving a titer of 51 gram per liter, yield of 0.43 gram per gram and a productivity of 0.71 gram per liter per hour. Corn cob molasses, a hemicellulose hydrolysate containing glucose, xylose and arabinose was used as a carbon source from the *P. acidipropionici*.

Now the three sugars in the corn cob molasses are simultaneously converted to a propionic acid titer of 71.8 grams per liter with the productivity of 0.28 grams per liter per hour in a fed-batch fermentation system with an initial cell density of 14.8 gram per liter. I think you can recall that we have discussed about what is a batch process, what is the fed-batch process, what is a continuous process. So, fed-batch process has its own advantages, the productivity actually increases.

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- In addition to the above food crop residues, *non-food crops* also can be used as low-cost biomass feedstock in biorefinery. For example, *Jerusalem artichoke* hydrolysate containing mainly fructose was also used for propionic acid fermentation, achieving a final titer of 68.5 g/L, yield of 0.48 g/g, and productivity of 1.55 g/L h (Liang *et al.*, 2012).
- In addition to the agricultural and food processing wastes mentioned above, *crude glycerol* as a low-value by-product from biodiesel production has also become an attractive substrate for propionic acid fermentation.
- Glycerol has a *higher reduction level* compared to glucose, sucrose, and lactate, and is thus beneficial to intracellular redox balance and propionic acid production.
- In general, a high P/A ratio of greater than 10-20 can be obtained with glycerol in propionic acid fermentation.

Liang Z, Li L, Li S, Gu Y, Yang S-T, Wang J (2012) Enhanced propionic acid production from Jerusalem artichoke hydrolysate by immobilized *Propionibacterium acidipropionici* in a Diazo-fed bioreactor. *Bioresource Eng.* 31: 915-921.



In addition to the above food crop residues, non-food crops can also be used as low-cost biomass feedstock in the biorefineries. For example, Jerusalem artichoke hydrolysate containing mainly fructose was also used for propionic acid fermentation achieving a final titer of 68.5 grams per liter, yield of 0.48 grams per gram and a productivity of 1.55 grams per liter per hour.

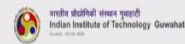
In addition to the agricultural and food processing wastes mentioned above, crude glycerol as a low-value byproduct from biodiesel production has also become an attractive substrate for propionic acid fermentation. Glycerol has a higher reduction level compared to glucose,

sucrose and lactate and is thus beneficial to intracellular redox balance and propionic acid production. In general, a high P/A ratio of greater than 10 to 20 can be obtained with glycerol in propionic acid fermentation.

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- High propionic acid production (106 g/L) was obtained in *immobilized-cell fermentation* in a fibrous bed bioreactor (FBB), with *glycerol* as carbon source (Zhang and Yang, 2009b).
- The fermentation performance with crude glycerol was comparable to that with pure glycerol, indicating *propionibacteria* was not inhibited by the impurity components, such as methanol, fatty acids, and salts, present in biodiesel wastes (Zhang and Yang, 2009b).
- Himmi *et al.* (2000) reported a propionic acid yield of 0.64 g/g and productivity of 0.42 g/L h from glycerol with *P. acidipropionici* ATCC 25562.
- Ruhul and Choudhury (2012) also reported the production of propionic acid and trehalose using *P. freudenreichii* subsp. *shermanii* and crude glycerol from biodiesel waste.

Zhang A, Yang S-T (2009). Propionic acid production from glycerol by metabolically engineered *Propionibacterium acidipropionici*. *Process Biochem*, 44:1346-1351.
Ruhul R, Choudhury B (2012). Use of an osmotically sensitive mutant of *Propionibacterium freudenreichii* subsp. *shermanii* for the simultaneous production of organic acids and trehalose from biodiesel waste based crude glycerol. *Bioresour Technol*, 109:131-139.



High propionic acid production of 106 gram per liter was obtained in immobilized-cell fermentation in a fibrous bed bioreactor with glycerol as sole carbon source. The fermentation performance with crude glycerol was comparable to that with pure glycerol indicating *Propionibacteria* was not inhibited by the impurity components such as methanol, fatty acids and salts present in the biodiesel wastes.

