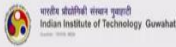


**Biomass Conversion and Biorefinery**  
**Prof. Kaustubha Mohanty**  
**Department of Chemical Engineering**  
**Indian Institute of Technology - Guwahati**

**Lecture – 33**  
**1, 3-Propanediol, 2, 3-Butanediol, PHA**

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Module	Module name	Lecture	Content
11	Organic Commodity Chemicals from Biomass	03	1,3-Propanediol, 2,3-Butanediol, PHA

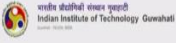


Good morning students. This is lecture 3 under module 11. As you know, we are discussing about the commodity chemicals from biomass. Today, we will discuss about precisely 3 very important commodity chemicals: 1, 3-Propanediol, 2, 3-Butanediol and then PHA. So, let us begin.

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- **1,3-Propanediol** can be used as an essential monomer to synthesize a new type of polyester, polytrimethylene terephthalate (PTT), and as an ingredient in other polymers, cosmetics, foods, lubricants, and medicine.
- 1,3-Propanediol is a three-carbon diol with hydroxyl groups at 1 and 3 carbon positions.
- It is one of the oldest known fermentation products, described as early as 1881 by August Freund in the Glycerol-fermenting anaerobic cultures containing *Clostridium pasteurianum* (Freund, 1881).
- Having two functional groups, 1,3-Propanediol is a high-value specialty chemical in polymer synthesis, in particular as a monomer for polyesters, polyethers, and polyurethanes.
- For instance, polyester associated with 1,3-Propanediol (i.e., PTT) exhibits unique features such as the elastic recovery of nylon and superior stretching (Kurian, 2005; Saxena et al., 2009), which are difficult to obtain from other glycol-based polyesters.

Freund A (1881). Über die Bildung und Darstellung von triäthylenglycol aus glycerin. Monats. Chem. 7: 651–661.  
Kurian PT (2005). A new polymer platform for the future—Kevlar® from corn-derived 1,3-propanediol. J Polym. Technol. 11: 139–167.  
Saxena RK, Anand P, Saini S, Jee J (2009). Microbial production of 1,3-propanediol: Recent developments and emerging opportunities. Biotechnol. Adv. 27: 895–913.




We will start our discussion about 1, 3-Propanediol. 1, 3-Propanediol can be used as an essential monomer to synthesize a new type of polyester that is polytrimethylene terephthalate (very well known as PTT), and as an ingredient in other polymers, cosmetics, foods, lubricants and medicines. 1, 3-Propanediol is a 3 carbon diol with hydroxyl groups at 1 and 3 carbon positions, that is why the name is 1, 3-Propanediol. It is one of the oldest known fermentation products described as early as 1881 by August Freund in the glycerol-fermenting anaerobic culture containing *Clostridium pasteurianum*.

Having two functional groups, 1, 3-Propanediol is a high-value speciality chemical in polymer synthesis, in particular as a monomer for polyesters, polyethers and polyurethanes. For instance, polyester associated with 1, 3-Propanediol exhibits unique features such as the elastic recovery of nylon and superior stretching which are difficult to obtain from other glycerol based products.

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- 1,3-Propanediol has been produced mainly from *petroleum derivatives* such as acrolein, using hydration and hydrogenation catalytic reaction (*Degussa-DuPont route*) and ethylene oxide using the hydroformylation and hydrogenation catalytic reactions (*Shell route*) (*Saxena et al., 2009*).
- In addition to the chemical catalytic processes, DuPont and its collaborator, Genencor International, Inc., have developed the glucose-to-1,3-propanediol biological process after an extensive effort was undertaken to make metabolically engineered *Escherichia coli* (*Um and Kim, 2013*).
- Considering problems with the conventional chemical process for 1,3-Propanediol production (*petroleum-based materials for sources, high pressure, and temperature in processes, as well as environmental aspects caused by toxic chemicals and intermediates*), the fermentation-based 1,3-Propanediol production as an economically competitive chemical process has been recognized as one of the novel biological processes representing a paradigm shift from petroleum products to biomass-based renewable products.

Saxena RK, Anand P, Saini S. Jan 11 2009 | Microbial production of 1,3-propanediol: Recent developments and emerging opportunities. Biotechnol. Adv. 27: 891-913.  
Um, Y. and Kim, K.D., 2013. Biotechnological Development for the Production of 1, 3-Propanediol and 2, 3-Butanediol. Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers, pp 339-414.



1, 3-Propanediol has been produced mainly from petroleum derivatives such as acrolein, using hydration and hydrogenation catalytic reactions (the well known Degussa-DuPont route) and ethylene oxide using the hydroformylation and hydrogenation catalytic reactions (the well known Shell route). Now, in addition to the chemical catalytic processes DuPont and its collaborator Genencor International have developed the glucose to 1, 3-Propanediol biological process after an extensive effort was undertaken to make metabolically engineered *Escherichia coli*. So, Genencor is a well known company which manufactures different types of enzymes. So, what they have done is that they have metabolically engineered the *E. coli* and both DuPont and Genecor have developed the process of making 1, 3-Propanediol using

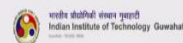
a biological route from glucose. Considering problems with the conventional chemical process for the 1, 3-propanediol production (so those are petroleum based material for sources, high pressure and temperature in processes as well as environmental aspects caused by the toxic chemicals and the intermediates), the fermentation based 1, 3-Propanediol production as an economically competitive chemical process has been recognized as one of the novel biological processes representing a paradigm shift from the petroleum products to biomass based renewable products.

So, this is actually true for all other chemicals which we have been discussing since last 2 classes that due to the problem, mostly environmental problems, then the depletion of the fossil based resources there is a need to look for biomass based platform to develop or produce such platform and commodity chemicals.

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- Considerable attention has been paid to 1,3-Propanediol production other than from glucose, especially *glycerol*, because a large amount of raw glycerol is generated as a by-product in the range of 10% (w/w) of biodiesel during the production of biodiesel (*Mu et al., 2006*).
- Unlike the chemical conversion of glycerol to 1,3-Propanediol, the biological conversion of glycerol to 1,3-Propanediol is an *environmentally friendly and economical process* due to the *low energy requirement with mild reaction conditions* compared to chemical processes and a higher yield of 1,3-Propanediol than that achieved via chemical reaction.
- 1,3-Propanediol can be produced naturally under anaerobic/microaerobic conditions by various microorganisms, including the genera *Klebsiella* (*Klebsiella pneumoniae*), *Clostridia* (*Clostridium butyricum* and *Clostridium pasteurianum*), *Enterobacter* (*Enterobacter agglomerans*), *Citrobacter* (*Citrobacter freundii*), and *Lactobacilli* (*Lactobacillus brevis* and *Lactobacillus buchneri*).

