Karlsruhe Institute of Technology

Lehrstuhl für
Technische Biologie

Industrial chemicals from waste materials and by products

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Research University · founded 1825

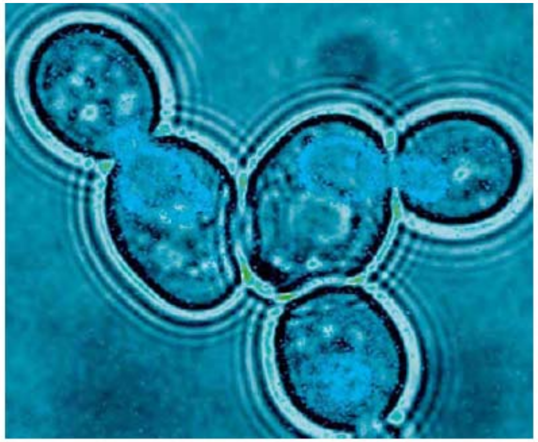
Outline

- White biotechnology today: main products and substrates
- Biorefinery concepts: future products and the demand for new substrates
- Limitations and R&D-challenges
- Conclusions

World Production and World Market Prices for Important Microbial Fermentation Products

Table 3. World production figures and prices for a number of fermentation products

	World production (ton/year)	World market price (€/kg)
Bio-ethanol	38 000 000	0.40
L-Glutamic acid (MSG)	1 500 000	1.50
Citric acid	1 500 000	0.80
L-Lysine	350 000	2
Lactic acid	250 000	2
Vitamin C	80 000	8
Gluconic acid	50 000	1.50
Antibiotics (bulk products)	30 000	150
Antibiotics (specialities)	5 000	1 500
Xanthan	20 000	8
L-Hydroxyphenylalanine	10 000	10
Dextran	200	80
Vitamin B_{12}	3	25 000



Pictures: BMBF, WBT (2008)

Reference:: W. Soetaert, E. Vandamme, Biotechnol. J. (2007)

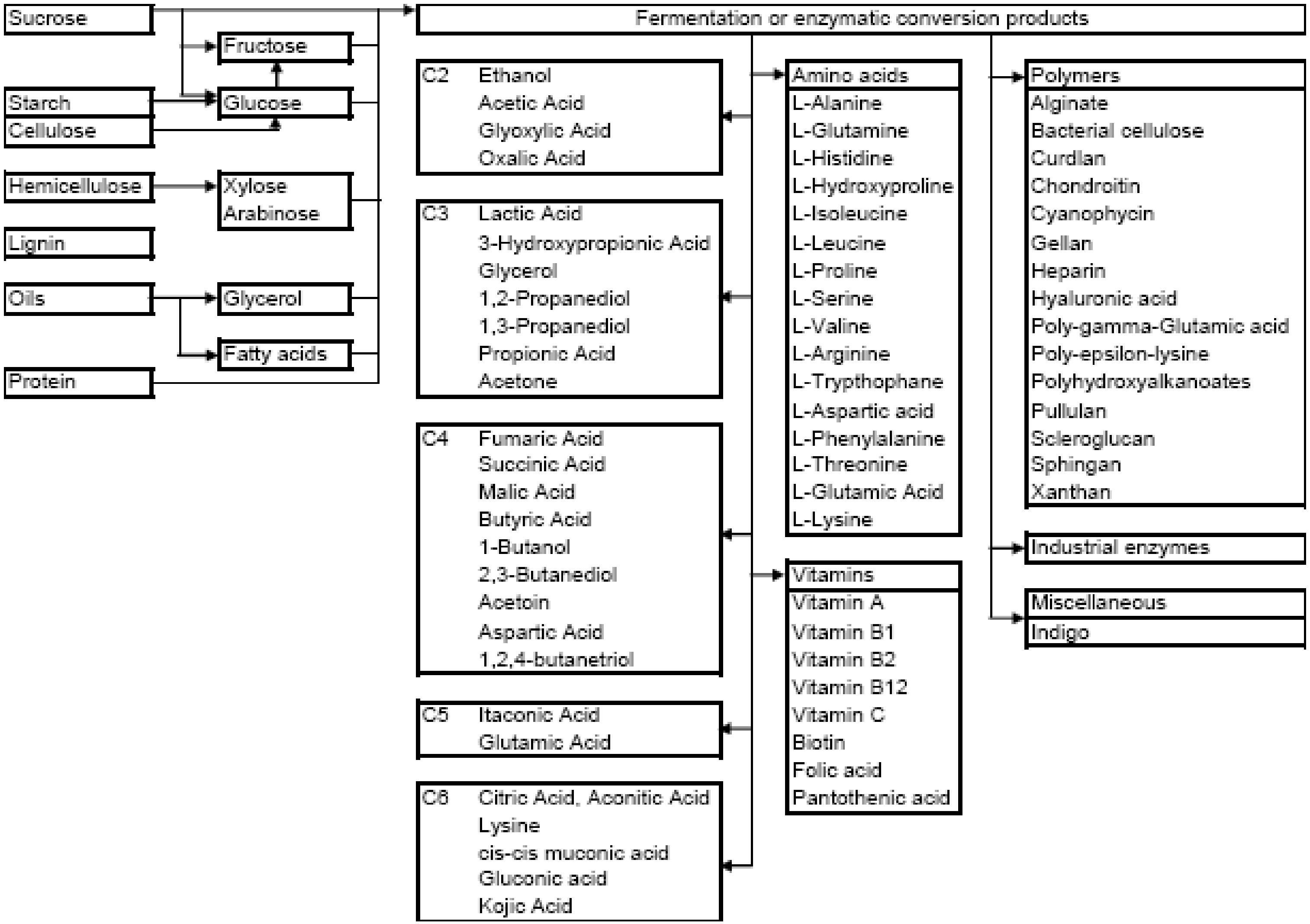
White Biotechnology – Products Available in Ton-Scale