So, this is the best part of this particular research work that is reported by Zhang and Yang and the reference is given below. Himmi *et al* in 2000 reported a propionic acid yield of 0.64 per gram per gram and a productivity of 0.42 gram per liter per hour from glycerol with *P. acidipropionici* ATCC 25562 strain. Ruhul and Choudhary also reported the production of propionic acid and trehalose using the *P. freudenreichii* subspecies *shermanii* and crude glycerol from the biodiesel waste.

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- Liu et al. (2011) reported propionic acid yield and productivity of 0.57 g/g and 0.15 g/L h, respectively, from the mixture of glycerol and glucose at a mass ratio of ~2 with *P. acidipropionici* ATCC 4965.
- Although free-cell fermentation of glycerol usually suffered from a low productivity due to in vivo NADH imbalance, a notably high productivity of 1.44 g/L h was reached by continuous fermentation with immobilized cells (Dishisha et al. 2012a).
- In summary, abundant low-cost renewable feedstocks are available for propionic acid fermentation, and they offer an opportunity to reduce the cost of bio-based propionic acid.



Liu et al in 2011 reported propionic acid yield and productivity 0.57 gram per gram and 0.15 gram per liter per hour respectively from the mixture of glycerol and glucose at a mass ratio of 2 with *P. acidipropionici* ATCC 4965. So it is a very important work in which actually they used two different types of substrates as a carbon source - a mixture of glycerol as well as glucose.

Although free-cell fermentation of glycerol usually suffered from low productivity due to the in vivo NADH imbalance, a notably high productivity of 1.44 gram per liter per hour was reached by continuous fermentation with immobilized cells. So, what is being done and reported by Dishisha et al is that, to balance the NADH inside the fermentation media, they have gone for the continuous fermentation process with the immobilized cells of the propionic acid bacteria.

So, in summary abundant low-cost renewable feedstocks are available for propionic acid fermentation and they offer an opportunity to reduce the cost of the bio-based propionic acid.

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- *Acetic acid* (CH₃COOH) is a colorless and weak organic acid with sour taste and pungent smell.
- Acetic acid is an important raw material in the chemical industry. The largest industrial application of acetic acid consists of the production of vinyl acetate monomer, acetic anhydride, and various esters. Acetic acid is also used as a solvent in chemical reactions such as the production of terephthalic acid, the raw material for polyethylene terephthalate.
- The global demand for acetic acid is around 6.5 million tons per year (Mt/a), of which approximately 1.5 Mt/a is recycled; the remainder is manufactured from petrochemical feedstock or from biological sources.
- About 75% of acetic acid in the chemical industry is produced by *carbonylation of methanol with carbon monoxide* (Yoneda et al., 2001).
- Only about 10% of acetic acid is produced by *fermentation, mainly as vinegar*.

Yoneda, N., Kawanishi, S., Yano, M., Fujimori, P., Wichterle, S. (2001). Recent advances in processes and catalysts for the production of acetic acid. Appl. Catal. A Gen. 211,219-265.



So, now we will discuss about the acetic acid, another very important commodity chemical and that is being now manufactured in a large scale from different biomasses. Acetic acid is a colorless and weak organic acid with sour taste and pungent smell. Acetic acid is an important raw material in the chemical industry. The largest industrial application of acetic acid consists of the production of the vinyl acetate monomer, acetic anhydride and various esters.

Acetic acid is also used as a solvent in chemical reactions such as the production of terephthalic acid, the raw material for the polyethylene terephthalate. The global demand for acetic acid is around 6.5 million tons per year of which approximately 1.5 million tonne per year is recycled, the remainder is manufactured from petrochemical feedstock or from biological sources.

The biological sources of course it is less than that of the petroleum or chemical stock, but as we have discussed it has to increase. So, about 75% of the acetic acid in the chemical industry is produced by carbonylation of methanol with carbon monoxide, the chemical pathway, chemical process. Only about 10% of acetic acid is only produced by the fermentation mainly as vinegar.