Ma Y, Tang H, Zhang D-J, Wang W, Xia Z-L (2006) Microbial production of 1,3-propanediol by *Klebsiella pneumoniae* using crude glycerol from biodiesel preparation. *Biotechnol Lett* 28: 1735–1739



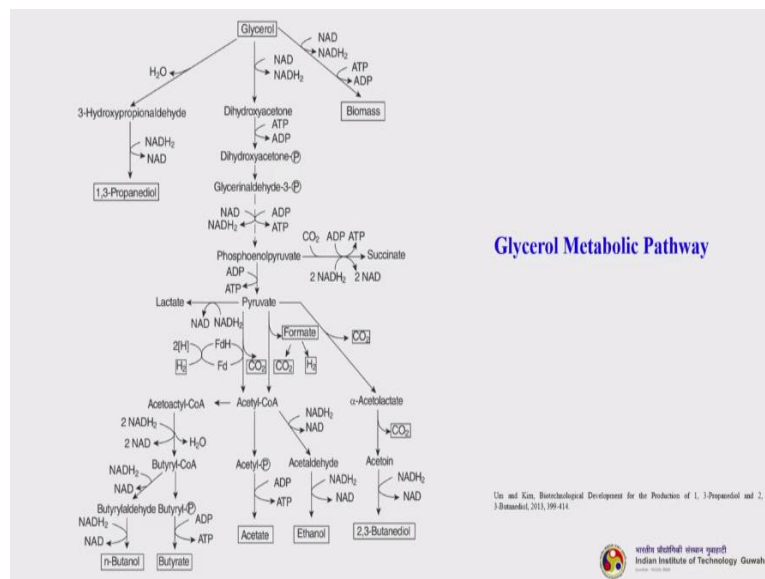
So, considerable attention has been paid to 1, 3-Propanediol production other than from glucose, especially glycerol because it is a huge amount as we have been discussing many times and you will recall that glycerol is one of the most important byproducts from the biodiesel processing unit. Only 10% has been used for some value addition, rest is a waste. So there is a huge demand to make the glycerol as a value-added product.

So a large amount of raw glycerol is generated in the range of almost 10% weight by weight of biodiesel during the production of biodiesel. Unlike the chemical conversion of glycerol to 1, 3-Propanediol, the biological conversion of glycerol to 1, 3-Propanediol is an environmental friendly and economical process due to the low energy requirement with mild

reaction conditions compared to that of chemical processes because in chemical processes you go for high temperature, pressure.

And there is also a higher yield of 1, 3-Propanediol than that is achieved via the chemical reaction; that can be one of the most significant result of this biological route. Now, 1, 3-Propanediol can be produced naturally under anaerobic as well as microaerobic conditions by various microorganisms including the genera *Klebsiella*, then *Clostridia*, *Enterobacter*, *Citrobacter* and *Lactobacilli* group.

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Now, this is the classical general metabolic pathway of the glycerol degradation. So, we can see how it actually happens. So, we can start with glycerol there. So, this is the usual route to produce butanol, acetate, ethanol, etc. from the glycerol. Now if we go for a dehydration reaction, then glycerol will result in 3-hydroxypropionaldehyde and that upon degradation will give us 1, 3-Propanediol, this is the route.

Now, this is the standard route of metabolic pathway through which we get many different types of products. So, glycerol is getting converted to phosphoenolpyruvate then to pyruvate using these particular steps. Then pyruvate to lactate, otherwise pyruvate straightaway to acetyl coenzyme A. Similar thing we have discussed during our fermentation pathway discussion. Now acetyl coenzyme A again goes back to acetoacetyl coenzyme A.

And this pathway will give us n-Butanol as well as Butyrate. Now acetyl coenzyme A also can be converted to acetate and acetaldehyde to ethanol. This is the usual route for the


eternal. Now pyruvate also can convert to acetoin and 2, 3-Butanediol. So, I hope you will understand that with glycerol itself only we are getting so many different types of products. But having said that please note that all these products whatever components, the chemicals that is being generated are produced from glycerol using this metabolic pathway are not in sufficient quantity. So, their concentrations will vary and some will be in higher concentration, some will be extremely low concentration that it is very difficult to purify. Now having said that metabolic pathway can also be engineered in such a way that you can target to produce 1, 3-Propanediol if you suppress this particular route.

Those technologies are already established and it has been done. Suppose you do not want 1, 3-Propanediol you can directly suppress this, you can go for any one of these. If you suppose you want ethanol, we can try to suppress this particular route. There will be some production of n-Butanol and butyrate, but we need to suppress the route. Similarly, any one of the product.

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- In addition to 1,3-Propanediol, the genera *Klebsiella* can produce ethanol, 2,3-butanediol, and acetate; *Clostridia* can produce butanol, butyrate, ethanol, and acetate.
- The best-known 1,3-Propanediol producing wild-type bacteria to date belongs to the genera *Klebsiella* and *Clostridium* because of their **tolerance** to 1,3-Propanediol and their **high yields and productivity**.
- Other bacteria such as *C. freundii*, *L. brevis*, and *C. pasteurianum* can also produce 1,3-Propanediol but at relatively low concentrations and productivity (Willke and Vorlop, 2008).
- There are two important enzymes involved in 1,3-Propanediol synthesis in *K. pneumoniae*: **glycerol dehydratase** (GDHt, vitamin B12-mediated dehydration) *converting glycerol to 3-hydroxypropionaldehyde* and **1,3-propanediol oxidoreductase** (PDOR) *reducing 3-hydroxypropionaldehyde to 1,3-propanediol* (Biebl et al., 1999).
- The accumulation of 3-hydroxypropionaldehyde, a microbial precursor of 1,3-Propanediol, has been known to **inhibit** 1,3-Propanediol production because of toxic effects.

Willke T, Vorlop K (2008) Bioremediation of glycerol into 1,3-propanediol. Eur J Lipid Sci Technol 110: 331–340  
Biebl H, Zeng AP, Meisel K, Deckwer WD (1999) Fermentation of glycerol to 1,3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae*. Appl Microbiol Biotechnol 50: 24–29



So in addition to 1, 3-Propanediol, the genera *Klebsiella* can produce ethanol, 2, 3-Butanediol, acetate and *Clostridia* can produce butanol, butyrate, ethanol and acetate. Now, we just saw that from glycerol we can get so many different products, but what are the products and all that again depends upon the type of species that you are using. The best known 1, 3-Propanediol producing wild-type bacteria to date belongs to the genera *Klebsiella* and *Clostridium* because of the high tolerance to 1, 3-Propanediol and their high yields and productivity. So other bacteria such as *Clostridium freundii*, *Lactobacillus brevis*, *Clostridium pasteurianum* can also produce 1, 3-Propanediol but at relatively low

concentrations as well as low productivity. There are two important enzymes involved in the 1, 3-Propanediol synthesis in *Klebsiella pneumoniae*, so one is glycerol dehydratase which is written as GDHt. So it is vitamin B12 mediated dehydration that is the route converting glycerol to 3-hydroxypropionaldehyde.

And then the next enzyme is 1, 3-Propanediol oxidoreductase/PDOR. What it does? It reduces 3-hydroxy propionaldehyde to 1, 3-Propanediol subsequent steps. Now, the accumulation of the 3-hydroxypropionaldehyde, a microbial precursor of the 1, 3-Propanediol has been known to inhibit 1, 3-Propanediol production because of the toxic effects.