*Produkte der Weißen Biotechnologie, die bereits heute im Tonnenmaßstab hergestellt werden (*enzymatisch hergestellt) nach DECHEMA, 2004.*

Produkt	Weltjahres produktion (t/a)	Anwendung	Produkt	Weltjahres produktion (t/a)	Anwendung
Säuren			**Antibiotika**		
Zitronensäure	1.000.000	Lebensmittel, Waschmittel	Penicilline	45.000	Medizin, Futtermittelzusatz
Essigsäure	190.000	Lebensmittel	Cephalosporine	30.000	Medizin, Futtermittelzusatz
Gluconsäure	100.000	Lebensmittel, Textil, Metall	Tetracycline	5.000	Medizin
Itaconsäure	15.000	Kunststoff, Papier, Klebstoff	**Biopolymere**		
L-Apfelsäure*	100	Säuerungsmittel	Polylactid	140.000	Verpackung
Aminosäuren			Xanthan	40.000	Erdölförderung, Lebensmittel
L-Glutamat	1500.000	Geschmacksverstärker	Dextran(-derivate)	2.600	Blutersatzstoff
L-Lysin	700.000	Futtermittel	**Vitamine**		
L-Threonin	30.000	Futtermittel	Ascorbinsäure (Vit. C)	80.000	Pharma, Lebensmittel
L-Asparaginsäure*	13.000	Aspartam-Herstellung	L-Sorbose	50.000	Pharma, Lebensmittel
L-Phenylalanin	10.000	Aspartam, Medizin	(Vit. C Vorstufe)		
L-Tryptophan	1.200	Ernährung, Futtermittel	Riboflavin (B_2)	30.000	Wirkstoff, Futterzusatz
L-Arginin	1.000	Medizin, Kosmetik	**Kohlenhydrate**		
L-Cystein	500	Pharma, Lebensmittel	Glucose*	20.000.000	Flüssigzucker
L-Alanin*	500	Infusionslösungen	High Fructose Syrup*	8.000.000	Getränke, Ernährung
L-Methionin	400	Infusionslösungen	Fructooligosaccharide*	10.500	Präbiotikum
Lösungsmittel			Cyclodextrine*	5.000	Kosmetik, Pharma, Lebensmittel
Bioethanol	18.500.000	Lösungsmittel, Energieträger			

Reference: Weiße Biotechnologie – Chancen für neue Produkte und umweltschonende Prozesse, BMBF (2008)

The Value Chain of White Biotechnology – Main Products



Reference: M. Patel et al. BREW-Report (2006)

The Value Chain of White Biotechnology – Intermediates?

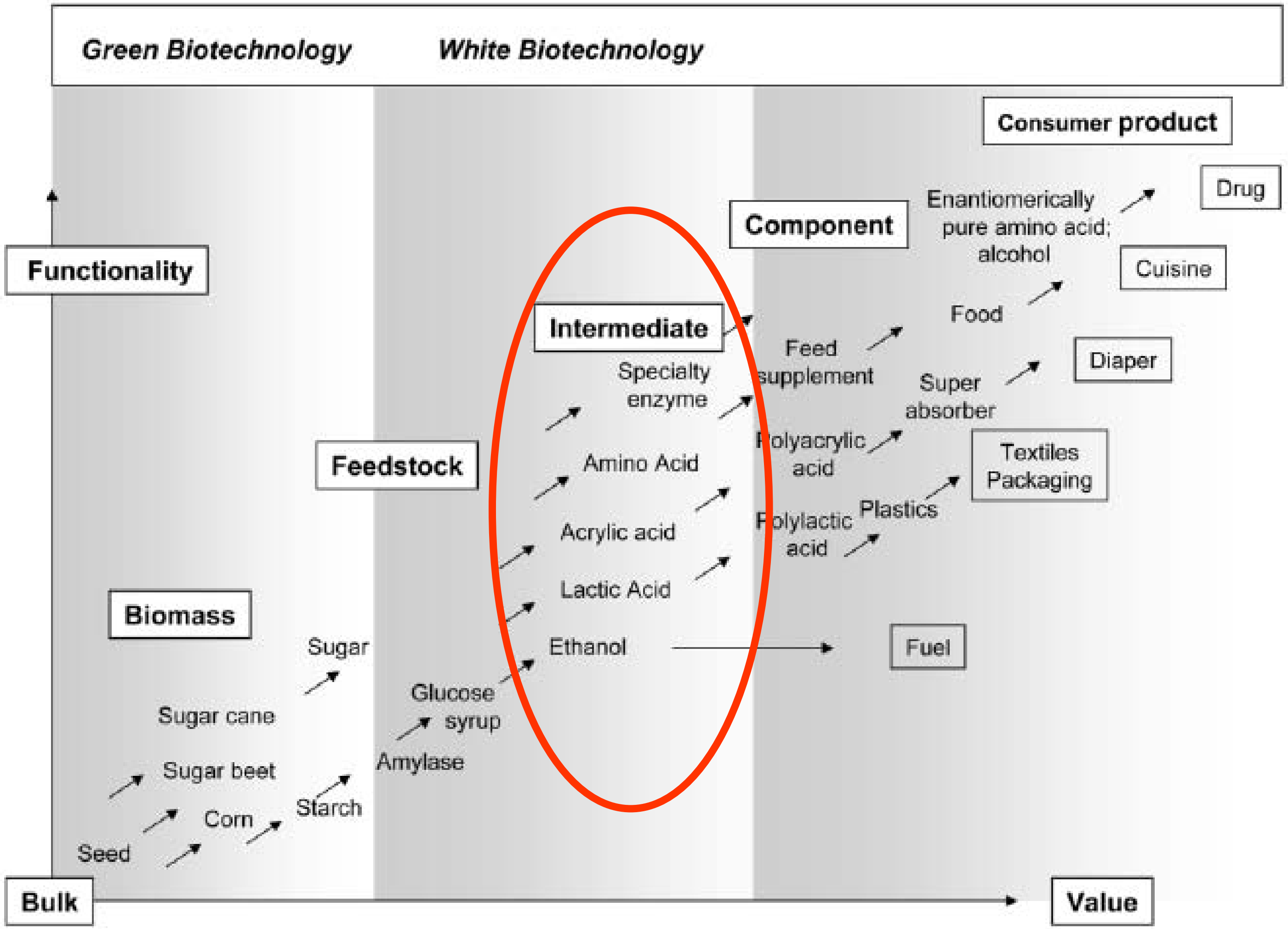


Figure 1. Products of white biotechnology in the value chain.

