Advanced Biomolecules and Transformations in Industrial Biotechnology

Lecture 12
Biofuels and Bioproducts

Bronx Community College - 2017

Chemistry and BioEnergy Technology for Sustainability NSF ATE 1601636

Outline

- From biofuels to ‘biorefining’
  - Biotech, BioEconomy, Value Chains
  - Commodity vs. Specialty Chemicals
  - Production Costs, General Considerations
  - NREL Report on Top Biomass Derived Commodity Chemicals
- Advanced Biomolecules
  - Natural Product Scaffolds
  - BioPolymers, Cell Free Syntheses

Slides and images adapted from:
Industrialization of Biology: A Roadmap to Accelerate the Advanced Manufacturing of Chemicals

Biotechnology Addresses Global Challenges

- Energy Security
- Climate Change
- Sustainable Agriculture
- Environmental Sustainability (petroleum is a limited resource, sugar is not)

Biotechnology Can Reduce

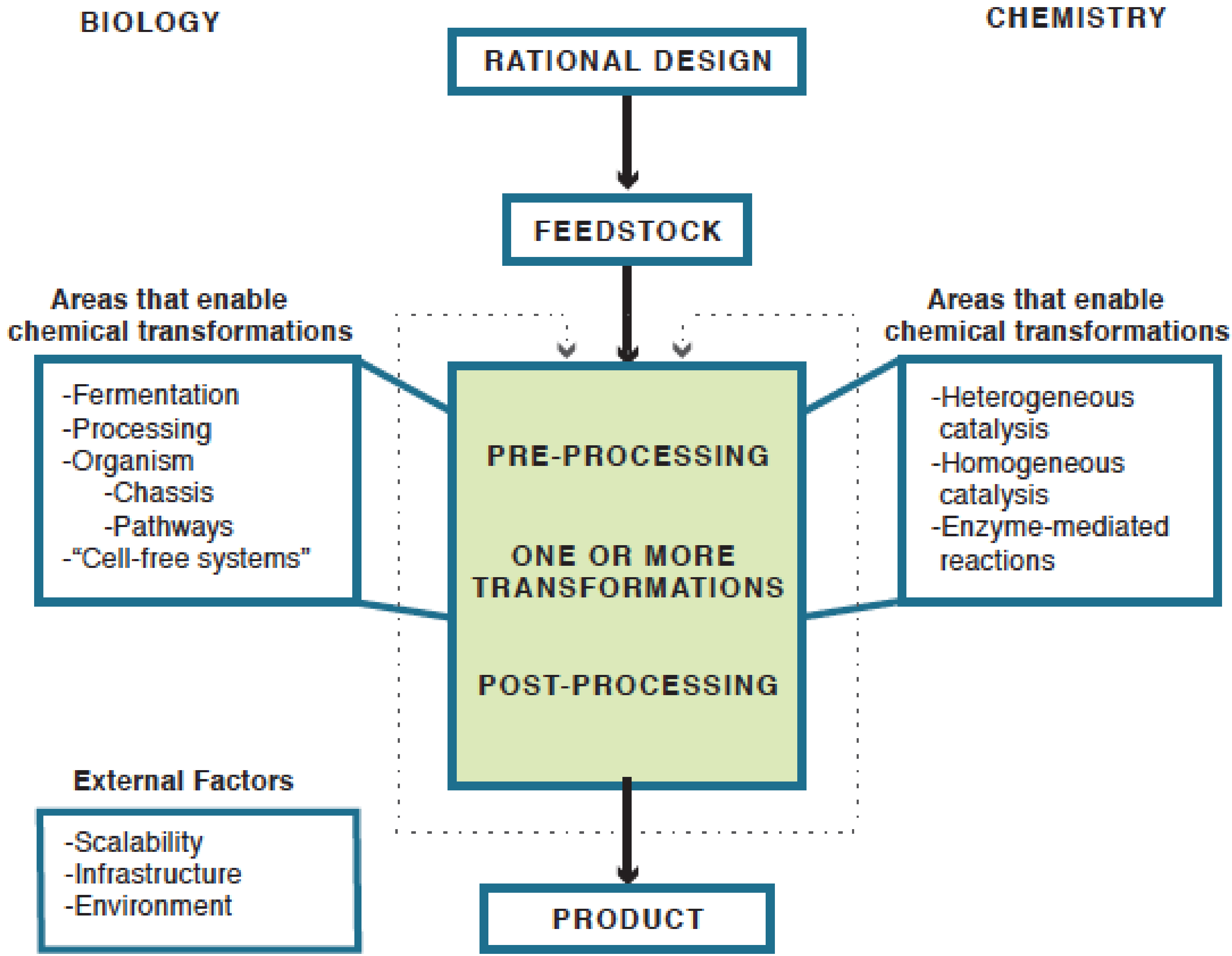
- Toxic by-products from fossil fuels
- Green House Gas emissions
- Fossil fuel consumption in production of petroleum based chemicals

Biotechnology can Provide

- Lower cost production
- Increased production speed and capacity
- Flexibility of manufacturing plants