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Microbial Production of Acetate

- Acetic acid is produced naturally as the sole or main fermentation product by anaerobic *homoacetogens* and aerobic *acetic acid bacteria*.
- Acetic acid bacteria contain 12 genera belonging to the family of *Acetobacteraceae*. They are aerobic, gram-negative or gram-variable, and ellipsoidal to rod-shaped microbes which oxidize ethanol to acetate in aerobic vinegar fermentation.
- A high acetate concentration of up to 20% (w/v) can be obtained in aerobic vinegar fermentation with about 60% (w/w) yield from sugar. The process generally involves two steps: (1) fermentation of sugar to ethanol by yeasts such as *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*, and (2) oxidation of ethanol to acetic acid by species of *Acetobacter*.
- In contrast, anaerobic *homoacetogens* produce only acetate from sugar at a theoretical yield of 3 mol acetate per mol glucose with an actual fermentation yield of higher than 85% (w/w).



So, let us talk about the microbial production of the acetate. Acetic acid is produced naturally as the sole or main fermentation product by anaerobic *homeacetogens* and aerobic acetic acid bacteria. Acetic acid bacteria contain 12 genera belonging to the family *Acetobacteraceae*. They are aerobic, gram-negative or gram-variable and ellipsoidal to rod-shaped microbes which oxidize ethanol to acetate in aerobic vinegar fermentation.

A high acetate concentration of up to 20% weight by volume can be obtained in aerobic vinegar fermentation with about 60% weight by weight yield from sugar. The process generally involves 2 steps. The first one is that fermentation of sugar to ethanol by yeasts such as *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* and second step is oxidation of ethanol to acetic acid by the species of *Acetobacter*.

In contrast, anaerobic *homoacetogens* produce only acetate from sugar at a theoretical yield of 3 mol of acetate per mole of glucose with an actual fermentation yield of higher than 85% weight by weight.

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Table 3. Homoacetogens and their optimal growth conditions and substrates

Microorganisms	Substrates	Optimal Temperature and pH	References
<i>Moorella thermoacetica</i> (<i>Clostridium thermoaceticum</i>)	Fructose, pyruvate, glucose, xylose, lactate, CO ₂ /H ₂ , CO/H ₂	60°C, 7.0	Andreesen et al. (1973); Kerby and Zeikus (1983)
<i>M. thermoautotrophica</i> (<i>Clostridium thermoautotrophicum</i>)	Fructose, glucose, galactose, glycerate, methanol, lactate, formate, CO ₂ /H ₂ , CO/H ₂	60°C, 5.7	Wiegel et al. (1981)
<i>Clostridium aceticum</i>	Fructose, pyruvate, CO ₂ /H ₂ , CO/H ₂	30°C, 8.3	Braun et al. (1981)
<i>Clostridium formicoaceticum</i>	Fructose, pyruvate, pectin, lactate, galacturonate	37°C, 7.6	Andreesen et al. (1970)
<i>Clostridium magnum</i>	Fructose, glucose, sucrose, xylose, citrate, malate	30°C, 7.0	Schink (1984)
<i>Acetobacterium woodii</i>	Fructose, glycerate, glucose, lactate, formate, CO ₂ /H ₂	30°C, 6.7	Balch et al. (1977)
<i>Acetobacterium carbinolicum</i>	Fructose, glucose, pyruvate, lactate, formate, aliphatic alcohols C ₁ -C ₅ , CO ₂ /H ₂	27°C, 7.0	Eichler and Schink (1984)
<i>Acetogenium kivui</i>	Fructose, pyruvate, formate, mannose, glucose, CO/H ₂	66°C, 6.4	Leigh et al. (1981)

Yang et al., *Acetobacter Fermentations for the production of acetic acid*, 2011, 351-374



So table 3 has listed the different types of *homoacetogens* and their optimal growth conditions and the respective substrates they could degrade to produce acetic acid. So we will go through one or two. So *Clostridium thermoaceticum*. So it can degrade fructose, pyruvate, glucose, xylose, lactate, even carbon dioxide and hydrogen. So, the optimal conditions is around 60 degrees centigrade and the pH is 7.

Similarly, there are many *Clostridium aceticum*, *Clostridium formicoaceticum*, *Clostridium magnum*. So all those can again degrade fructose, pyruvate, pectin, lactate, then malate, xylose, citrate all these things and their respective optimal conditions are also given. So, just we can understand that there are many different types of microorganisms are there and they are also efficient enough to degrade various types of substrate at a particular optimal condition.