Reference: M. Kircher, Biotechnol. J. (2007)

White Biotechnolgy – 13 key plant carbohydrate-derived building blocks identified in the EU „BREW“ project

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Acetic Acid	**Fumaric Acid**	**Malic Acid**
Acetone	**Glutamic Acid**	**Propionic Acid**
Butanol	**Gluconic Acid**	**Succinic Acid**
Citric Acid	**Itaconic Acid**	
Ethanol	**Lactic Acid**	

Pictures: BMBF, WBT (2008)

Reference: D.B. Turley in: Chemicals from Biomass, Wiley (2008)

The Value Chain of White Biotechnology – Substrates?

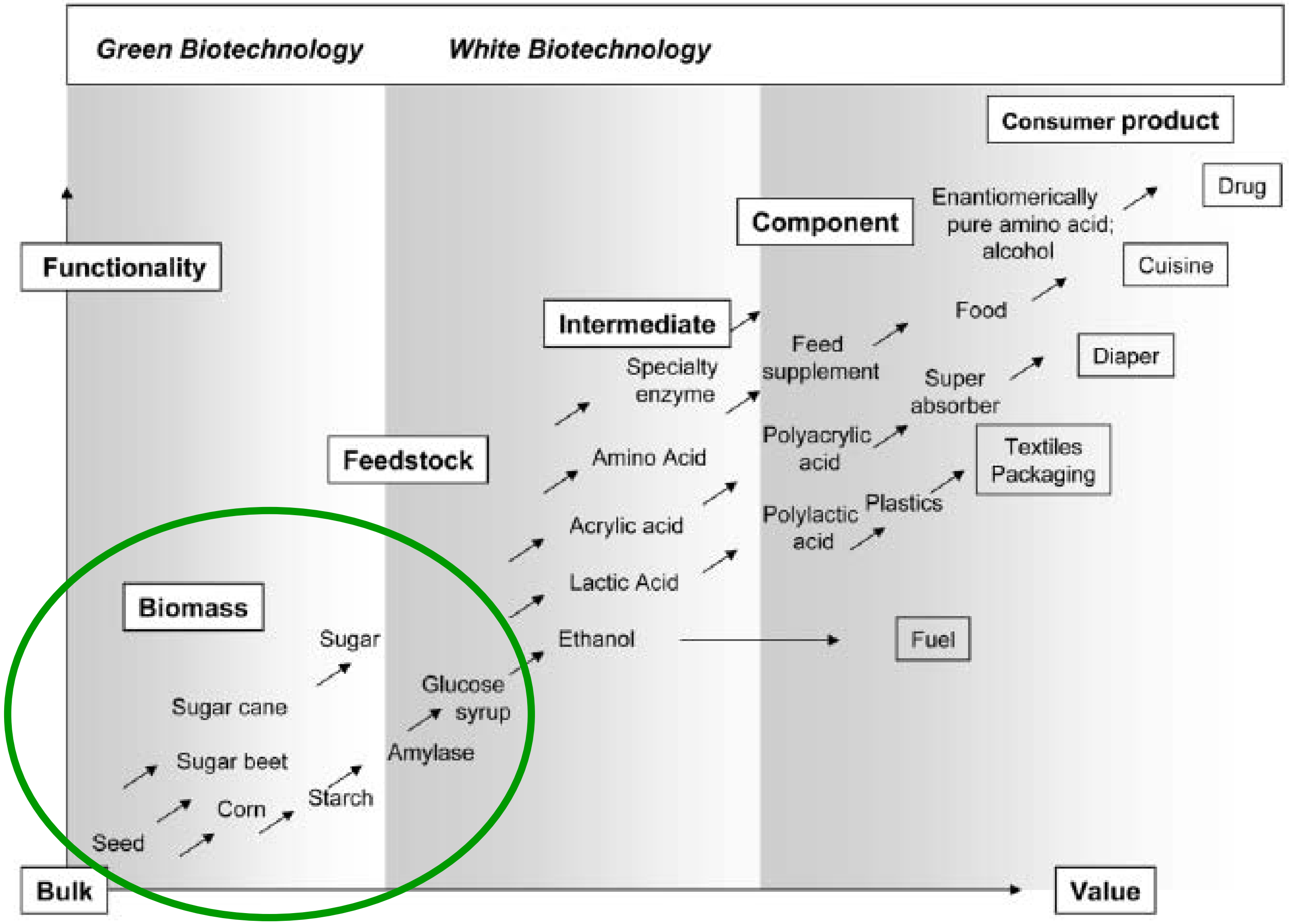


Figure 1. Products of white biotechnology in the value chain.

Reference: M. Kircher, Biotechnol. J. (2007)

White Biotechnology today –
What are the main substrates used today?