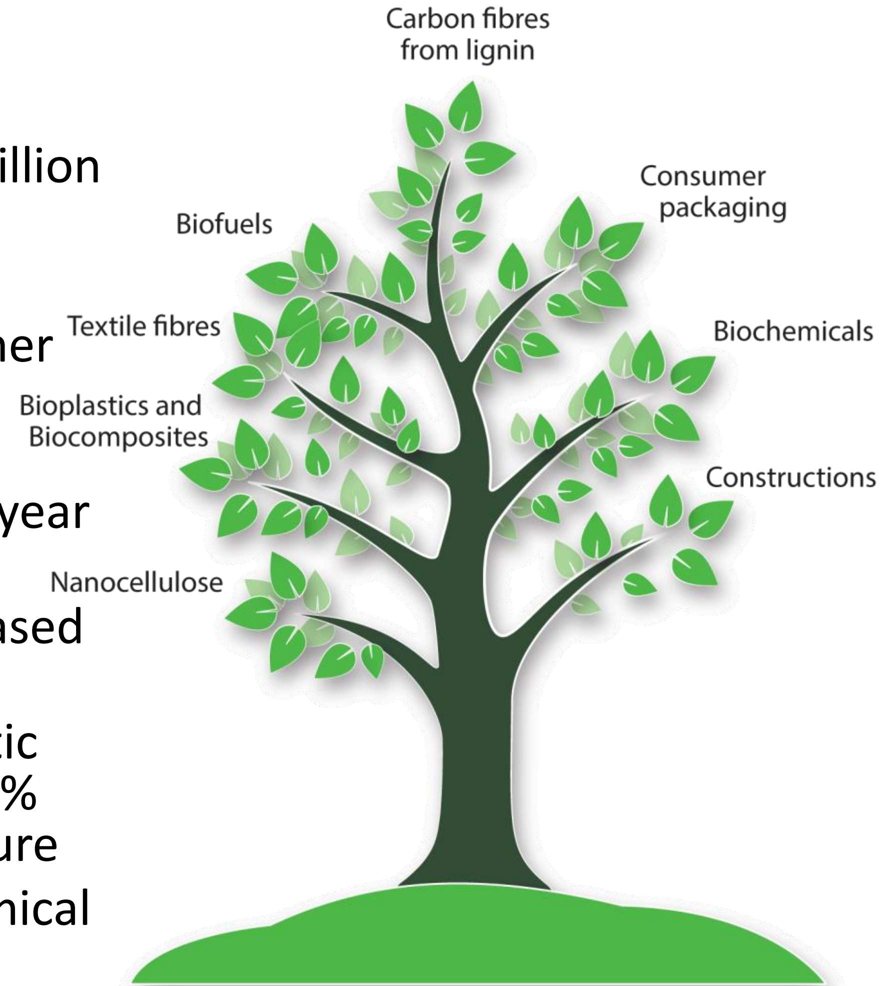
- Compared to traditional chemical synthesis or extraction from natural sources (e.g. taxol)

- Both HIGH VOLUME and HIGH VALUE CHEMICALS

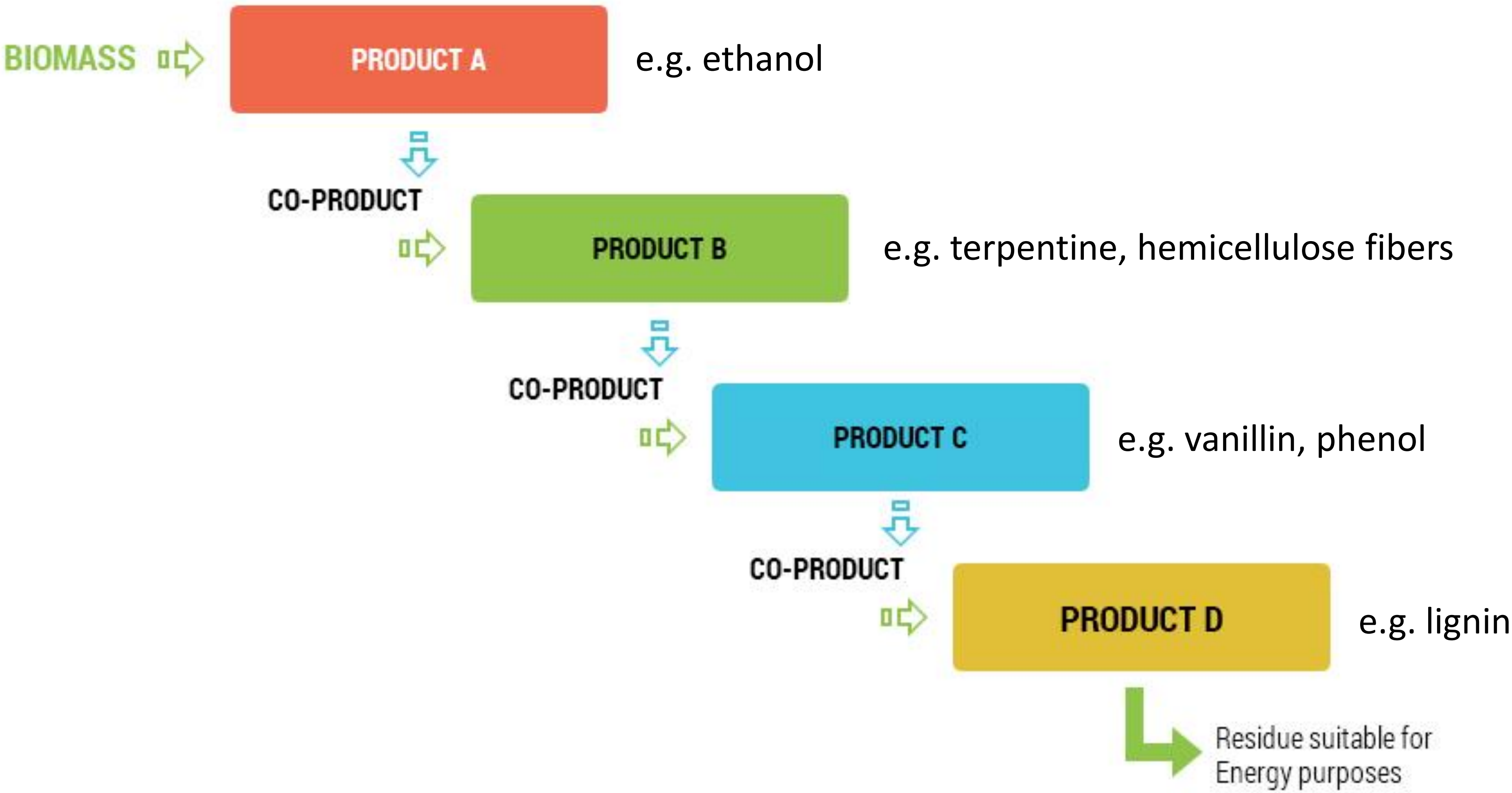

BIOLOGY
CHEMISTRY
RATIONAL DESIGN
FEEDSTOCK
Areas that enable chemical transformations
-Fermentation
-Processing
-Organism
-Chassis
-Pathways
-"Cell-free systems"
PRE-PROCESSING
ONE OR MORE TRANSFORMATIONS
POST-PROCESSING
Areas that enable chemical transformations
-Heterogeneous catalysis
-Homogeneous catalysis
-Enzyme-mediated reactions
External Factors
-Scalability
-Infrastructure
-Environment
PRODUCT