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- *2,3-Butanediol* is also of commercial interest as an anti-freeze agent due to its low freezing point and as a feedstock for the formation of methyl ethyl ketone by dehydration, which can be used as a liquid fuel additive.
- 2,3-Butanediol is a four-carbon diol with hydroxyl groups at 2 and 3 carbon positions.
- This compound is also known as 2,3-butylene glycol, 2,3-dihydroxybutane, or dimethylethylene glycol. 2,3-Butanediol has a high boiling point of 177 °C and a low freezing point of -60 °C (levoform of 2,3-butanediol), and is promising as an *anti-freezing agent* (Soltys *et al.*, 2001).
- There are three stereoisomeric forms of 2,3-butanediol: l-(+), d(-), and mesoform

$$\begin{array}{c} \text{CH}_3 \\ | \\ \text{HO}-\text{C}-\text{H} \\ | \\ \text{H}-\text{C}-\text{OH} \\ | \\ \text{CH}_3 \end{array}$$

D-(-)-2,3-Butanediol  
(Levorotatory form)

$$\begin{array}{c} \text{CH}_3 \\ | \\ \text{H}-\text{C}-\text{OH} \\ | \\ \text{HO}-\text{C}-\text{H} \\ | \\ \text{CH}_3 \end{array}$$


L-(+)-2,3-Butanediol  
(Dextrorotatory form)

$$\begin{array}{c} \text{CH}_3 \\ | \\ \text{H}-\text{C}-\text{OH} \\ | \\ \text{H}-\text{C}-\text{OH} \\ | \\ \text{CH}_3 \end{array}$$

Meso-2,3-Butanediol  
(Optically inactive form)

Soltys KA, Batta AK, Kumar B (2001) Successful anaerobic, substrate preservation of rat liver with 2,3-butanediol and type I anti-freeze protein. *J. Sup. Bio. 30*: 30-34

Lin and Kim. Biotechnological Development for the Production of 1,3-Propanediol and 2,3-Butanediol. 2011, 399-414



Now the next class of the commodity chemical is 2, 3-Butanediol. 2, 3-Butanediol is also of commercial interest as an anti-freezing agent due to its low freezing point and as a feedstock for the formation of methyl ethyl ketone by dehydration, which can be used as a liquid fuel additive. 2, 3-Butanediol is a 4-carbon diol with hydroxyl groups at 2 and 3 carbon positions.

This compound is also known as 2, 3-butylene glycol, 2, 3-dihydroxybutane or dimethylethylene glycol. Now, 2, 3-Butanediol has a high boiling point of 177 degrees centigrade and a low freezing point of -60 degrees centigrade, so the levoform of 2, 3-Butanediol and basically, and is a promising anti-freezing agent. Now, there are 3 different stereoisomeric forms of 2, 3-Butanediol, one(L)plus, d minus and a mesoform.



So, you can see this. These are the 3 different forms. So, this form is that 2, 3-Butanediol, it is the levorotatory form. And this is L+ 2, 3-Butanediol, this is dextrorotatory form. And this is the optically inactive form.

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- 2,3-Butanediol is an industrially useful chemical with numerous applications in the manufacture of *printing inks, synthetic perfumes, softening, and moistening agents, a solvent for resins and lacquers, and a carrier for drugs and pharmaceuticals.*
- Furthermore, the derivatives of 2,3-Butanediol, such as *methyl ethyl ketone and 1,3-butadiene*, have attracted much attention recently because those chemicals, which are now produced by a petroleum refinery process, can be produced from biologically produced 2,3-Butanediol.
- Methyl ethyl ketone can be produced from 2,3-Butanediol by dehydration, and it can be used as an *effective fuel additive* because of a higher heat of combustion than ethanol.
- An interest in 1,3-butadiene production from 2,3-Butanediol increased significantly during World War II because 1,3-butadiene is an essential monomer for synthetic rubber.
- In addition, 1,3-butadiene can be converted to styrene by dimerization (Diels–Alder reaction), which is an important aromatic building block for polymer.



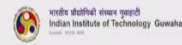
2, 3-Butanediol is an industrially useful chemical with numerous applications in the manufacture of printing inks, synthetic perfumes, softening and moistening agents, a solvent resins and lacquers and a carrier for drugs and pharmaceuticals. Furthermore, the derivatives of 2, 3-Butanediol such as methyl ethyl ketone and 1, 3-butadiene have attracted much attention recently.

Because those chemicals which are now produced by a petroleum refinery process can be produced from biologically produced 2, 3-Butanediol. Methyl ethyl ketone can be produced from 2, 3-Butanediol by dehydration. It is a simple process and it can be used as an effective fuel additive because of a higher heat of combustion than ethanol. An interest in 1, 3-butadiene production from 2, 3-Butanediol increased significantly during World War 2.

Because 1, 3-butadiene is an essential monomer for synthetic rubber. In addition, 1, 3-butadiene can be converted to styrene by dimerization which is an important aromatic building block for polymer.

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- Due to the shortage of fossil fuel supplies and rising oil prices, the biotechnology for the production of 2,3-Butanediol has received much attention recently.
- Numerous microorganisms have been known to produce 2,3-Butanediol from a variety of carbohydrates such as *glycerol*, *hexose*, *pentose*, and *lignocelluloses* through a microbial fermentation processes.
- These include a number of species of the genera *Klebsiella* (*K. oxytoca*, *K. pneumoniae*, *Klebsiella terrigena*), *Enterobacter* (*Enterobacter aerogenes*, *Enterobacter cloacae*), *Bacillus* (*B. polymyxa*, *Bacillus subtilis*, *Bacillus licheniformis*), *Lactobacillus* (*L. brevis*, *Lactobacillus casei*), and others.
- *K. oxytoca* and *K. pneumoniae* are facultative anaerobic bacteria, which obtain the energy for growth and maintenance through aerobic respiration and anaerobic fermentation.
- The *Klebsiella* species is also found to utilize various substrates including *glycerol*, *glucose*, *xylose*, *mannose*, *galactose*, *arabinose*, and *lactose*, making it feasible to produce 2,3-Butanediol with cheap substrates (i.e., biodiesel waste, lignocellulosic waste).



Due to the shortage of fossil fuel supplies and rising oil prices, the biotechnology for the production of 2, 3-Butanediol has received much attention recently. Numerous microorganisms have been known to produce 2, 3-Butanediol from a variety of carbohydrates such as glycerol, hexose and pentose as well as lignocelluloses through a microbial fermentation processes.

These include a number of species of the genera *Klebsiella*, *Enterobacter*, *Bacillus* and *Lactobacillus*. *Klebsiella oxytoca* and *Klebsiella pneumoniae* are facultative anaerobic bacteria which obtain the energy for growth and maintenance through aerobic respiration and anaerobic fermentation.

The *Klebsiella* species is also found to utilize various substrates including glycerol, glucose, xylose, mannose, galactose, arabinose, and lactose making it feasible to produce 2, 3-Butanediol with cheap substrates or low cost substance such as biodiesel waste, lignocellulosic waste, etc.

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- Usually, 2,3-Butanediol is synthesized as a mixture of two isomer types of 2,3-Butanediol by different microorganisms. For example, *B. subtilis* can produce d-(–)-form and mesoform in almost equal amounts. On the other hand, *B. polymyxa* produces only the optically active d-(–)-form of 2,3-Butanediol. There are only a few reports on stereoisomer analysis of 2,3-Butanediol produced from different microorganisms.
- During the fermentation, microorganisms produce 2,3-Butanediol along with other by-products, such as ethanol, lactic acid, and acetic acid.
- It is crucial to use all of the available cheap substrates for the cost-effective production of biofuel and bio-based chemicals because the cost of raw material significantly influences the final cost of the product.
- *Wood and agricultural biomass* (i.e., lignocellulosic materials) are popular candidates for low-price biomass resources, and there has been an increased interest in research on their use for 2,3-Butanediol production.



Usually, 2, 3-Butanediol is synthesized as a mixture of two isomer types of 2, 3-Butanediol by different microorganisms. For example, *Bacillus subtilis* can produce the d form and mesoform in almost equal amounts. Whereas on the other hand, *Bacillus polymyxa* produces only the optically active form of 2, 3-Butanediol. There are only a few reports on stereoisomer analysis of 2, 3-Butanediol produced from different microorganisms.

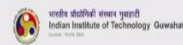
During the fermentation, microorganisms produce 2, 3-Butanediol along with other byproducts such as ethanol, lactic acid and acetic acid. It is crucial to use all of the available cheap substrates for the cost effective production of the biofuels and bio-based chemicals because the cost of raw material significantly influences the final cost of the product.