- Main C-sources: glucose, sucrose, starch, glycerol, acetate
- Main waste substrates: sugar cane and sugar beet molasses, corn steep liquor, deproteinised whey, waste streams from food and paper industries
- Main complex medium components: yeast extract, malt extract, peptones
- Chemical precursors for more complex products

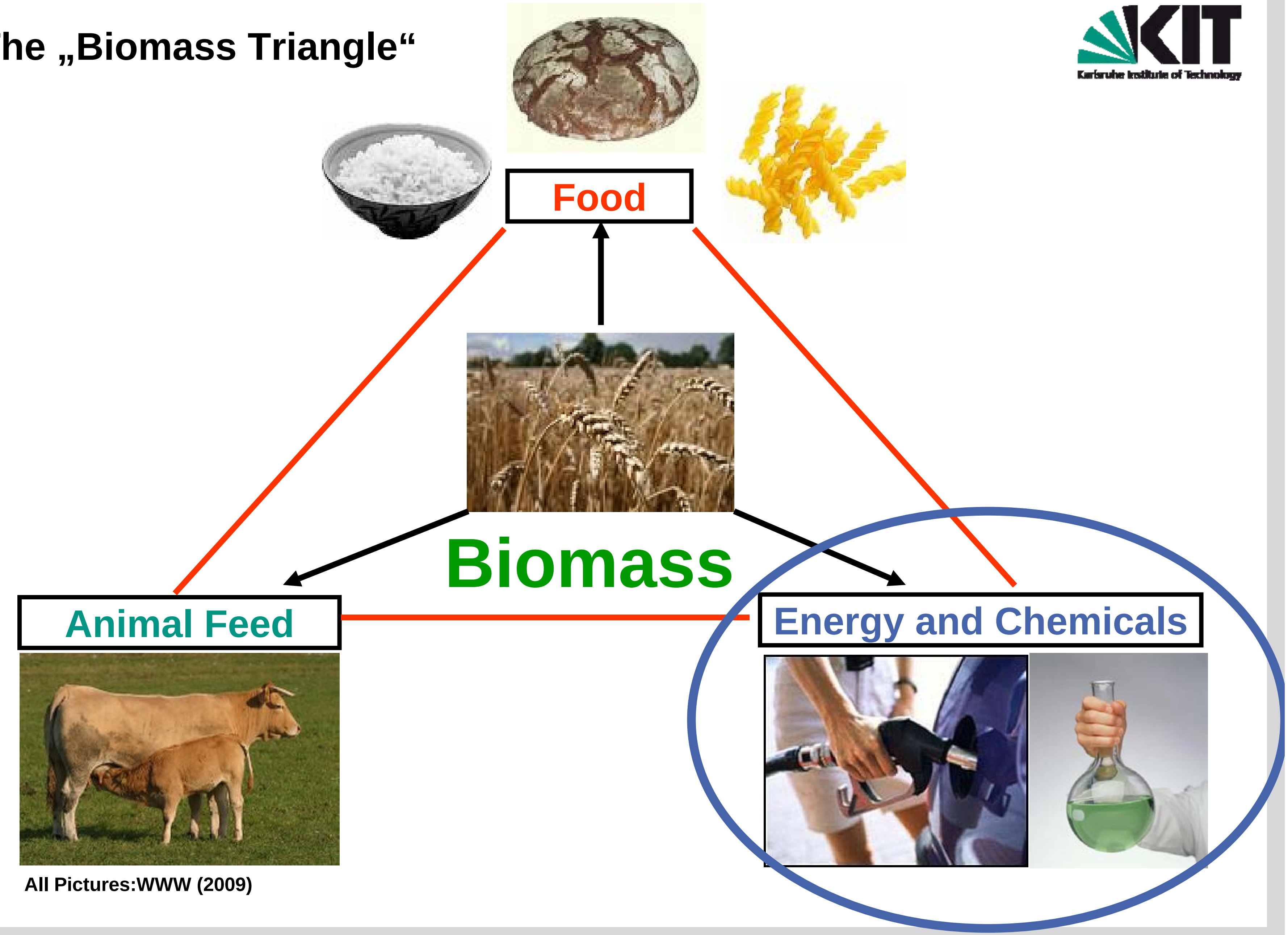
White Biotechnology – „The Vision 2025“ (EuropaBio and ESAB, 2007)

Our vision for industrial or white biotechnology in 2025:

- An increasing number of chemicals and materials will be produced using biotechnology in one of its processing steps. Biotechnological processes are used for producing chemicals and materials, otherwise not accessible by conventional means, or existing products in a more efficient and sustainable way.
- Biotechnology allows for an increasing eco-efficient use of renewable resources as raw materials for the industry
- Industrial biotechnology will enable a range of industries to manufacture products in an economically and environmentally sustainable way.
- Biomass derived energy, based on biotechnology, is expected to cover an increasing amount of our energy consumption.
- Rural biorefineries will replace port-based oil refineries wherever it is economically feasible.
- European industry will be innovative and competitive, with sustained cooperation and support between the research community, industry, agriculture and civil society.
- Green Biotechnology could make a substantial contribution to the efficient production of biomass raw materials.

Reference: Dirk Carrez, Wim Soetaert, EuropaBio and ESAB (2007)

The „Biomass Triangle“



All Pictures:WWW (2009)

White Biotechnology –
Will it be possible to use alternative substrates?

- Substrates used for industrial microbiology processes have been optimised since many years.
- At the moment no better substrates are available according to optimal microbial growth, product yields and prices.
- Substrate availability is sufficient for today´s microbial production processes, but will not meet the demands for biofuel and chemical production.
- It will be necessary to use substrates not in competition with food or animal feed and to evaluate waste and by-product streams as substrates.