The "Bio-Economy"

- ***The BIO-ECONOMY is all economic activity resulting from scientific research focused on biotechnology***
- In 2012, U.S. bio-based product markets comprised 2.2% of GDP ($353B)
- European BioEconomy = €2 trillion (21.5 million employees)
- Largely in Human Health and Agriculture
- Global Market for enzymes used in consumer products = $8 billion
- Global bio-based chemical and polymer production is estimated at 50 million tons/year
- Business-to-business revenues in U.S. estimated at $125B in 2012 ($66B in bio-based chemicals and $30B in biofuels)
- Industrial Chemicals made through synthetic biology = $1.5B market expanding at 15-25% annual growth rate for the foreseeable future
- This represents approximately 10% of chemical market

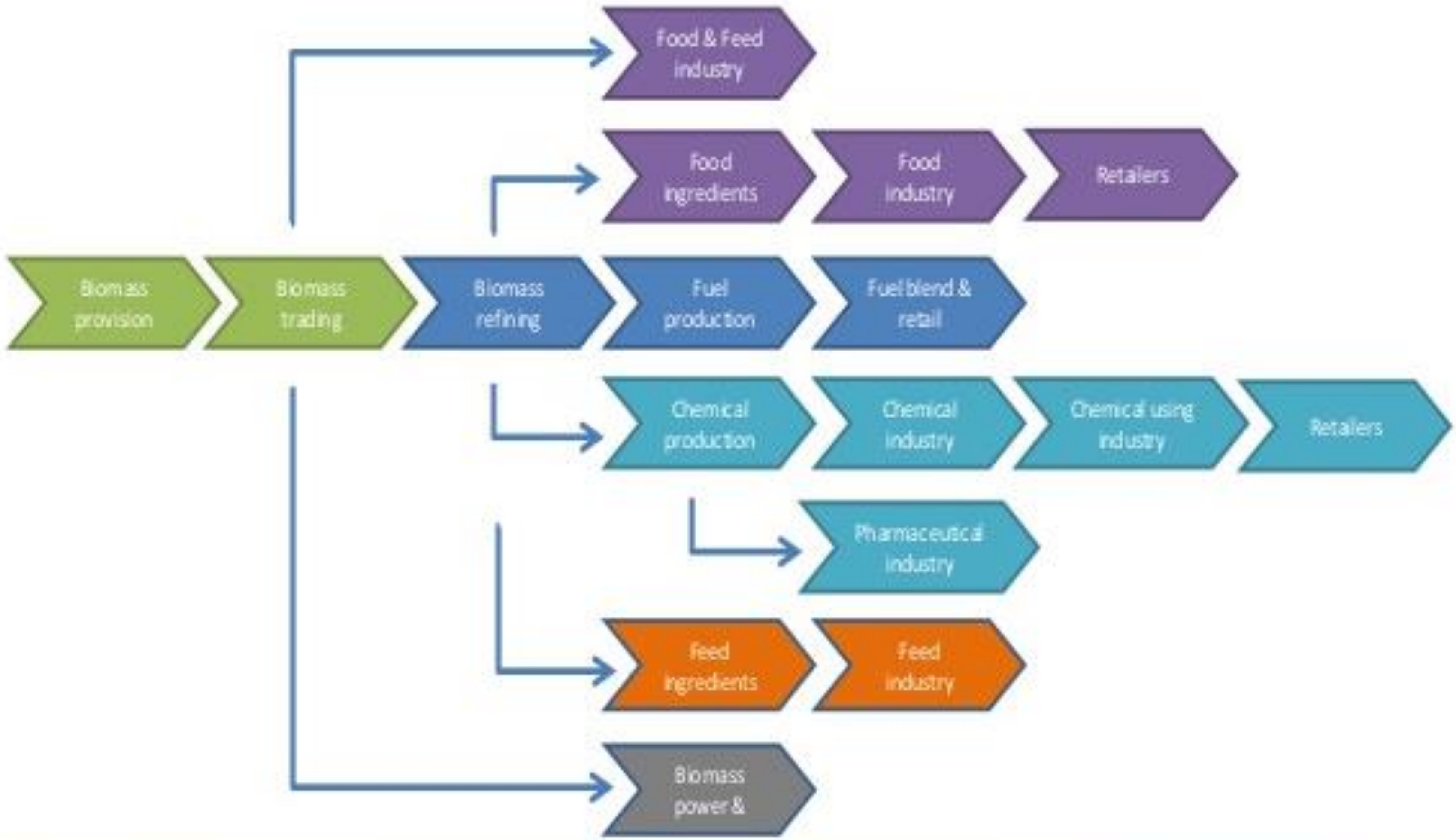


The Biomass Value Chain

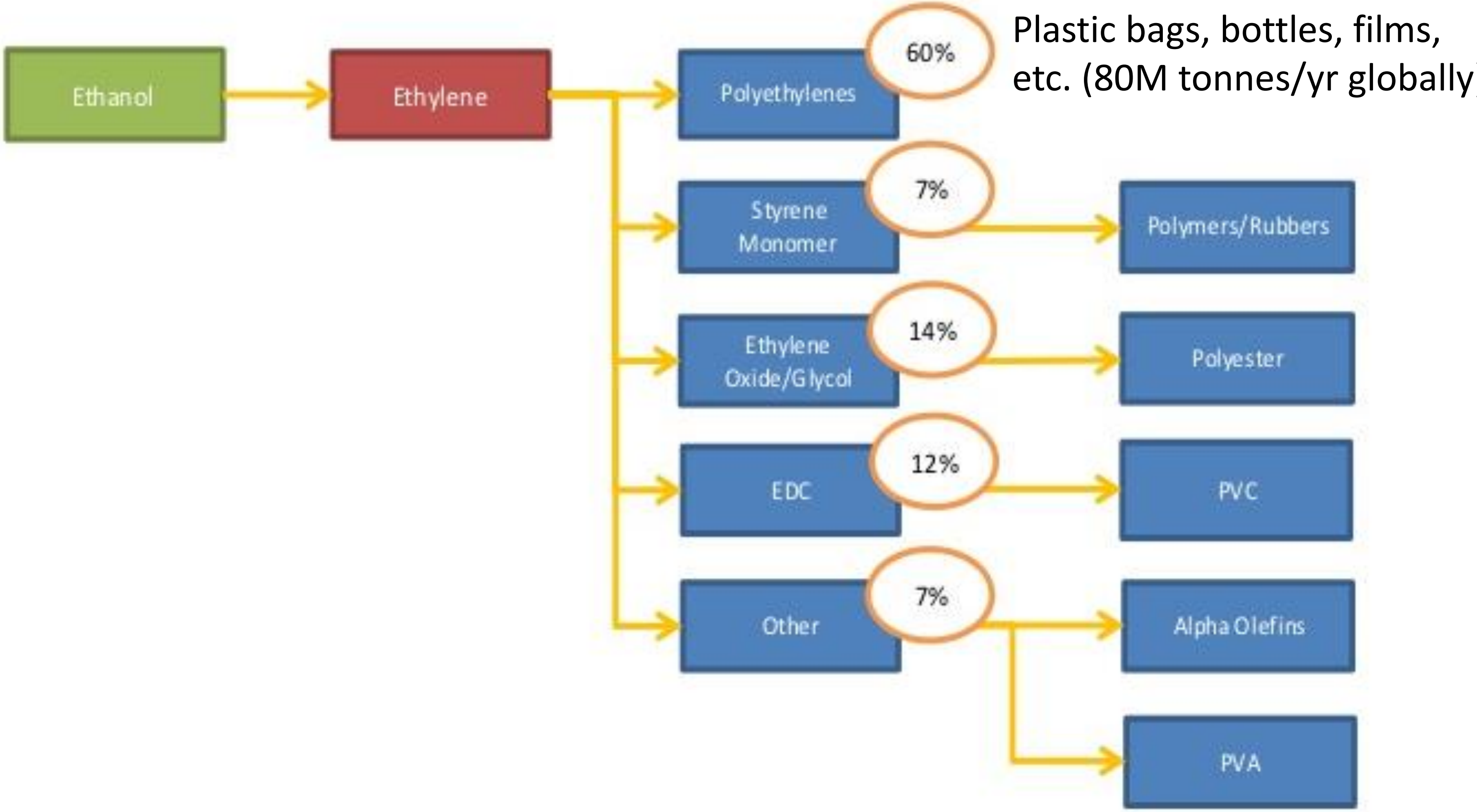


val•ue chain: (noun) *"The process or activities by which a company adds value to a [good or service], including production, marketing and the provision of after-sales service"*

Bio-Economy Value Chains

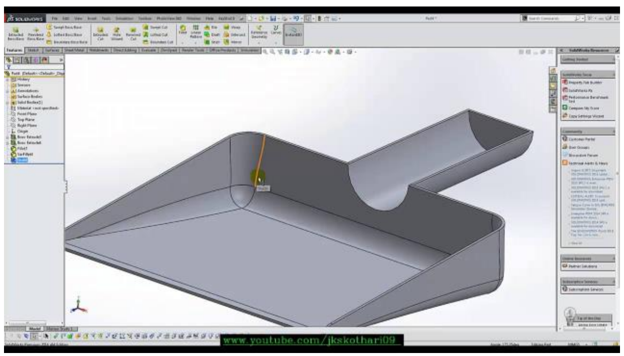


Ethanol Value Chain



Source: Nexant ChemSystems

Localized production can decentralize production processes and shift the value chain



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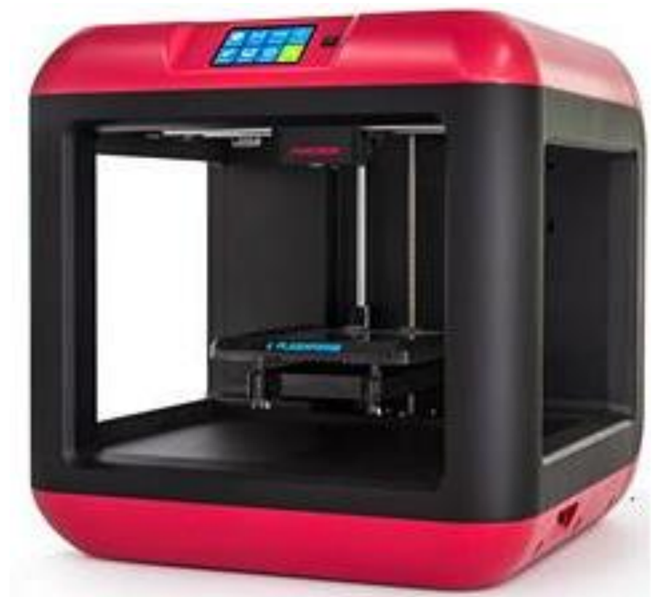