Wood and agricultural biomass mostly the lignocellulosic materials are popular candidates for low-price biomass resources and that has been an increased interest in research on their use for 2, 3-Butanediol production.

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- Among components in lignocellulosic materials, cellulose is a polymer of glucose, and hemicellulose contains hexose (glucose) and pentose (xylose, arabinose, mannose, galactose, and rhamnose).
- According to previous results, 2,3-Butanediol can be produced from various types of carbohydrates, including hexose and pentose sugars, which are liberated from lignocellulosic materials by hydrolytic method.
- Each hexose and pentose or mixed substrate has been examined for 2,3-Butanediol production. Among them, *glucose* has been the substrate most often examined for 2,3-Butanediol production.
- *Aerobacter aerogenes* NRRL B199 yielded 0.45 g of 2,3-Butanediol/g of glucose at the initial glucose concentration of 195 g/L (*Sablajrolles and Goma, 1984*).
- *B. polymyxa* utilized glucose as a carbon source for the production of 2,3-Butanediol. The inhibition of growth occurred at substrate concentrations exceeding 150 g/L (*De Mas et al., 1988*).

Sablajrolles JM, Goma G (1984) Butanediol production by *Aerobacter aerogenes* NRRL B199—Effects of initial substrate concentration and aeration agitation. *Biotechnol. Bioeng.* 26: 148–155.  
De Mas C, James NB, Tsao GT (1988) Production of optically active 2,3-butanediol by *Bacillus polymyxa*. *Biotechnol. Bioeng.* 31: 366–377.



Among components in lignocellulosic materials, cellulose is a polymer of glucose and hemicellulose contains hexose and pentose. According to previous results, 2, 3-Butanediol can be produced from various types of carbohydrates including hexose and pentose sugars which are liberated from lignocellulosic materials by hydrolytic method. Each hexose and pentose or mixed substrate has been examined for 2, 3-Butanediol production.

Among them, glucose has been the substrate most often examined for 2, 3-Butanediol production. *Aerobacter aerogenes* NRRL B199, this is the strain name, yielded 0.45 gram of 2, 3-Butanediol per gram of glucose at the initial glucose concentration of 195 grams per liter. Then *Bacillus polymyxa* utilized glucose as a carbon source for the production of 2, 3-Butanediol.

The inhibition of growth occurred at substrate concentration exceeding 150 grams per liter. These are very classical studies. The references are given, you can browse through later on if you are interested to read more into detail.

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- *B. licheniformis* produced 2,3-Butanediol from glucose when grown either in air or under N<sub>2</sub> (Nilegaonkar et al., 1992). After 72 hours of fermentation on 2% (w/v) glucose at pH 6.0 and 37 °C, the optimum yield of 2,3-Butanediol was 47 g/100 g glucose.
- In addition to glucose, the use of various pentoses and hexoses has been examined for 2,3-Butanediol production.
- Jansen and Tsao (1983) reported that *K. pneumoniae* was able to utilize various pentoses including xylose, arabinose, mannose, galactose, and cellobiose for growth and 2,3-Butanediol production.
- The maximum specific growth rate of 1.05/h occurred at a xylose concentration of 20 g/L by *K. oxytoca* under aerobic conditions (Jansen et al., 1984a).
- Jansen et al. (1984b) performed 14-batch fermentation for 2,3-Butanediol production and simulated 2,3-Butanediol production with respect to the oxygen transfer rate and the initial xylose concentration.

Nilegaonkar S, Bhosale SB, Kulkarni DC, Kapadi AH (1992) Production of 2,3-butanediol from glucose by *Bacillus licheniformis*. *World J. Microbiol. Biotechnol.* 5: 378–381.  
 Jansen NB, Tsao GT (1983) Bioreconversion of pentoses to 2,3-butanediol by *Klebsiella pneumoniae*. *Adv. Biochem. Eng. Biotechnol.* 27: 83–99.  
 Jansen NB, Pichayng MC, Tsao GT (1984a) Production of 2,3-butanediol from D-xylose by *Klebsiella pneumoniae* ATCC 3722. *Biotechnol. Bioeng.* 26: 362–369.  
 Jansen NB, Pichayng MC, Tsao GT (1984b) Application of bioreactors to modeling the microbial conversion of D-xylose to 2,3-butanediol. *Biotechnol. Bioeng.* 26: 373–382.



*Bacillus licheniformis* produced 2, 3-Butanediol from glucose when grown either in air or under nitrogen. After 72 hours of fermentation on a 2% weight by volume glucose at pH 6 and 37 degrees centigrade, the optimum yield of 2, 3-Butanediol was 47 gram per 100 grams of glucose. In addition to glucose, the use of various pentoses and hexoses has been examined for 2, 3-Butanediol production.

Jansen and Tsao reported that *Klebsiella pneumoniae* was able to utilize various pentoses including xylose, arabinose, mannose, galactose and cellobiose for growth and 2, 3-Butanediol production. The maximum specific growth rate of 1.05 per hour occurred at a xylose concentration of 20 grams per liter by *Klebsiella oxytoca* under aerobic conditions.

Janssen et al performed a 14-batch fermentation for 2, 3-Butanediol production and simulated 2, 3-Butanediol production with respect to the oxygen transfer rate and initial xylose concentration.

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- With an initial xylose concentration of 100 g/L and an oxygen transfer rate of 0.027 mol/L/h, the average 2,3-Butanediol production rate was 1.35 g/L/h.
- The production of 2,3-Butanediol from lignocellulosic wastes other than purified hexose and pentose has been considered as an economical method.
- Laube *et al.* (1984) attempted to use *acid-hydrolyzed aspen wood, larchwood, and oat spelts*; but only a low 2,3-Butanediol yield was obtained even with the additional xylose, implying that there was an inhibitory effect of hydrolyzate on 2,3-Butanediol production.
- Frazer and McCaskey (1991) investigated the effect of selected components in *acid-hydrolyzed hardwood* on 2,3-Butanediol production by *K. pneumoniae*. According to their results, sulfate up to 0.2% (w/v) and furfural up to 0.2% (w/v) did not affect growth; but sulfate reduced 2,3-Butanediol by 30% while furfural induced growth slightly. Phenolic compounds including syringaldehyde and vanillin significantly inhibited growth and 2,3-Butanediol production to as low as 0.1% (w/v) and 0.05% (w/v), respectively.

Laube VM, Gotschal D, Maras SM (2004). 2,3-Butanediol production from xylose and other hexose-derived components by *Bacillus polymyxa*. *Bioresource*, *Let*. 6: 217–242.

Frazer FR, McCaskey TA (1991). Effect of components of acid-hydrolyzed hardwood on conversion of d-xylose to 2,3-butanediol by *Klebsiella pneumoniae*. *Enzyme Microb. Technol.* 13: 110–113.



With an initial xylose concentration of 100 grams per liter and an oxygen transfer rate of point 0.027 moles per liter per hour, the average 2, 3-Butanediol production rate was 1.35 grams per liter per hour. The production of 2, 3-Butanediol from lignocellulosic wastes other than purified hexose and pentose has been considered as an economical method.

Laube et al attempted to use acid-hydrolyzed aspen wood, larchwood and oat spelts; but only a low 2, 3-Butanediol yield was obtained even with the additional xylose implying that there was an inhibitory effect of hydrolyzate on 2, 3-Butanediol production. As you know that beyond certain concentration, all the products as well as substrates can inhibit the entire fermentation process, so that care must be taken. Frazer and McCaskey investigated the effect of the selected components in acid-hydrolyzed hardwood on 2, 3-Butanediol production by the *Klebsiella pneumoniae* species.