Production of chemicals from plant saccharides

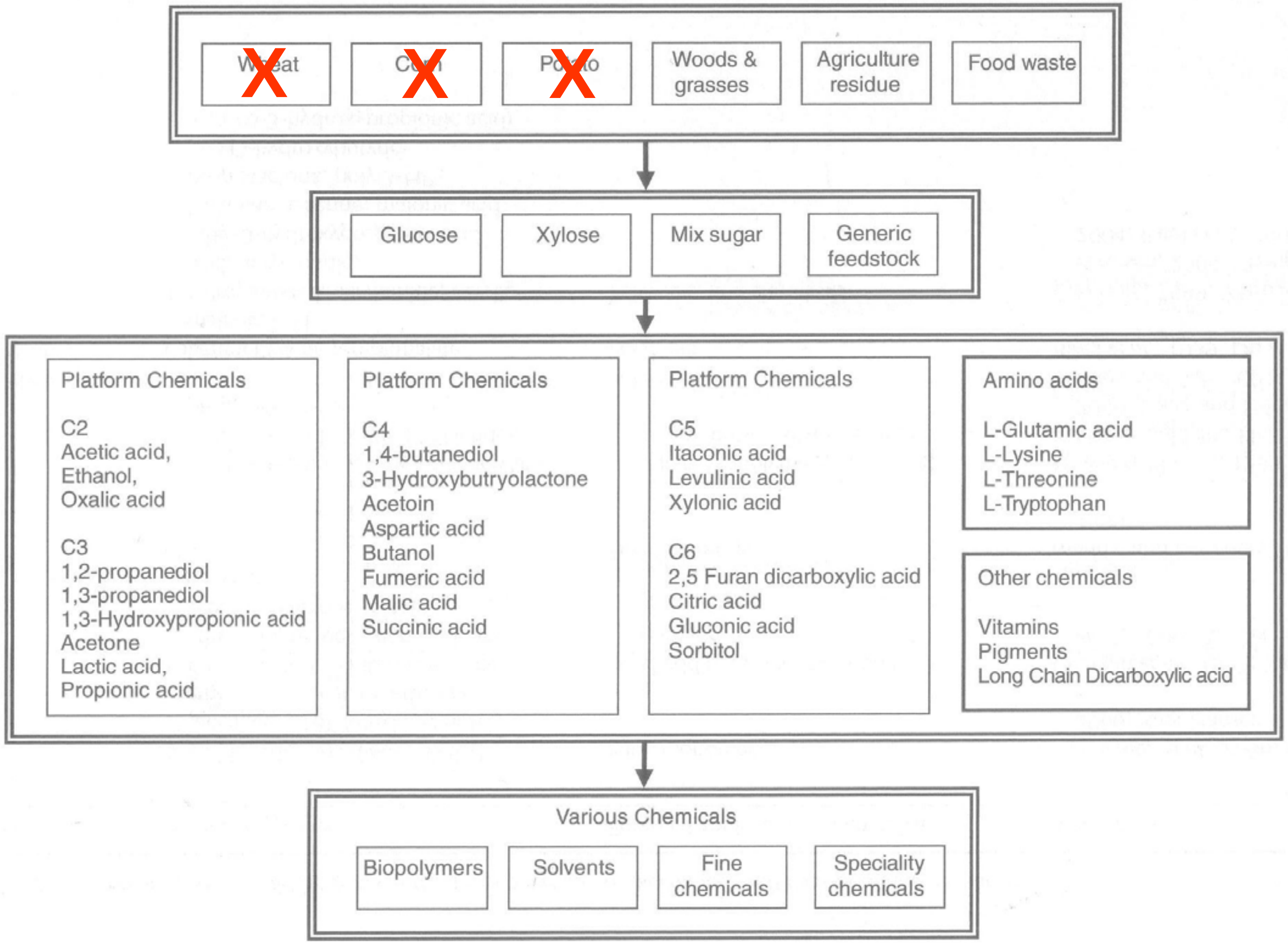
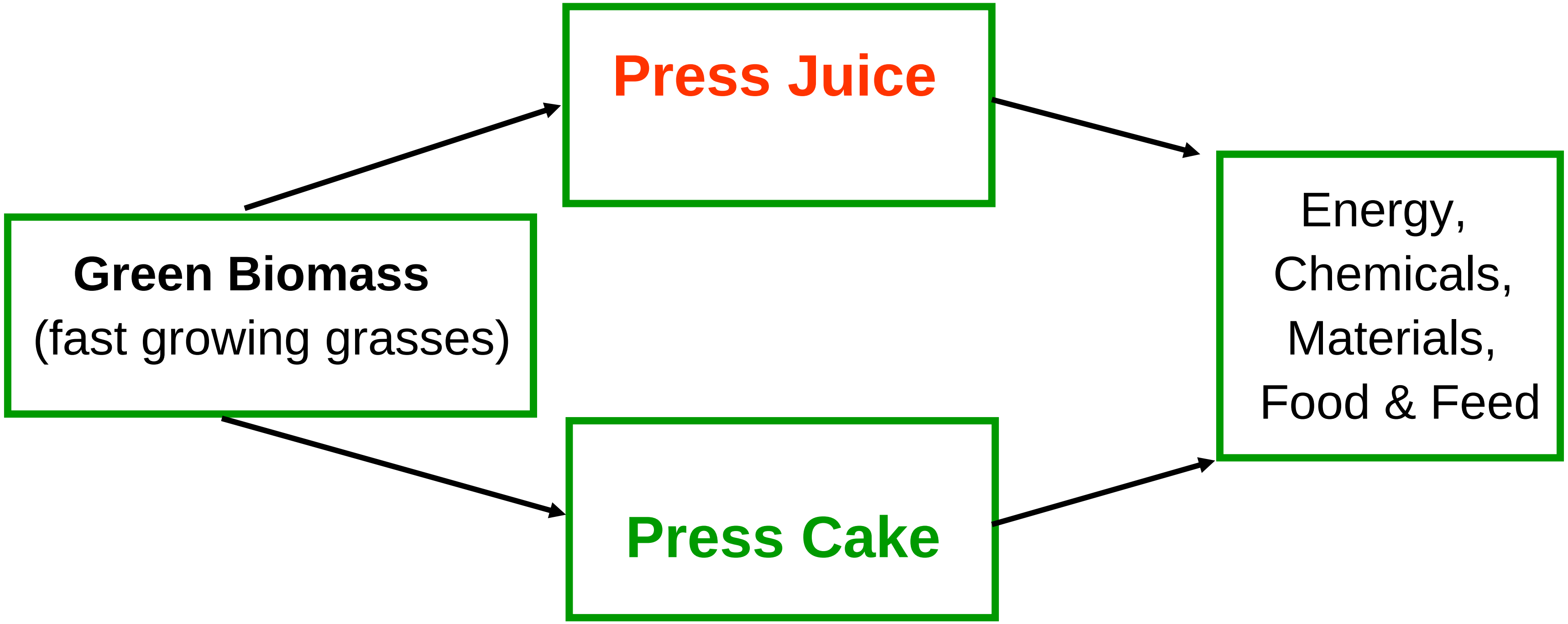