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- **Butyric acid** ($\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$) is a four-carbon carboxylic acid, present as an oily colorless liquid that is soluble in water, ethanol, and ether.
- Butyric acid has an unpleasant smell and acidic taste.
- It is naturally found in butter, parmesan cheese, vomit, and as a product of some anaerobic fermentation.
- Butyric acid and its derivatives have been widely used in food, chemical, and pharmaceutical industries. The acid form is used to enhance the *butter-like flavor in food*, and the ester form can serve as *flavor in food and perfume*.
- Butyric acid can also be used to synthesize *cellulose butyrate plastics* for textile fiber production.
- As butyrate can provide protection against colon cancer and colitis (*Archer et al., 1998*); various butyrate derivatives are considered as antineoplastic drugs for treating hemoglobinopathies, cancer, and gastrointestinal diseases (*Hamer et al., 2008*).

Archer S, Meng SF, Wu J, Johnson J, Tang R, Hahn R (1998). Butyrate inhibits colon carcinoma cell growth through two distinct pathways. *Surgery* 124:249-253.
Hamer BM, Jenkins D, Yamada K, Vashistha S, Tsoo FJ, Brasseur PJ (2008). Review article: The role of butyrate on colonic function. *Aliment Pharmacol Ther* 22:114-119.



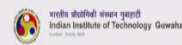
Now, we will talk about butyric acid. So, butyric acid is a four carbon carboxylic acid present as an oily colorless liquid that is soluble in water, ethanol and ether. Butyric acid has an unpleasant smell and acidic taste. It is naturally found in butter, parmesan cheese, vomit and as a product of some anaerobic fermentation. Butyric acid and its derivatives have been widely used in food, chemical and pharmaceutical industries.

The acidic form is used to enhance the butter like flavor in food and the ester form can serve as a flavor in the food and perfume. Butyric acid can also be used to synthesize cellulose butyrate plastics for the textile fiber production. As butyrate can provide protection against colon cancer and colitis, various butyrate derivatives are considered as antineoplastic drugs for treating hemoglobinopathies, cancer and gastrointestinal diseases. It has a wide medicinal application.

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- Butyric acid producing anaerobic bacteria have also been proposed as a novel probiotic treatment for inflammatory bowel disease (Van Immerseel *et al.*, 2010).
- Moreover, acids produced from a fermentation process are more favorable than chemically synthesized counterparts for *applications in food and pharmaceutical industries*.
- There are many bacterial strains producing butyric acid, which belong to the genera *Clostridium*, *Butyribacterium*, *Butyrivibrio*, *Sarcina*, *Eubacterium*, *Fusobacterium*, *Megasphaera*, *Roseburia*, and *Coprococcus*.
- The genera *Clostridium*, *Butyribacterium*, and *Butyrivibrio* are the most studied butyric acid producing microorganisms. However, the preferred strains for potential commercial uses are in the genus *Clostridium* due to their high and stable productivity.
- Table 4 lists butyrate producing *Clostridium* species with their substrates and fermentation products. They are gram-positive, chemo-organotrophic, strict anaerobes, and spore formers.

Van Immerseel F, Decanlis E, De Vos M, Sassi N, Van De Wiele T, Verbeke K, Raemdonck P, Sani B, Lenoir P, Floridi H (2010) Butyric acid-producing anaerobic bacteria as a novel probiotic treatment approach for inflammatory bowel disease. *J Med Microbiol*; 59:141-143.



Butyric acid producing anaerobic bacteria have also been proposed as a novel probiotic treatment for inflammatory bowel disease. Moreover, acids produced from a fermentation process are more favorable than chemically synthesized counterparts for applications in food and pharmaceutical industries. There are many bacterial strains producing butyric acid which belong to the genera *Clostridium*, *Butyribacterium*, *Butyrivibrio*, *Sarcina*, *Eubacterium*, *Fusobacterium*, *Megasphaera*, *Roseburia*, and *Coprococcus*.