According to their result, sulfate up to 0.2% weight by volume and furfural up to 0.2% again weight by volume did not affect growth, but sulfate reduced 2, 3-Butanediol by 30% while furfural induced growth slightly. So phenolic compounds including syringaldehyde and vanillin significantly inhibited growth and 2, 3-Butanediol production to as low as 0.1% and 0.05% respectively.

So both these components syringaldehyde as well as vanillin are great inhibitors in the entire fermentation process to produce 2, 3-Butanediol using this particular strain.

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- Grover *et al.* (1990) used *wood hydrolysate* after neutralization with  $\text{Ca}(\text{OH})_2$  and filtration. In flask cultures, 2,3-Butanediol was produced at 12 g/L with the yield of 0.27 g/g of sugars.
- Glycerol is also considered to be a cost-effective and efficient carbon source for the production 2,3-Butanediol. *K. pneumoniae* DMS 2026 has been known to be a 1,3-Propanediol producing strain as well as a 2,3-Butanediol producing strain using glycerol (Menzel *et al.*, 1997).
- *K. pneumoniae* GT1 produced 2,3-Butanediol from glycerol at low pH and microaerobic conditions (Biebl *et al.*, 1998). When the pH was lowered stepwise from 7.3 to 5.4 in a continuous culture, 2,3-Butanediol formation started at pH 6.6 and reached a maximum yield at pH 5.5 under *microaerobic conditions*, while production of acetate, ethanol, and 1,3-propanediol decreased.

Grover BP, Gang SK, Varma J (1990). Production of 2,3-butanediol from wood hydrolysate by *Klebsiella pneumoniae*. *World J. Microbiol. Biotechnol.* 6: 328–332.  
Menzel K, Zeng AP, Deckwer WD (1997). High concentration and productivity of 1,3-propanediol from continuous fermentation of glycerol by *Klebsiella pneumoniae*. *Enzyme Microb. Technol.* 20: 12–16.  
Biebl H, Zeng AP, Menzel K, Deckwer WD (1998). Fermentation of glycerol to 1,3-propanediol and 2,3-butanediol by *Klebsiella pneumoniae*. *Appl. Microbiol. Biotechnol.* 50: 24–28



Grover et al used wood hydrolysate after neutralization with calcium hydroxide and filtration. In flask cultures, 2, 3-Butanediol was produced at 12 grams per litre with a yield of 0.27 gram per gram of sugar. Glycerol is also considered to be a cost effective and efficient carbon source for the production of 2, 3-Butanediol. *Klebsiella pneumoniae* DMS 2026 has been known to be a 1, 3-Propanediol producing strain as well as a 2, 3-Butanediol producing strain using glycerol.

This is what we have already seen when we discussed at the beginning of our class about the 1, 3-Propanediol production. So this *Klebsiella pneumoniae* GT1 produced 2, 3-Butanediol from glycerol at low pH and microaerobic conditions. When the pH was lowered stepwise from 7.3 to 5.4 in a continuous culture, 2, 3-Butanediol formation started at pH 6.6 and reached a maximum yield at pH 5.5 under microaerobic conditions, while production of acetate, ethanol and 1, 3-Propanediol decreased.

This is what we want. I was just talking if you recall our discussion during metabolic pathway, so if you want to maximize 2, 3-Butanediol production then you have to suppress the production of other components, whether it is acetate, ethanol, 1, 3-Propanediol or any other such components, like even butanol also.

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- While *K. pneumoniae* can produce 1,3-propanediol and hydrogen only under strictly anaerobic conditions, the microaerobic conditions caused little 1,3-propanediol production and higher 2,3-butanediol production in the presence of oxygen and glycerol.
- Fed-batch cultures of *K. pneumoniae* G31 was carried out by Petrov and Petrova (2009). By optimizing the medium conditions without pH control, 49.2 g/L of 2,3-Butanediol was produced at an initial pH of 8 under microaerobic conditions with the concentration of glycerol about 30 g/L.
- The addition of organic acids such as acetic acid and succinic acid has been demonstrated to be advantageous for 2,3-Butanediol production.

Petrov K, Petrova P (2009) High production of 2,3-butanediol from glycerol by *Klebsiella pneumoniae* G31. *Appl. Microbiol. Biotechnol.* 84: 659–663.



So while this *Klebsiella pneumoniae* can produce 1, 3-Propanediol and hydrogen only under strictly anaerobic condition, the microaerobic conditions caused little 1, 3-Propanediol production, and higher 2, 3-Butanediol production in the presence of oxygen and glycerol. There is a very interesting study. Fed-batch culture of this particular strain was carried out by Petrov and Petrova.

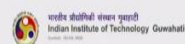
By optimizing the medium conditions without pH control, 49.2 grams per liter of 2, 3-Butanediol was produced at an initial pH of 8 under microaerobic conditions with the concentration of glycerol about 30 grams per liter. The addition of organic acids such as acetic acid and succinic acid has been demonstrated to be advantageous for 2, 3-Butanediol production.

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- The addition of succinic acid at 10 g/L to the medium containing xylose was found to improve 2,3-Butanediol productivity from xylose by *K. oxytoca*, but higher concentrations of succinate were inhibitory (Eiteman and Miller, 1995).
- Nakashimada *et al.* (2000) investigated the effect of organic acids on 2,3-Butanediol production of *B. polymyxa* and found that 150 mM of acetate gave the highest 2,3-Butanediol yield (0.87 mol of 2,3-butanediol/mol of glucose) and the concentration of 248 mM in a batch culture.
- When glucose and acetic acid were fed together in the ratio of 0.35 mol acetate/mol glucose in the fed-batch cultures, 2,3-Butanediol production reached 637 mM and 566 mM at pH 6.8 and pH 6.3, respectively.

Eiteman MA, Miller RH (1995) Effect of succinic acid on 2,3-butanediol production by *Klebsiella oxytoca*. *Biotechnol. Lett.* 17: 1037–1042.

Nakashimada Y, Maruyama B, Katochikawa T, Kakimoto T, Nishio N (2000) Enhanced 2,3-butanediol production by addition of acetic acid in *Pantoea agglomerans* J. *Biosci. Biotech.* 90: 661–664.

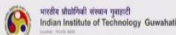




The addition of succinic acid at 10 grams per liter to the medium containing xylose was found to improve 2, 3-Butanediol productivity from xylose by *Klebsiella oxytoca*, but higher concentration of succinate were inhibitory. Then Nakashimada et al., investigated the effect of organic acids on 2, 3-Butanediol production of *Bacillus polymyxa* and found that 150 millimolar of acetate gave the highest 2, 3-Butanediol yield, that is 0.87 moles of 2, 3-Butanediol per mole of glucose which is a very good actually, and the concentration of 248 millimolar in a batch culture. When glucose and acetic acid were fed together in the ratio of 0.35 mole acetate per mole of glucose in the fed-batch cultures 2, 3-Butanediol production reached 637 millimolar and 566 millimolar at pH 6.8 and pH 6.3 respectively.