Figure 4.2 Generic biorefining schemes for chemical production from saccharides

Reference: A.A. Koutinas et al. in: Chemicals from Biomass, Wiley (2008)

The concept of the „Green Biorefinery“:



Reference: J.F. Clarke and F.E.I. Deswarte in: Chemicals from Biomass, Wiley (2008)

The concept of the „Green Biorefinery“

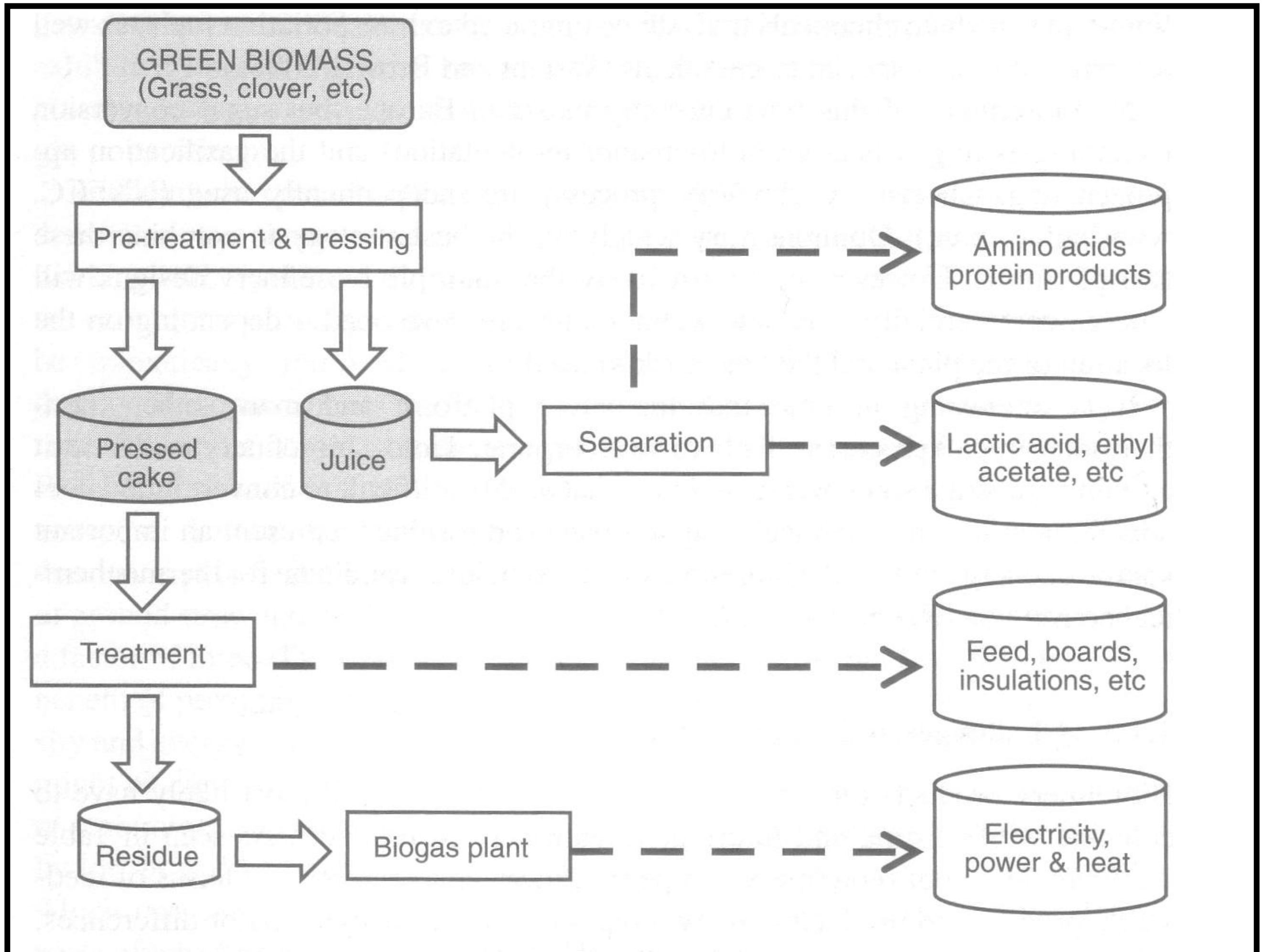


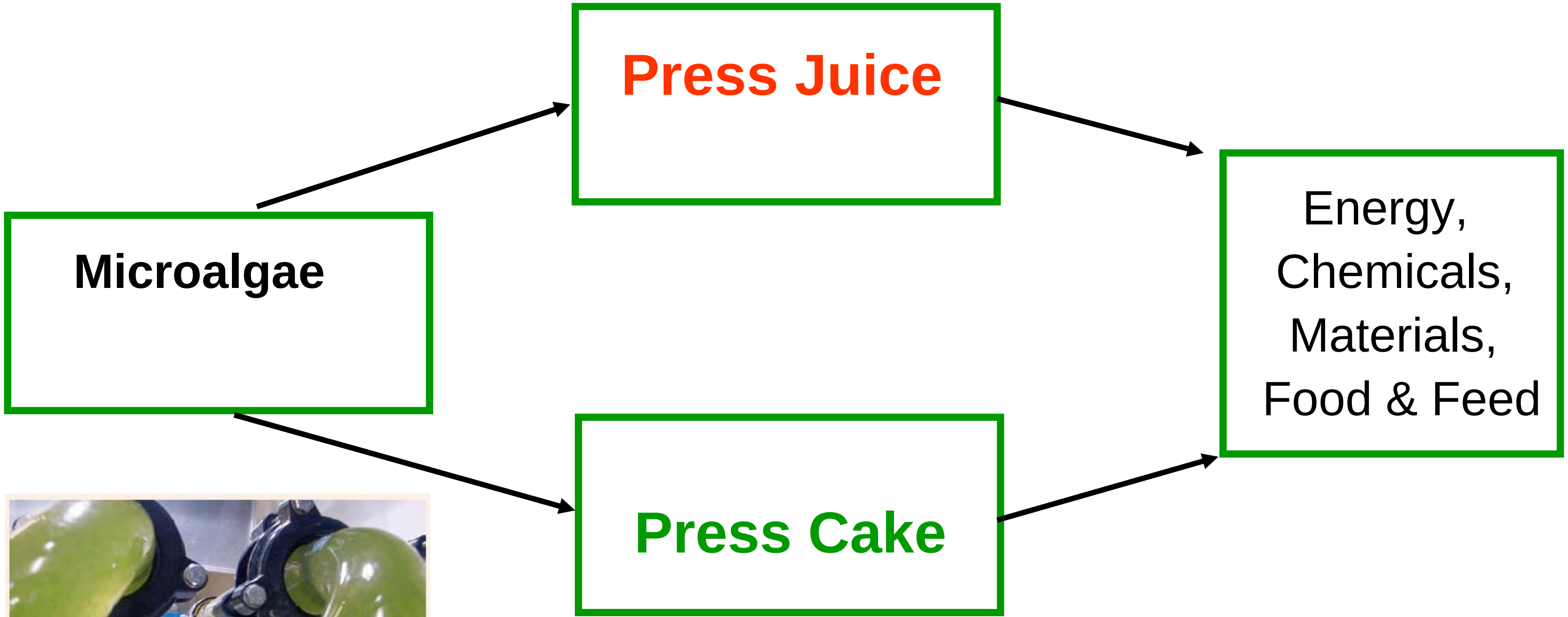
Figure 1.10 *A simplistic schematic of the 'Austrian Green Biorefinery' (Based on Schnitzer, 2006)*

Reference: J.F. Clarke and F.E.I. Deswarte in: Chemicals from Biomass, Wiley (2008)

The „Green Biorefinery“ – A future key role for „Green Biotechnology“?

- „If plants would be easily degradable to sugars, there would be no plants.“ (Weyman, 2008)
- The development of „energy plants“ optimal for the production of biofuels and chemicals will take at least a decade of time.
- An interesting alternative could be a high cell density cultivation and use of microalgae.
- Another alternative, which already is under investigation, is the use of waste biomass containing cellulose, hemicellulose and lignin.

The concept of a „Microalgae Biorefinery“:



Picture: BMBF, WBT (2008)

Reference: J.F. Clarke and F.E.I. Deswarte in: Chemicals from Biomass, Wiley (2008)

White Biotechnology –
Composition of selected plants and biomasses

Table 4.1 Composition of selected plant biomass (after Wyman, 2008)

Content of compound [%]	Agricultural Waste	Woody Plants	Municipal Solid Waste	Herbaceous energy crops
Cellulose	43	45	45	45
Hemicellulose	27	25	9	30
Lignin	17	22	10	15
Other Carbohydrates	n.s.	n.s.	9	n.s.
Protein	n.s.	n.s.	3	n.s.
Ash	n.s.	3	n.s.	n.s.
Other	13	5	9	10

Reference: D. Ernst, A. Neumann and C. Syldatk (in preparation)

World production and recent world market prices for different feedstocks

Table 2. Estimated world production figures and indicative world market price of a number of renewable and petrochemical raw materials

	World production (million tons/year)	World market price (€/ton)
Renewable raw material		
Cellulose	320	500
Sugar	140	250
Starch	55	250
Glucose	30	300
Bio-ethanol	38	400
Glutamic acid	1	1500
Petrochemicals		
Ethylene	85	500
Propylene	45	350
Benzene	23	400
Terephthalic acid	12	700
Isopropanol	2	700
Caprolactam	3	2000

Reference: W. Soetaert, E. Vandamme, Biotechnol. J. (2007)