The genera *Clostridium*, *Butyribacterium* and *Butyrivibrio* are the most studied butyric acid producing microorganisms. However, the preferred strains for potential commercial uses are in the genus *Clostridium*. The reason is that they have high and stable productivity performance. The next table gives us the butyrate producing *Clostridium* species with their substrates and the fermentation products. They are gram-positive, chemo-organotrophic, strict anaerobes and spore formers.

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Table 4. *Clostridium* species producing butyrate as the major product

<i>Clostridium</i> Species	Substrates	Products*	References
<i>Clostridium aminobutyricum</i>	γ -Aminobutyrate	ACETATE, BUTYRATE, NH ₃	Hardman and Stadman (1960)
<i>Clostridium butarii</i>	Glucose, cellobiose, fructose, galactose, lactose, mannose, sucrose, maltose	ACETATE, BUTYRATE, lactate, H ₂	Cato et al. (1982); Nakamura et al. (1973)
<i>Clostridium barkeri</i>	Glucose, fructose, ribose, mannitol, pyruvate	BUTYRATE, ACETATE, lactate, propionate, H ₂	Stadman et al. (1972)
<i>Clostridium butyricum</i>	Glucose, starch, disaccharides, sugars	BUTYRATE, ACETATE, succinate, formate, lactate, CO ₂ , H ₂	Cummins and Johnson (1971)
<i>Clostridium carboxidivorans</i>	CO, CO ₂ /H ₂ , glucose, galactose, fructose, xylose, mannose, cellobiose, trehalose, cellulose, starch	ACETATE, ETHANOL, butyrate, butanol	Liou et al. (2005)
<i>Clostridium cellulovorans</i>	Cellulose, xylan, pectin, cellobiose, glucose, maltose, galactose, sucrose, lactose, mannose	BUTYRATE, ACETATE, formate, lactate, CO ₂ , H ₂	Sleat et al. (1984)
<i>Clostridium cochlearium</i>	Glutamate, histidine	ACETATE, BUTYRATE, CO ₂ , H ₂ , NH ₃	Laanbroek et al. (1979)
<i>Clostridium herbivorans</i>	Cellulose, cellobiose, maltose, starch	BUTYRATE, FORMATE, ethanol, H ₂	Varel et al. (1995)
<i>Clostridium kluyveri</i>	Ethanol, acetate (+CO ₂), propanol, succinate	BUTYRATE, CAPROATE, H ₂	Schobert and Gottscha (1969)
<i>Clostridium longiporum</i>	Glucose, cellobiose, cellulose, fructose, galactose	BUTYRATE, ACETATE, FORMATE, ethanol	Varel et al. (1995)
<i>Clostridium lortetii</i>	Amino acids, glucose, fructose, maltose, starch	ACETATE, propionate, butyrate, isobutyrate, isovalerate, H ₂ , NH ₃	Oren (1983)
<i>Clostridium mangenotii</i>	Amino acids, proteins, pyruvate	ACETATE, ISOVALERATE, propionate, butyrate, isobutyrate, H ₂ , NH ₃	Elsden and Hilton (1978)

Yang et al., *Acetate: Intermediates for the production of acetic and butyric acids*, 2013, 211-214.



You can see that various types of *Clostridium* species producing butyrate as the major product, we will just see one or two. The first one *Clostridium aminobutyricum*. So gamma aminobutyrate is the substrate. The products are acetate, butyrate and ammonia. Similarly, if you will come down and see *Clostridium barkeri*, so it can degrade glucose, fructose, ribose, mannitol, pyruvate and finally produce butyrate, acetate, lactate, propionate and hydrogen.

So, there are many others also there. So you can later on go through it and have a feeling that what are the different types of *Clostridium* species have been used to produce various products such as acetate, butyrate from different types of substrates.

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- Most butyric acid producing *clostridia* ferment glucose, xylose, lactose, starch, and glycerol for cell growth and butyric acid production.
- Other substrates such as cellulose and CO₂ can also be used for butyrate production.
- *Clostridium carboxidivorans* (Datar et al., 2004) and *Butyrivibrio methylotrophicum* (Worden et al., 1989) can utilize CO, and CO₂ and H₂ to produce butyric acid.
- *Clostridium cellulovorans*, *C. carboxidivorans*, *Clostridium polysaccharolyticum*, and *Clostridium populeti* can use cellulose for butyrate synthesis.
- *C. kluyveri* can produce butyric acid as a major product from ethanol and acetate as substrates (Ljungdahl et al., 1989).
- Most *Clostridium* species produce butyrate and acetate along with additional products such as ethanol and lactate.