**(Refer Slide Time: 19:31)**

- *Polyhydroxyalkanoates (PHAs)* are a family of bio-polyesters synthesized by many bacteria for energy and carbon storage.
- The natural polymers can be melted and molded like petrochemical thermoplastics but degraded completely into benign metabolic products by microorganisms in the environment. *Poly(3-hydroxybutyrate)* or *P3HB* is the most common PHA.
- More than 100 hydroxyalkanoic acids (HAs) have now been identified as the possible PHA monomers. They are usually 3-hydroxyalkanoates (3HAs) with R configuration, resulting in high stereoregularity and optical activity of bio-polyesters.
- The monomeric hydroxyalkanoates can be roughly divided into two groups, *short-chain-length hydroxyalkanoates (scl-HA)* with 3–5 carbons and *medium-chain-length hydroxyalkanoates (mcl-HA)* with 6–14 carbons.
- The size of side chain can, to a great extent, *affect the material properties*.



Now we will talk about the other one of the most important commodity chemicals, PHA, that is polyhydroxalkanoate. So, PHAs are a family of bio-polyesters synthesized by many bacteria for energy and carbon storage purposes. The natural polymers can be melted and molded like petrochemical thermoplastics but degraded completely into benign metabolic products by microorganisms in the environment. P3HB is the most common PHA.

More than 100 hydroxyalkanoic acids have now been identified as the possible PHA monomers. They are usually 3-hydroxyalkanoates with R configuration resulting in high stereoregularity and optical activity of the biopolyesters. The monomeric hydroxyalkanoates can be roughly divided into 2 groups: Short-chain length hydroxylalkanoates with 3 to 5

carbons and medium-chain-length hydroxyalkanoates with 6 to 14 carbons. The size of side chain can to a great extent affect the material properties.

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- Small side chains, such as methyl and ethyl groups of scl-P3HAs, result in a *stiff material* with *high crystallinity, high tensile modulus, and low ductility*, while large side chains (C3–C14) of mcl-P3HAs make the material *elastic* with *low crystallinity, low melting temperature, and high ductility*.
- Controlling the size and content of side chains in P3HA copolymers can therefore make a bio-polyester with desired properties.
- The material properties can also be modified by incorporating longer monomers, such as 4-hydroxybutyrate (4HB) and 4-hydroxyvalerate (4HV), in the PHA backbone.
- For instance, as 4HB content in a co-polyester, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB), increases, the *crystallinity of the copolymer declines and its ductility increases*.
- In microbial PHA biosynthesis, precursors of 4HB (e.g., 1,4-butanediol) and 4HV (e.g., 4-ketovaleric acid or levulinic acid) are supplied either as the sole carbon source or as a co-substrate of glucose.



Small side chains such as methyl and ethyl groups of scl-P3HAs result in a stiff material with high crystallinity, high tensile modulus and low ductility whereas large side chains of mcl-P3HA make the material more elastic with lower crystallinity, low melting temperature as well as high ductility. So, actually the length of the side chains matters. So controlling the size and content of the side chains in P3HA copolymers can therefore make a bio-polyester with desired properties.

So, basically you can tailor make a particular PHA. The material properties can also be modified by incorporating longer monomers such as 4-hydroxybutyrate and 4-hydroxyvalerate in the PHA backbone. For instance, as the 4HB content in a copolyester, that is P3HB4HB, increases, the crystallinity of the copolymer declines and its ductility increases. In microbial PHA biosynthesis, precursors of 4HB are supplied either as the sole carbon source or as a co-substrate of glucose.

**(Refer Slide Time: 21:30)**

- PHA bio-polyesters, because of their *biodegradability and biocompatibility*, have attracted great research and industrial interests.
- They have been produced via microbial fermentation from various renewable feedstocks such as *glucose, starch, sucrose, vegetable oils, animal fat, and food processing residues*.
- The *production cost*, however, is still relatively high in comparison with petrochemical plastics. It is attributed to the costs of raw materials, microbial fermentation, and polymer recovery.
- Cellulosic biomass such as *corn stover and cane bagasse* is a potentially inexpensive and renewable feedstock for bio-based fuels, chemicals, and materials.
- Compared to starchy grains, cellulosic biomass contains much less glucan, more hemicellulose, lignin, and other plant components.
- A substantial amount of organic residues are left from ethanol fermentation, posing a high treatment cost or environmental liability to biomass refining.



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Cellulosic biomass such as corn stover and cane bagasse is a potentially inexpensive and renewable feedstock for bio-based fuels, chemicals as well as materials. Compared to starchy grains, cellulosic biomass contents much less glucan, more hemicellulose, lignin and other plant components. A substantial amount of organic residues are left from ethanol fermentation, posing a high treatment cost or environmental liability to the biomass refining processes.

**(Refer Slide Time: 22:26)**

- A general PHA bioprocess consists of three basic operations: *biomass treatment, microbial synthesis, and polyester recovery and purification*.
- Microbial synthesis of bio-polyesters on complex and many times inhibitory substrates plays a key role in PHA bioprocess.
- Under controlled conditions, bio-polyesters are accumulated inside the cells as inclusion bodies that may count for 60-80% of dry cell mass.
- Glucose is a popular carbon source for PHA fermentation in laboratory as well as in industry. In a laboratory fed-batch culture, a very high productivity of 4.6 g P3HB/L/h and a high P3HB content (80 wt %) was achieved (Kim *et al.*, 1994).
- This high-cell density culture, however, may be impossible in biomass refining because of a much lower glucose concentration and presence of other inhibitory substances in the feeding solution.
- *Fed-batch fermentation* is a useful tool in controlling substrate inhibition for high cell density cultures.

Kim BS, Lee SC, Lee SY, Chang HN, Chang YK, Woo SI (1994). Production of poly(3-hydroxybutyric acid) by fedbatch culture of *Clostridium* sp. with glucose concentration control. *Biomol. Bioproc.* 4: 181-188.

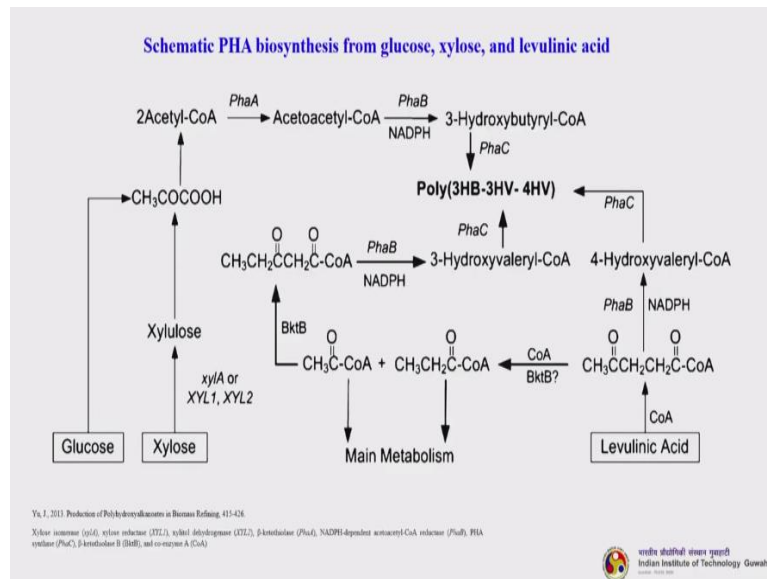


A general PHA bioprocess consists of three basic operations. What are those? The first is the biomass pretreatment, second is the microbial synthesis, and then third is the polyester recovery and purification step. Now, microbial synthesis or bio-polyesters are complex and many times inhibitory substrates play a key role in the PHA bioprocess. Under controlled conditions, bio polyesters are accumulated inside the cells as inclusion bodies that may account for 60 to 80% of the dry cell mass.

Glucose is a popular carbon source for PHA fermentation in laboratory as well as in industry. In a laboratory fed-batch culture a very high productivity of 4.6 grams P3HB per liter per hour and a high P3HB content up almost 80 weight percent was achieved. It was a very significant report actually. The reference is given below, you can browse through it if you are interested to learn more.