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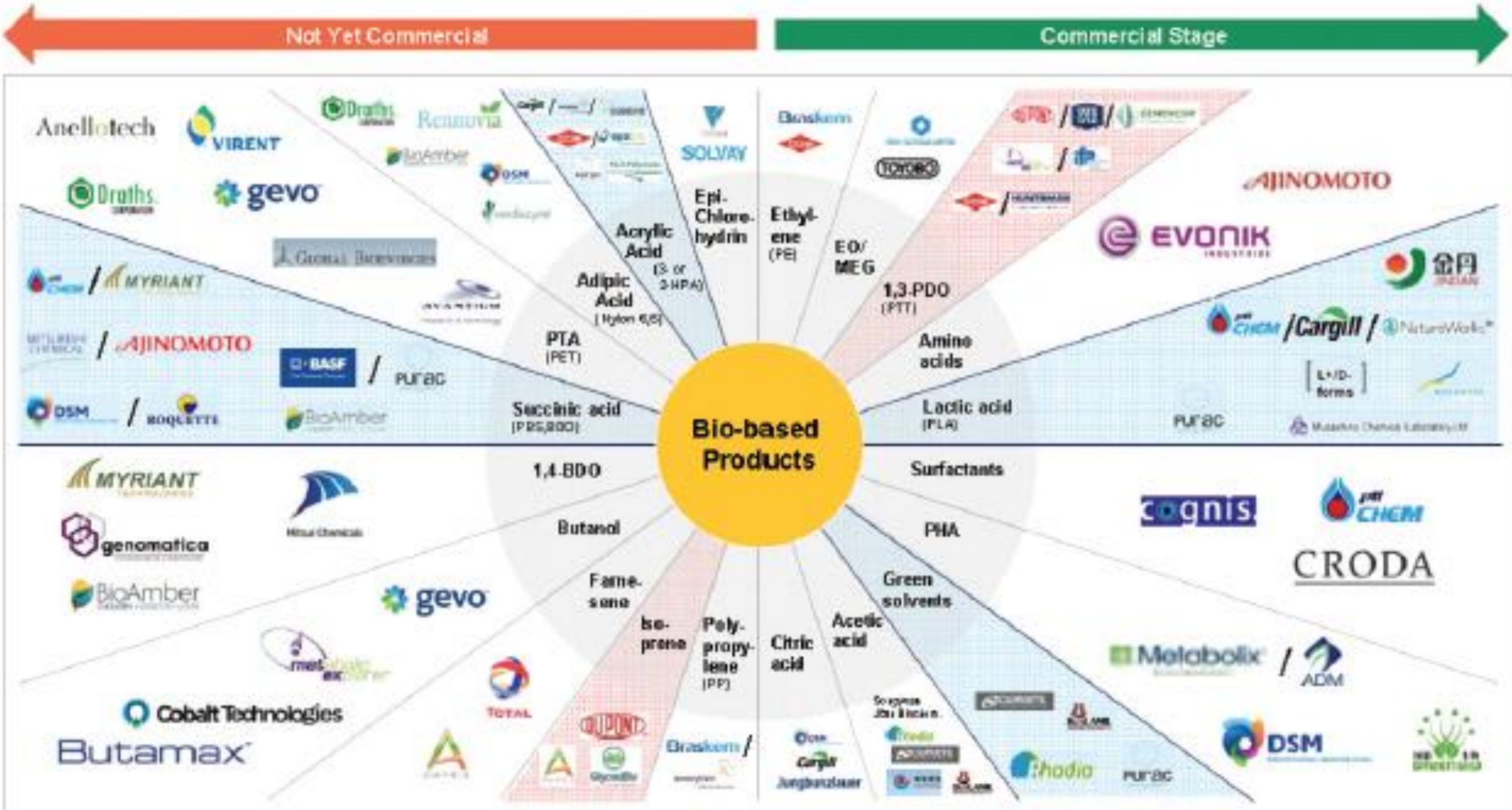
Start-Up and Commercial Biotech Companies

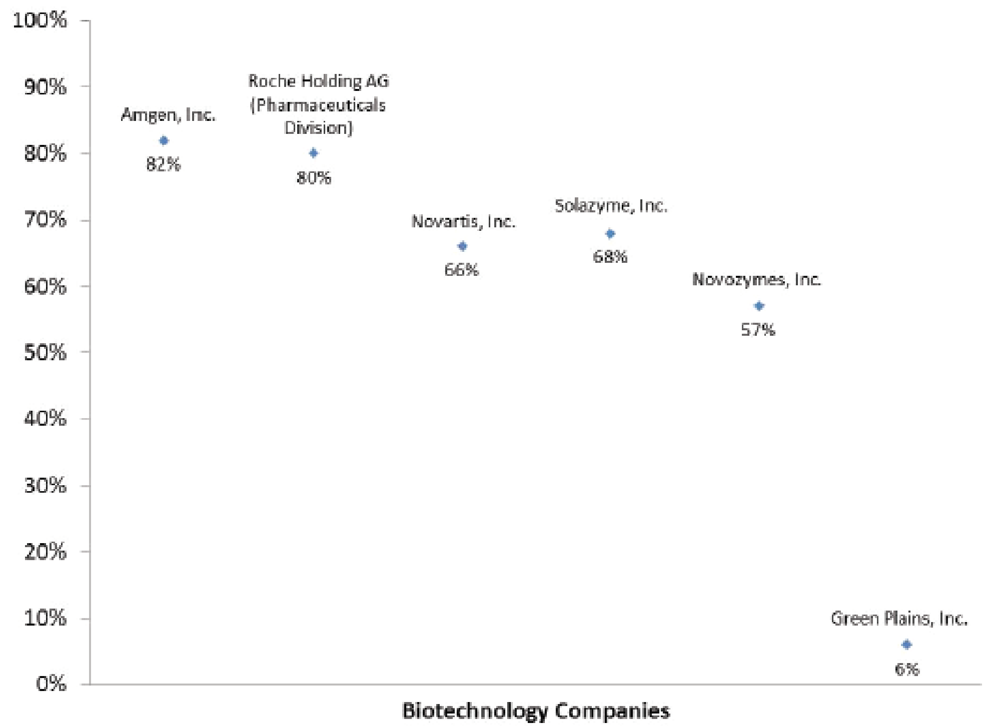


"Only 5% of the $4 trillion global market of products made by chemical transformations have been addressed [by biological production]"

- Organization for Economic Co-operation and Development

Bridging the Gap: Biofuels and Bioproducts

- Biofuels must reach an "economy of scale"
- Reactors > 600 m^3 (10^6 fold scale up from lab)
- Choice of host and co-products are essential for a successful biorefinery



Percent gross profit margins obtained by selected biomanufacturing companies (2014) producing pharmaceuticals, industrial enzymes, high value oils and ethanol

Industrial Readiness and Feedstocks

- Current feedstocks = starch from corn and sugar cane
- Cellulosic biomass (present)
- Syngas, methane and CO_2 (future)

Commodity Chemicals

- Chemicals produced at a global scale (hundreds to billions of tons)
- Often low priced (~$1-20/kg)
- Prices fluctuate daily in complex markets
  - https://www.chemweek.com/CW
  - https://www.icis.com/

- Approximately 50-100 commonly traded commodity chemicals including e.g. acetic acid, acetone, benzene, biodiesel, fatty acids, glycerol, hexane, methanol, polyethylene, phenol, sorbitol, water, etc.