Datar SP, Shrivastava KM, Choudhury BG, Haldar RL, Levin RS (2004) Fermentation of biomass-generated producer gas to ethanol. *Biotechnol. Bioeng.* 86:51-54.
 Worden RM, Gerstein AJ, Zeman KS, Datta R (1989) Butyrate production from carbon monoxide by *Butyrivibrio methylotrophicum*. *Appl. Environ. Microbiol.* 55:61-69.
 Ljungdahl LG, Hagerholm J, Wergé J (1989) Acetogenic and acid-producing *Clostridia*. In: *Microb. Syst. Cyclic D. 6th. Chloride*. Plenum Press, New York, pp. 145-193.



Most butyric acid producing clostridia ferment glucose, xylose, lactose, starch and glycerol for the cell growth and butyric acid production. Other substrates such as cellulose and carbon

dioxide can also be used for butyrate production, *Clostridium carboxidivorans* and *Butyribacterium methylotrophicum* can utilize carbon monoxide and carbon dioxide and hydrogen to produce butyric acid.

Clostridium cellulovorans, then *Clostridium carboxidivorans*, *Clostridium polysaccharolyticum* and *Clostridium populeti* can use cellulose for butyrate synthesis. Similarly, *Clostridium kluyveri* can produce butyric acid as a major product from ethanol and acetate as substrates. Most *Clostridium* species produce butyrate and acetate along with additional products such as ethanol and lactate, but they are in very low concentration.

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- Some *Clostridium* species such as *Clostridium acetobutylicum*, *Clostridium saccharolyticum*, and *Clostridium thermocellum* can be manipulated to form either solvents or acids as the main products depending on culture conditions.
- Several species, including *Clostridium butyricum*, *Clostridium tyrobutyricum*, and *Clostridium thermobutyricum* produce butyrate as the main product with a relatively high productivity and yield, and they are thus of high interest for potential industrial production of butyrate.
- The optimal temperature and pH for *C. butyricum* and *C. tyrobutyricum* are 30~37 °C and 4.5~7.0, respectively (Zigova and Sturdik, 2000).
- *C. thermobutyricum* is a moderate thermophile with the optimal growth temperature of 55 °C and pH 7.0 (Canganella et al., 2002).
- *C. butyricum* is classified into non-toxigenic and toxigenic species. Only non-toxigenic *C. butyricum* can be used for butyric acid production.

Zigova J, Sturdik E (2000). Advances in biotechnological production of butyric acid. J Ind Microbiol Biotechnol. 24:133-140.
Canganella F, Kok S, Magan H, Wapel J (2002). Clostridial Biotechnology: Growth studies and stimulation of butyrate formation by acetate supplementation. Microbiol. Rev. 157:149-156.



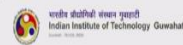
Some *Clostridium* species such as *Clostridium acetobutylicum*, *Clostridium saccharolyticum*, and *Clostridium thermocellum* can be manipulated to form either solvents or acids as the main product depending on the culture conditions. Several species including *Clostridium butyricum*, *Clostridium tyrobutyricum* and *Clostridium thermobutyricum* can produce butyrate as the main product with a relatively high productivity and yield and they are thus of high interest for potential industrial production of butyrate.

The optimal temperature and pH for the *Clostridium butyricum* and *Clostridium tyrobutyricum* are 30 to 37 degrees centigrade and 4.5 to 7 respectively. *Clostridium thermobutyricum* is a moderate thermophile with the optimal growth temperature of 55 degrees centigrade and a pH of 7. *Clostridium butyricum* is classified into non-toxigenic and toxigenic species. Only non-toxigenic *C. butyricum* can be used for butyric acid production.