This high-cell density culture however may be impossible in biomass refining because of a much lower glucose concentration and presence of other inhibitory substances in the feeding solution. Fed-batch fermentation is a useful tool in controlling substrate inhibition for high-cell density cultures. If you recall our discussions during fermentation, I have clearly told you how fed-batch is an advantageous mode of operation with respect to batch operation or batch mode of fermentation.

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So, this is the classical schematic PHA biosynthesis from glucose, xylose and levulinic acid route. So, let us understand. Glucose and xylose, so both are giving acetic acid. So, xylose is giving an intermediate before it goes to acetic acid, xylulose. So, now this acetic acid is getting converted to 2-Acetyl coenzyme A, then to Acetoacetyl coenzyme A, then to 3-Hydroxybutyryl coenzyme A, then poly 3HB-3HV-4HV.

And you can see that this is the main metabolism pathway which has not been described here, and has been left out. So, this particular component is giving again to the 3-hydroxyvaleryl coenzyme A. And from the levulinic acid also that particular step is going through two different intermediate compounds and finally we are getting poly 3HB-3HV-4HV as the product. Now there are some intermediate steps which have been skipped here. This is just a simple understanding of the schematic biosynthesis pathway.

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- The inhibitory compounds in hydrolysates solution, however, may not be utilized by microbial cells and could be accumulated with feeding, resulting in quick deterioration of microbial activity.
- A *continuous feeding system consisting of two bioreactors in series* may provide a solution. In the *first bioreactor*, cells grow with a balanced nutrient supply, and in the *second one*, PHA is formed under limited nutrients at a constant level of inhibitory compounds.
- For example, one system operated at a dilution rate of 0.08/h produced 1.23 g P3HB/L/h with a yield of 0.36 g P3HB/(g glucose). The PHA content was 72 wt % of the dry cell mass (Du et al., 2001).
- Xylose is a predominant hemicellulosic sugar of biomass hydrolysates. Because of its poor utilization by yeast, it may also be a primary residual sugar in ethanol fermentation.

Di G, Chen J, Yu J, Liu S (2001) Kinetic studies on poly-3-hydroxybutyrate formation by *Rubrobacterales* in a two-stage continuous culture system. *Process Biochem*. 37 (3): 219–227.



The inhibitory compounds in hydrolysates solution however may not be utilized by microbial cells and could be accumulated with feeding resulting in quick deterioration of the microbial activity. A continuous feeding system consisting of two bioreactors in series may provide a solution. So, how it can help us basically? In the first bioreactor, you grow the cells. The cells grow with a balanced nutrient supply, basically you are growing the biomass.

And in the second one PHA is formed under limited nutrient conditions at constant level of inhibitory compounds because what happens when you are growing and producing PHA simultaneously in a single reactor, during the cell growth, there are some inhibitory compounds production that actually inhibit the growth of the cell as well as inhibit that PHA formation. So, that will not happen if you are going to use a continuous feeding system having two separate bioreactors in series.

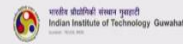
For example, one system operated at a dilution rate of 0.08 per hour produced 1.23 gram P3HB per liter per hour with a yield of 0.36 gram P3HB per gram of glucose. The PHA content was 72 weight percent of the dry cell mass. Xylose is a predominant hemicellulosic sugar of biomass hydrolysates. Because of its poor utilization by yeast, it may also be a primary residual sugar in ethanol fermentation.

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- An early study demonstrated P3HB accumulation on xylose and arabinose in *Pseudomonas pseudoflava* (Bertrand *et al.*, 1990). In a well-controlled bioreactor, the specific P3HB formation rate, cell yield, and polymer yield on xylose were 0.03 (g/g/h), 0.17 (g/g), and 0.04 (g/g), respectively, in comparison with 0.11 (g/g/h), 0.36 (g/g), and 0.08 (g/g), respectively, on glucose.
- Two problems are encountered in PHA biosynthesis on levulinic acid: the *acid toxicity* and the *composition control of co-polyesters*.
- With a strain of *Alcaligenes sp.* grown on levulinic acid and glucose, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HB3HV) was formed with about 40 mol % of 3HV (Jang and Rogers, 1996).
- The cell growth and PHA synthesis were inhibited at a low concentration of levulinic acid (1 g/L).
- This fact indicates that the acid has a higher toxicity than propionic acid does, a popular precursor substrate of 3HV.

Bertrand D., Ramay BA, Ramay JA, Charrier C (1990) Biosynthesis of poly-β-hydroxyalkanoates from pentoses by *Pseudomonas pseudoflava* Appl. Environ. Microbiol. 56: 3333 – 3338.  
Jang JH, Rogers PL (1996) Effect of levulinic acid on cell growth and poly-β-hydroxyalkanoate production by *Alcaligenes sp.* 501-69. Biotechnol. Lett. 18: 219 – 224.



An early study demonstrated P3HB accumulation on xylose and arabinose in *Pseudomonas pseudoflava*. In a well-controlled bioreactor the specific P3HB formation rate, cell yield and polymer yield on xylose were 0.03 grams per grams per hour, 0.17 gram per gram and 0.04 gram per gram respectively in comparison with 0.11 gram per gram per hour, 0.36 gram per gram and 0.08 gram per gram respectively on glucose.

Two problems are encountered in the PHA biosynthesis on levulinic acid. The first is the acid toxicity and the second is the composition control of the co-polyesters. With a strain of *Alcaligenes* species grown on levulinic acid and glucose, this PHA that is P3HB3HV was formed with about 40 mole percent of the 3HV. The cell growth and PHA synthesis were inhibited at a low concentration of levulinic acid that is 1 gram per liter.

So this fact indicates that the acid has a higher toxicity than propionic acid does, and a popular precursor substrate of the 3HV.

**(Refer Slide Time: 27:26)**

- A strain of *Ralstonia eutropha* (formerly *Alcaligenes eutropha*) was inhibited with levulinic acid at concentrations from 0.5 to 8 g/L (Chung et al., 2001).
- The co-polyesters P3HB3HV formed on fructose and levulinic acid contains 35 mol % of 3HV or lower.
- No 4-hydroxyvalerate (4HV) is detected in the polyester backbone from its precursor, levulinic acid.
- PHA with a high mole percentage of 4HV (19–30 mol %) could be synthesized on levulinic acid with a recombinant strain (Gorenflo et al., 2001).

Chung SH, Choi GG, Kim HY, Han YH (2001). Effect of levulinic acid on the production of poly(3-hydroxybutyrate-3-hydroxyvalerate) by *Ralstonia eutropha* KCTC 8902. *J. Microbiol.* 39: 79–82.  
Gorenflo V, Schneck C, Vogel R., Strauchel A (2001). Development of a process for the biotechnological large-scale production of 4-hydroxyvalerate-containing polyesters and characterization of their physical and mechanical properties. *Biomacromolecules* 2: 41–57.



A strain of *Ralstonia eutropha*, so formerly it was known *Alcaligenes eutropha*, was inhibited with levulinic acid at concentrations from 0.5 to 8 grams per liter. The co-polyesters P3HB3HV formed on fructose and levulinic acid contains 35 mole percent of 3HV or lower. No 4HV is detected in the polyester backbone from its precursor levulinic acid. PHA with the high mole percentage of 4HV (almost 19 to 30%) could be synthesized on levulinic acid with a recombinant strain. So the two references are very classical studies particularly on the PHA formation from the levulinic acid. So, if you are interested to learn more you can go through it.