Bio-Based Commodity Chemicals

- For chemicals less than \$20/kg market must be 1000 ton/year to justify production
- For chemicals of value of \$2-5/kg market must be 50,000 ton/year

General Process Considerations

- What are the chemicals to be produced?
- What is the target price of the finished chemical? ($/kg)
- What is the target mass/volume? (metric tons/yr)
- What is the target feedstock, and cost to produce at a purity that works? (i.e. glucose from lignocellulosic pretreatment)

Specialty vs. Commodity Chemicals

SPECIALTY CHEMICALS

-Examples
- -Industrial enzymes
- -Pharma Intermediaries

-Small volume
-Small assets
-Aerobic fermentation
-High value add
-High purity, high separations cost

LARGE-VOLUME CHEMICALS

-Examples
- -Biofuels
- -Polymer intermediates

-Moderate to large volume
-Asset intensive
-Anaerobic and aerobic fermentation
-Feedstock costs important
-Large fermentation scale
-Large volumes of water and cell-mass co-product

SHARED ENABLING SCIENCE AND ENGINEERING

Production Considerations

- Largest Production Cost = Feedstock and Capital Expenditure (CapEx)
  - Specialty chemical = 0.1-1 g/L-hr (reactor = $200K/m^3$)
  - Commodity Chemical = 1-5 g/L-hr (reactor = $50-100K/m^3$)
  - Corn EtOH 3-5 g/L-hr (reactor/plant = $7500/m^3$)
  - Chemical reactions (by comparison) = 30-50 g/L-hr
- Host Organism = most important determinant for economics.
  - Titer = Amount of product in unit volume (e.g. grams EtOH/Liter)
  - Rate = Titer in unit time (e.g. grams EtOH/L-hr)
  - Yield = grams EtOH/grams glucose equivalent

Fermentation

- Large capital expense
- Scale up ability is critical to project success
- Batch or Fed-Batch Mode (Current)
- Continuous Fermentation (Needed)
- Continuous Product Removal (Needed)
- Cell Free Processing (Needed)

Downstream Processing: Product Separation

- Filtration/Centrifugation of Cells
- Excretion of Product or Sonication of Cells
- Extraction (solvent or resin)
- Continuous removal of product ☺
- Concentration/Purification of Product (distillation, chromatography…)
- Packaging (sterile vs. bulk)
- Costly steps, must be considered!

Top Value Added Chemicals from Biomass[1]

- Produced from sugars via biological conversion
- Converted to higher-value chemicals or materials via chemical conversion
- This list was vetted by PNNL and NREL (from 300 candidate molecules) based on market, chemical properties/technical complexity and performance data

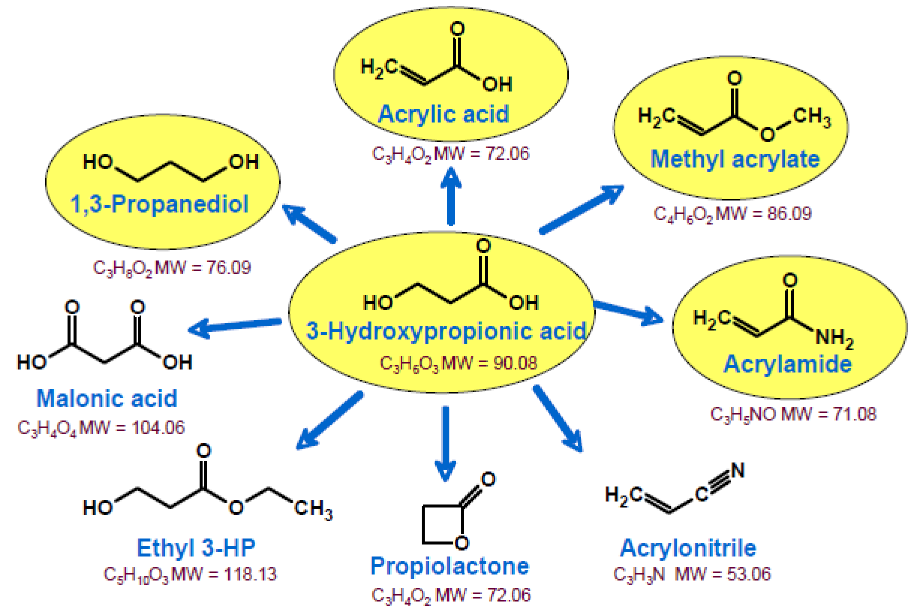
Building Blocks
1,4 diacids (succinic, fumaric and malic)
2,5 furan dicarboxylic acid
3 hydroxy propionic acid
aspartic acid
glucaric acid
glutamic acid
itaconic acid
levulinic acid
3-hydroxybutyrolactone
glycerol
sorbitol
xylitol/arabinitol

Second Tier Group: gluconic acid, lactic acid, malonic acid, Propionic acid, citric acid, aconitic acid, xylonic acid, acetoin, furfural, levoglucosan, lysine, serine, threonine

[1] http://www.nrel.gov/docs/fy04osti/35523.pdf (2004)

Star Diagrams to Rank Derivatives

- Derivatives at points o the “star”
- Derivatives ranked by:
  - Replacement potential for currently used petrochemicals
  - Novel material and performance properties that would allow for new applications and/or create a new market segment



Circled derivatives = currently in commercial use *and* produced in commodity-scale volume

Applications, e.g.

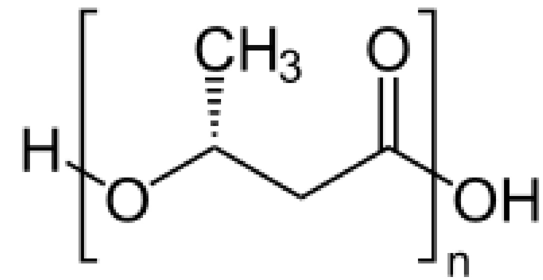
- *1,3-propanediol* -> residential carpet (Sorona^{TM})
- *acrylates* -> nail polish, Plexiglas, house paints, superabsorbents (diapers), super glue, flocculation agent (water treatment)

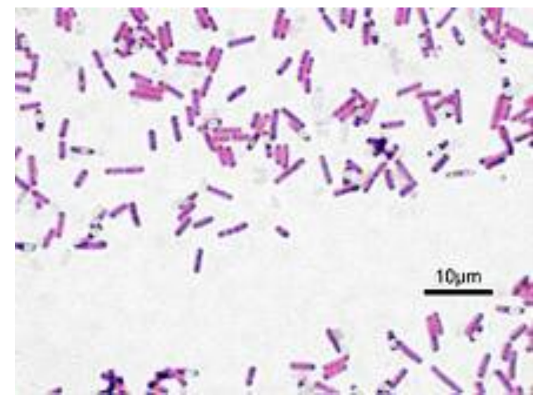
BioPolymers

- Wide ranging applications (plastics, rubbers, fibers, paints, controlled drug release)
- Properties controlled by many variables (monomer structure and bonding between monomers, average MW/polydispersity, branching patterns
- Co-polymers have additional considerations (e.g. monomer arrangement)
- Polymerases – make protein polymers (e.g. silks, wool, collagen)