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- Butyric acid bacteria can use a wide variety of carbon sources. To date, various feedstocks have been studied for butyric acid production by fermentation (see Table 5).
- Butyric acid production from *cheese whey*, a by-product from cheese manufacturing containing mainly lactose and whey protein, by *C. beijerinckii* at pH 5.0 gave a final concentration of 12 g/L, yield of 0.25 g/g, and productivity of 0.6 g/L h (Alam *et al.*, 1988).
- *Cane molasses*, a by-product of the sugar industry, containing approximately 45–50% (w/w) total sugars, was used as the substrate to produce 26.2 g/L butyric acid with yield of 0.47 g/g and productivity of 4.13 g/L h (Jiang *et al.*, 2009).
- *Cornmeal* after hydrolysis of starch with amylases was used for butyric acid production by *C. tyrobutyricum* immobilized in an FBB at 37 °C, pH 6.0, giving a high butyric acid productivity of 6.78 g/L h, yield of 0.45 g/g, and final concentration of 46 g/L (Huang *et al.*, 2002).

Alam S, Stevens D, Bagan R (1988). Production of butyric acid by batch fermentation of cheese whey with *Clostridium beijerinckii*. J. Ind. Microbiol. 2:359-364
 Jiang L, Wang L, Liang S, Wang X, Cai P, et al. (2009). Butyric acid fermentation in a fibrous bed bioreactor with immobilized *Clostridium tyrobutyricum* from cane molasses. Bioprocess Technol. 100:1403-1409
 Huang YL, Wu Z, Zhang L, Chang CM, Yang S-T (2002). Production of carboxylic acids from hydrolyzed corn feed by immobilized cell fermentation in a fibrous bed bioreactor. Bioprocess Technol. 82:51-59



Butyric acid bacteria can use a wide variety of carbon sources. To date, various feedstocks have been studied for butyric acid production by fermentation. It is presented in table 5, we will come back to it later. So butyric acid production from cheese whey, a byproduct from the cheese manufacturing containing mainly lactose and whey protein by *Clostridium beijerinckii* at pH 5 gave a final concentration of 12 grams per liter (it is a very good concentration actually), yield of 0.25 gram per gram and a productivity a 0.6 gram per liter per hour.

Cane molasses, a byproduct of the sugar industry containing approximately 45 to 50% weight by weight total sugars was used as substrate to produce 26.2 grams per liter butyric acid with a yield of 0.47 gram per gram and a productivity of 4.13 grams per liter per hour, it is a very good productivity.

Corn meal after hydrolysis of starch with amylases was used for butyric acid production by *Clostridium tyrobutyricum* immobilized in an FBB at 37 degrees centigrade, pH 6 giving a high butyric acid productivity of 6.78 grams per liter per hour and a yield of 0.45 gram per gram and a final concentration of 46 gram per liter.

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Table 5. Butyric acid production from different substrates

Substrate	Strain	Concentration (g/L)	Productivity (g/L-h)	Yield (g/g)	References
Cheese whey	<i>Clostridium beijerinckii</i>	12.0	0.6	0.25	Alam et al., (1988)
Brown algae	<i>Clostridium tyrobutyricum</i>	11.0	0.1	0.11	Song et al. (2011)
Cane molasses	<i>C. tyrobutyricum</i>	26.2	4.13	0.47	Jiang et al. (2009)
Corn fiber/CSL	<i>C. tyrobutyricum</i>	44.1	2.91	0.47	Zhu et al. (2002)
Cornmeal	<i>C. tyrobutyricum</i>	46.0	6.78	0.45	Huang et al. (2002)
Jerusalem artichoke	<i>C. tyrobutyricum</i>	27.5	2.75	0.44	Huang et al., (2011a)
Wheat flour	<i>C. tyrobutyricum</i>	62.8	1.25	0.45	Fayolle et al. (1990)

CSL, corn steep liquor.

Yang et al., Anaerobic fermentations for the production of acetic and butyric acids, 2013, 333-374.



Yes, so we can go through this table which lists some of the butyric acid production from different substrates, the strains, the concentration, productivity and yield. Let us see the cheese whey by using the *Clostridium beijerinckii*. So, the concentration is 12 grams per liter, productivity is 0.6 gram per liter per hour, yield is 0.25 grams per gram. So, if you look at the third one the cane molasses using the *Clostridium tyrobutyricum* you can see the concentration is 26.2, productivity has increased many fold 4.13. Similarly, if you look at cornmeal, again the same *Clostridium* species, you can see that productivity has increased many fold again 6.78 grams per liter per hour. So, these are some of the classical works that are reported by various authors and the main references given here Yang et al. So, you can browse it if you are interested more in detail. So, let us move ahead.