**(Refer Slide Time: 28:11)**

Monomer Composition, Average Molecular Weight, and IR Absorbance of PHA Formed on Glucose, Valeric Acid, and Levulinic Acid

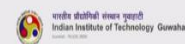
Carbon Source	Mw (kDa)	3HB (mol %)	3HV (mol %)	4HV (mol %)	Abs <sup>a</sup> (-)	Abs <sup>b</sup> (-)	Abs <sup>a</sup> /Abs <sup>b</sup>
Glucose	1500–2400	100.0	0.0	0.0	0.013	0.014	0.93
Valeric acid	1350–1650	21.8	78.2	0.0	0.024	0.012	2.0
Levulinic acid	1570–2100	55.4	43.1	1.3	0.057	0.024	2.38

<sup>a</sup>FTIR absorbance at wave number 1180 cm<sup>-1</sup>.

<sup>b</sup>FTIR absorbance at wave number 1380 cm<sup>-1</sup>.

- Although the average molecular weight of PHAs formed on three different carbon sources is quite similar, the *monomeric composition varies*.
- A homopolymer poly(3-hydroxybutyrate) (P3HB) is formed on glucose.
- On valeric acid, a co-polyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HB3HV) is formed with 3HV being the predominant monomer (~78 mol %) and 3HB the only secondary monomer (~22 mol %).

Yu, J., 2013. Production of Polyhydroxyalkanoates in Biomass Refining. 415-426.



So, this table tells you about the monomer composition, average molecular weight, IR absorbance of the PHA formed on glucose, valeric acid as well as levulinic acid. So, 3 different carbon sources are given, their molecular weight, how much 3HV is getting formed.

You can see from glucose it is 100% 3HB, 0 3HV, 0 4HV. When you come to valeric acid you see that 3HB is less whereas 3HV is more and no 4HV.

And when you talk about levulinic acid there is a balanced formation of 3HB as well as 3HV. So although the average molecular weight of PHAs formed on 3 different carbon sources is quite similar, the monomeric composition varies. A homopolymer P3HB is formed on glucose. On valeric acid, a co-polyester that is P3HB3HV is formed with 3HV being the predominant monomer. You can see that 78.2 mole percent and 3HB is the only secondary monomer, roughly it is 22%.

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- On levulinic acid, poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-4-hydroxyvalerate) (P3HB3HV4HV) is formed in which both 3HB (~55 mol %) and 3HV (~43 mol %) are the predominant monomers while 4HV is a minor monomer (~1 mol %).
- The IR absorption intensity at wave number around  $1180\text{ cm}^{-1}$  is reversal to the crystallinity of PHA matrix. The IR absorbance at wave number  $1380\text{ cm}^{-1}$  is attributed to the vibration energy of methyl group (-CH<sub>3</sub>) of bio-polyesters and often used as a reference.
- P3HB is a well-known brittle PHA because of its high stereoregularity and high crystallinity (60–70%). Its relative IR absorbance is 0.93, the smallest among the three PHAs.
- The copolymer P3HB3HV has a *lower crystallinity and higher ductility* than P3HB does, but the effect of the second monomer on matrix crystallinity and material ductility is not high because of co-crystallization of 3HB and 3HV.



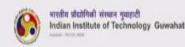
On levulinic acid, this P3HB3HV4HV is formed in which both 3HB and 3HV are the predominant monomers while 4HV is a minor monomer. The IR absorption intensity at wavelength number around 1180 centimeter inverse is reversal to the crystallinity of the PHA matrix. The IR absorbance at wave number 1380 centimeter inverse is attributed to the vibration energy of methyl group of bio-polyesters and often used as a reference.

P3HB is a well known brittle PHA because of its high stereoregularity and high crystallinity, almost 60 to 70%. Its relative IR absorbance is 0.93, the smallest among all the 3 PHAs. The copolymer P3HB3HV has a lower crystallinity and higher ductility than the P3HB does, but the effect of the second monomer on matrix crystallinity and material ductility is not high because of co-crystallization of the 3HB and 3HV.

**(Refer Slide Time: 30:24)**

- The terpolyester P3HB3HV4HV has the lowest crystallinity and highest relative IR absorbance (2.38) because of the content of 3HV (43 mol %) as well as the irregularity caused by 4HV.
- Indeed, the cast film of this terpolyester exhibits a very high elongation at break (~500%) in comparison with P3HB (<5%) and P3HB3HV (<50%). The melting point of the terpolyester is between 120–130 °C in comparison with P3HB (170–180°C) and 3HB3HV (150–160 °C).
- In addition to glucose, xylose, or levulinic acid, many organic hydrolysates exist in the solutions of biomass refining. They may not be the primary carbon source because of relatively low concentrations, but can apply a great inhibitory effect on PHA fermentation.
- For instance, *Pseudomonas pseudoflava* is able to grow and form P3HB on xylose and arabinose or the hydrolysates from hemicellulosic fraction of poplar wood. The strain, however, is inhibited completely in hydrolysates solution of high concentrations (>30% v/v) (Bertrand *et al.*, 1990).

Bernard E., Ramirey BA., Ramirey DA., Charrier C (1990). Biosynthesis of poly-β-hydroxybutanoate from pentoses by *Pseudomonas pseudoflava*. *Appl. Environ. Microbiol.* 56: 3133–3138.



The terpolyester P3HB3HV4HV has the lowest crystallinity and highest relative IR absorbance because of the content of 3HV as well as the irregularity caused by the 4HV. Indeed, the cast film of this terpolyester exhibit a very high elongation at break close to 500% in comparison with P3HB which is less than 5% and P3HB3HV which is less than 50%. The melting point of the terpolyester is between 120 to 130 degrees centigrade in comparison with the two other things.

Like P3HB is 170 to 180, 3HB3HV is 150 to 160 degrees centigrade, almost similar. So, in addition to glucose, xylose and levulinic acid, many organic hydrolysates exist in the solutions of biomass refining. They may not be primary carbon source because of relatively low concentrations, but can apply a great inhibitory effect on the PHA fermentation.

For instance, *Pseudomonas pseudoflava* is able to grow and form P3HB on xylose and arabinose or the hydrolysates from hemicellulosic fraction of the poplar wood. The strain however is inhibited completely in hydrolysates solution of high concentrations, almost greater than 30% volume by volume.

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- The toxicity of hydrolysates depends on three factors: *cell density, hydrolysates concentration, and inoculum preparation*. A high initial cell density ranging from 3 to 6 g/L could effectively relieve the inhibitory effect of the hydrolysates. The hydrolysates and glucose are simultaneously utilized with cell growth and PHA formation.



The toxicity of hydrolysates depend on the three factors. First is cell density, then hydrolysates concentration and the inoculum preparation. A high initial cell density ranging from 3 to 6 gram per liter could effectively relieve the inhibitory effect of the hydrolysates. The hydrolysates and glucose are simultaneously utilized with cell growth and PHA formation.

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**(Overview of next lecture)**

Module	Module name	Lecture	Title of lecture
12	Integrated Biorefinery	01	Concept, corn/soybean/sugarcane biorefinery, lignocellulosic biorefinery

**Thank you**

For queries, feel free to contact at: [kmohanty@iitg.ac.in](mailto:kmohanty@iitg.ac.in)



So with this, I wind up today's class and we are done with our discussion on commodity chemicals production from the biomass. Though biomass gives us so many different types of commodity chemicals, I have restricted our discussions to only those which are commercially being produced and are of high value. So, thank you very much. In case you have any query register in the Swayam portal. You can always drop a mail to me at [kmohanty@iitg.ac.in](mailto:kmohanty@iitg.ac.in).