BioPolymers Contd

- Ethylene has a huge market (140 million tons/yr) and can be made from ethanol
- Price of ethanol dependent on sugar price
- Butadiene (from 1,4-butanol, Genomatica), acrylic acid (Cargill), lactate (Myriant), isoprene (DuPont and Goodyear) are “in the pipeline”
- Polylactate (from lactic acid) – many organisms convert glucose in near quantitative yields, bio-sourced, biodegradable polyester.
- Create your own! small changes to the monomer structure (e.g. stereochemistry, substitution patterns, spacing between functional groups can greatly influence polymer performance)





Polyhydroxyalkanoates (PHAs) above made by engineered *Bacillus subtilis* below can be used in high value medical devices due to their biodegradable nature

Oils and Aromatics

- The NREL list only includes sugars and syngas, not lignin and oils
- Aromatics have a large commodity market for polymers and surfactants
- Polysaccharides (hemicelluloses) have potential markets in enhanced oil recovery and paper/metal finishing
- Oils have broad range of markets/applications
- Lignin can produce the entire family of aromatics that can not be made from oils or sugars

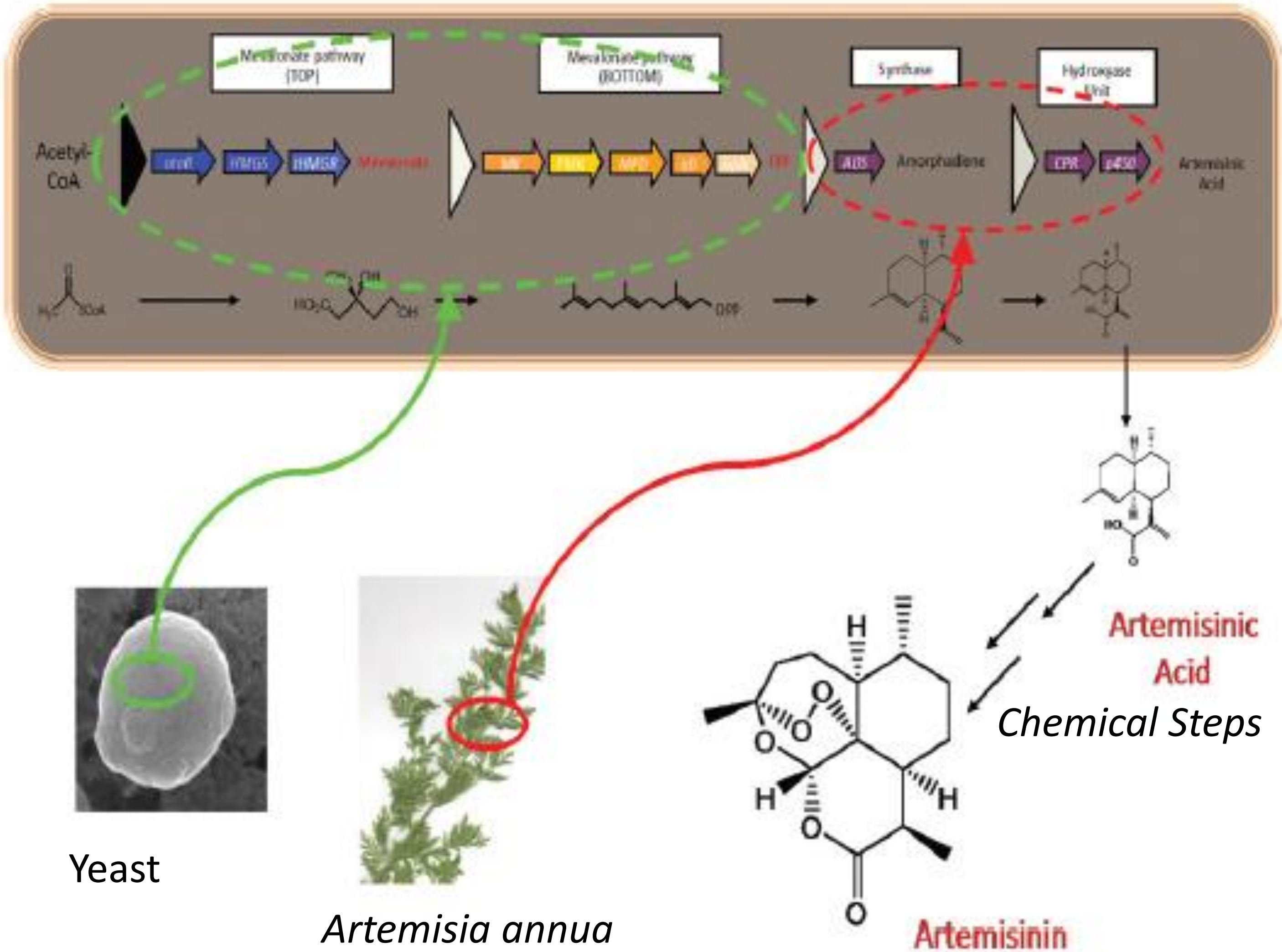
Chemistry for Consideration

- Milder oxidations, selective reductions and dehydrations
- Better control of bond cleavage
- Improvements to direct polymerization of multifunctional monomers
- Comprehensive database on biopolymer performance characteristics

Biology for Consideration

- Better pathway engineering of industrial host organisms
- Better understanding of metabolic pathways and cell biology
- Lower downstream recovery costs (of products)
- Increased utility of mixed sugar streams
- Improved enzyme thermal stability