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- Butyric acid production from *wheat flour* gave a high final butyric acid concentration of 62.8 g/L, productivity of 1.25 g/L h, and yield of 0.45 g/g glucose (Fayolle *et al.*, 1990).
- *Jerusalem artichoke* after treating with dilute acid to hydrolyze its carbohydrates, mainly inulin, to fructose and glucose, was used for butyric acid production in repeated-batch fermentations (Huang *et al.*, 2011). The butyric acid productivity, yield, and final concentration from the fermentation were 2.75 g/L h, 0.44 g/g, and 27.5 g/L, respectively.
- Most of the *clostridial* species are also capable of fermenting pentoses such as xylose.
- Butyric acid fermentation with *corn fiber hydrolysate* (CFH) containing glucose, xylose, and arabinose as the carbon source and CSL as the nitrogen source gave a high butyrate yield of 0.47 g/g and productivity of 2.91 g/L h (Zhu *et al.*, 2002).

Fayolle F, Machet R, Beldrea D (1990). Effect of controlled substrate feeding on butyric acid production by *Clostridium tyrobutyricum*. J. Ind. Microbiol. 6:179-183.
Huang J, Cai L, Wang J, Zhu X, Huang L, Yang S-T, Wu Z (2011). Efficient production of butyric acid from Jerusalem artichoke by immobilized *Clostridium tyrobutyricum* in a stirred-bed bioreactor. Biotechnol. 112:3923-3928.
Zhu Y, Wu ZT, Yang ST (2002). Butyric acid production from acid hydrolysate of corn fiber by *Clostridium tyrobutyricum* in a stirred-bed bioreactor. Process Biochem. 38:617-666.




So, butyric acid production from wheat flour gave a high final butyric acid concentration of 62.8 grams per liter, productivity of 1.25 grams per liter per hour and a yield of 0.45 gram per gram of glucose. Jerusalem artichoke after treating with dilute acid to hydrolyze its carbohydrate, mainly inulin, to fructose and glucose was used for butyric acid production in repeated batch fermentations.

The butyric acid productivity, yield and final concentration from the fermentation were 2.75 grams per litre per hour, 0.44 gram per gram and 27.5 grams per liter respectively. Most of the *Clostridial* species are also capable of fermenting pentose such as xylose. Butyric acid fermentation with corn fiber hydrolysate containing glucose, xylose and arabinose as the carbon source and corn steep liquor as the nitrogen source gave a high butyrate yield of 0.47 gram per gram and the productivity of 2.91 gram per liter per hour.

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- In addition, butyric acid production from brown algae treated with dilute H₂SO₄ for hydrolysis of its polysaccharides, mainly galactan, have also been studied (Song et al., 2011).
- These studies demonstrated that butyric acid can be produced from various biomass, including processing wastes and agricultural residues.
- However, further improvements in the fermentation process are needed for economic production of bio-butyrac acid.

Song J-H, Verman J-RS, Lee C-H, Jang D (2011). Butyric acid production from brown algae using *Clavibacterium xyloxyticum* ATCC 25735. *Bioresource Eng.* 16:42-49



In addition, butyric acid production from brown algae treated with dilute sulfuric acid for hydrolysis of its polysaccharides mainly the galactan have also been studied. It is also a very classical work. These studies demonstrated that butyric acid can be produced from various biomasses including processing waste and agricultural residues. However, further improvements in the fermentation processes are needed for economic production of the bio-butyrac acid.


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(Overview of next lecture)

Module	Module name	Lecture	Title of lecture
11	Organic Commodity Chemicals from Biomass	03	1,3-propanediol, 2,3-butanediol, PHA

Thank you

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So with this, I wind up today's lecture. If you have any query, please register it in the Swayam portal or you can always drop a mail to me at kmohanty@iitg.ac.in. In our next lecture under this module, we will be discussing about 2-3 more such commodity chemicals; 1, 3-propanediol, 2, 3-butanediol and the PHA. Thank you very much.