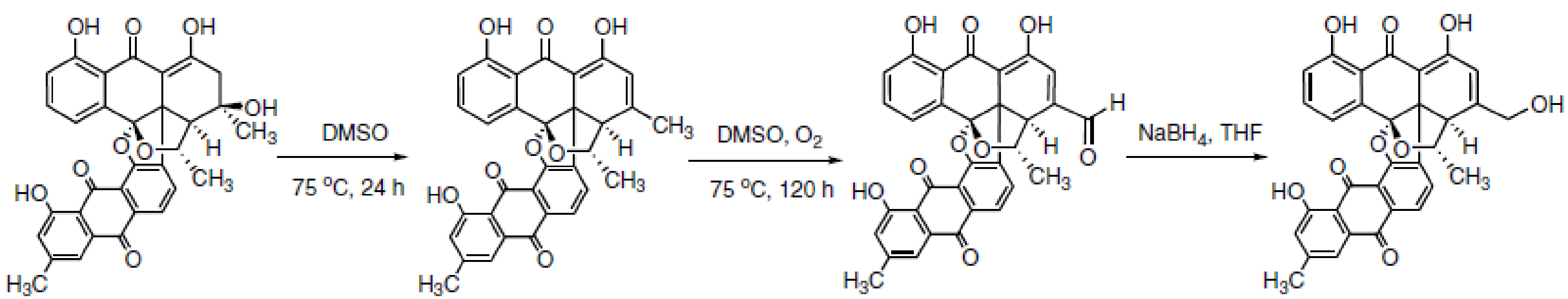
Natural Product Scaffolds



Metabolic Engineering 14 (3) 189-195

Natural Product Analogs

- Variations in core structure
  - New chemical "handles" or linkage locations (e.g. halogens, aldehydes, amines) for downstream functionalization (semi-synthesis)



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MBC vs. VRE = 29 μM

MBC vs. VRE = 0.91 μM

Bioorg Med Chem **2006** 14, 8446

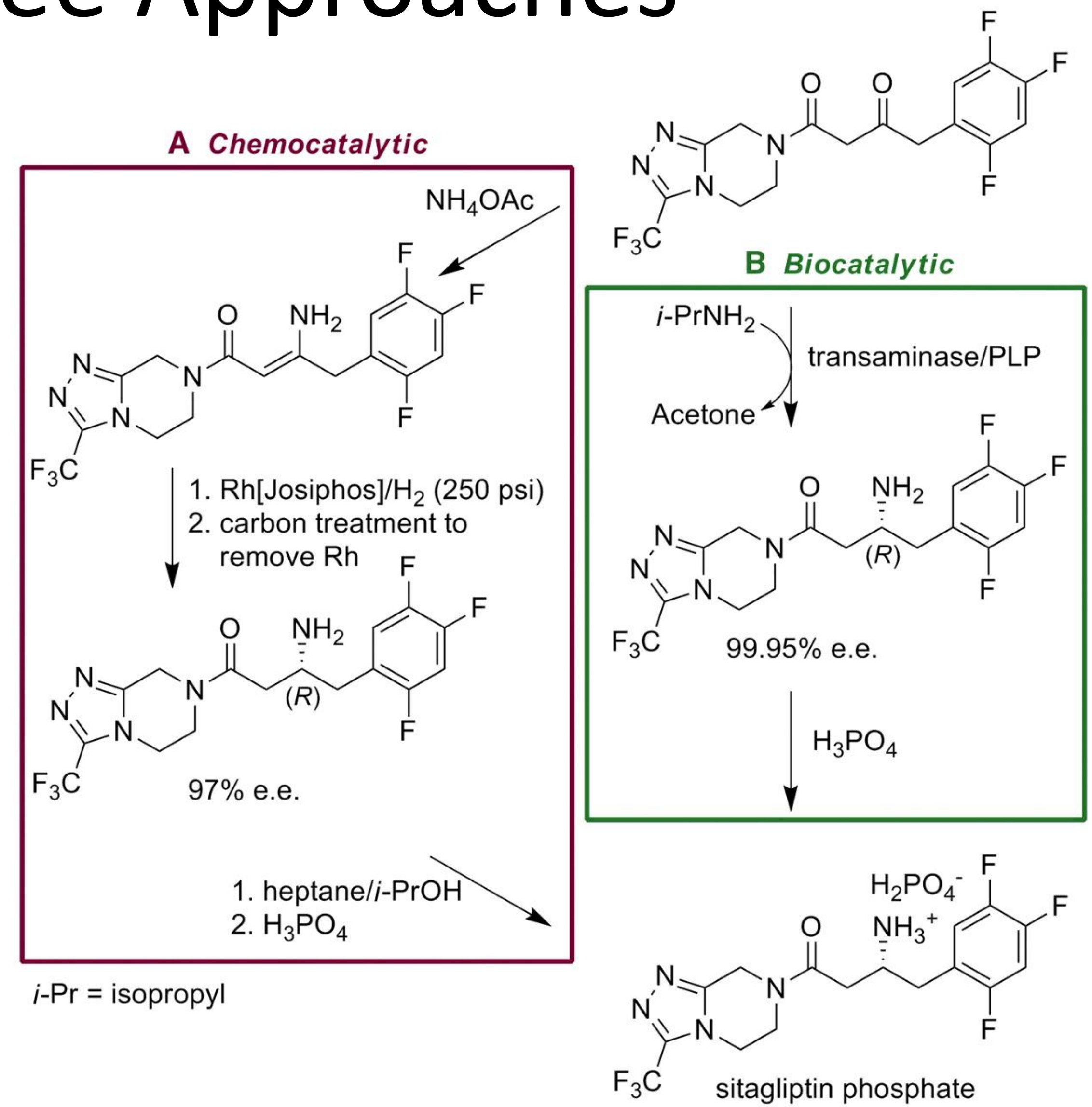
Polysaccharides

- Synthesis
  - Laborious protection/deprotection (traditional synthesis)
  - Regioselectivity
  - Glycosyl transferase enzymes can assemble
- Important modifiers of bioactive agents
  - Non-toxic, biodegradable and biocompatible
  - Drug delivery enhancement (e.g. tissue specificity, lower dosage requirements, improved encapsulation, hydrogels)
  - Diverse natural features (polyanionic, polycationic, sulfated and carboxylated)
  - Marine derived (algae, chitin)
  - Extend life of DNA and Protein therapeutics

Marine Drugs **2016**, 14, 34

Cell Free Approaches

- Pure enzymes can conduct single transformations (e.g. ketoreductases, esterases, peptidases, transaminases)
- Now only a few enzyme families can accommodate a wide range of substrates



Syn Comm 2013, 43, 24, 3281