The concept of the „Lignocellulosic Feedstock Biorefinery“

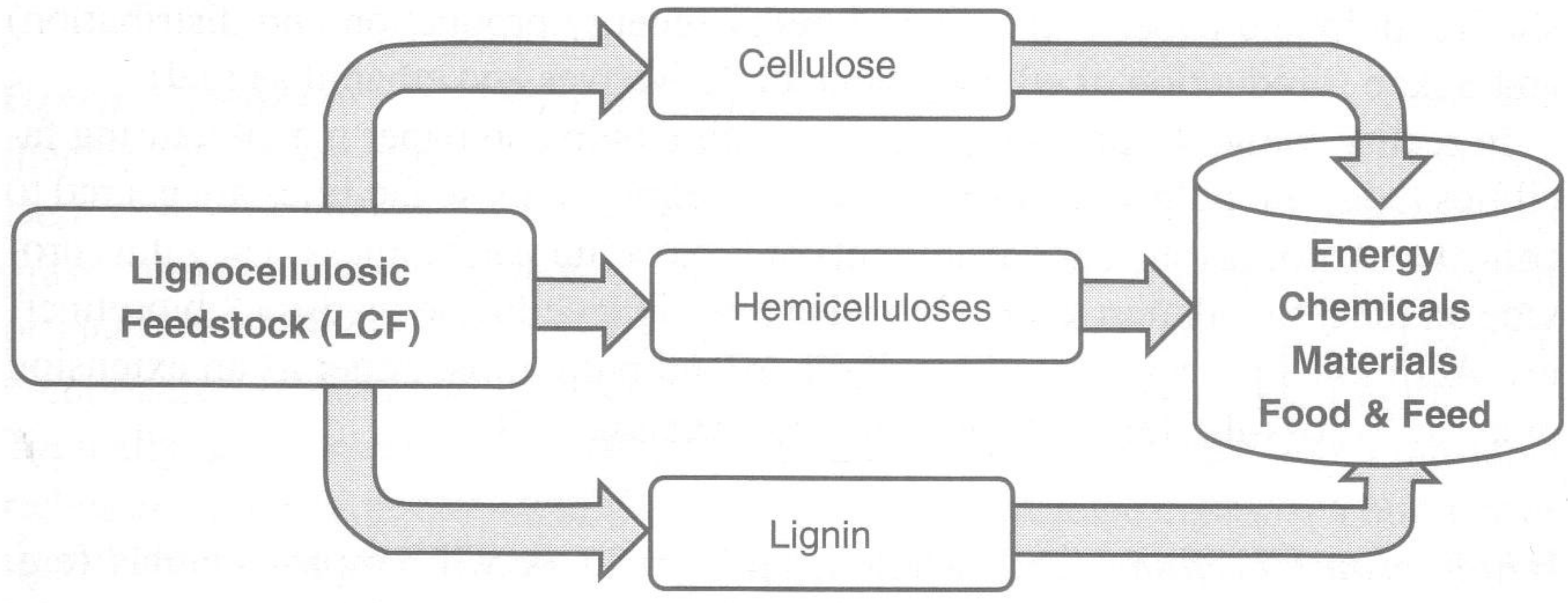
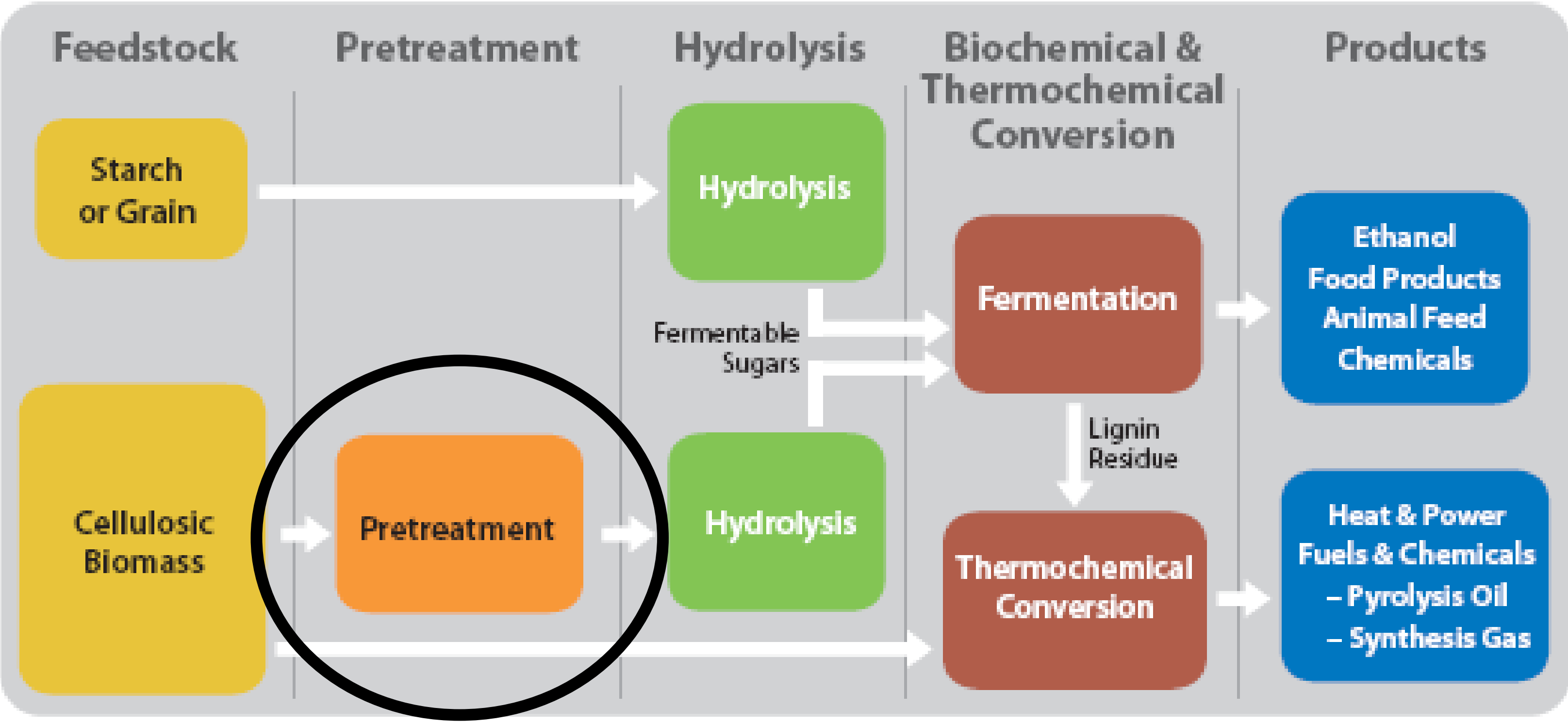


Figure 1.5 Simplified schematic diagram of a lignocellulosic feedstock biorefinery

Reference: J.F. Clarke and F.E.I. Deswarte in: Chemicals from Biomass, Wiley (2008)

Starch or grain biorefineries versus lignocellulose biorefinery – a pretreatment of the substrate is needed



Schematic of a hypothetical integrated ethanol biorefinery.

Reference: Top Value Added Chemicals from Biomass, NREL (2007)

The lignocellulose feedstock biorefinery – The necessity of pretreatment of lignocellulose

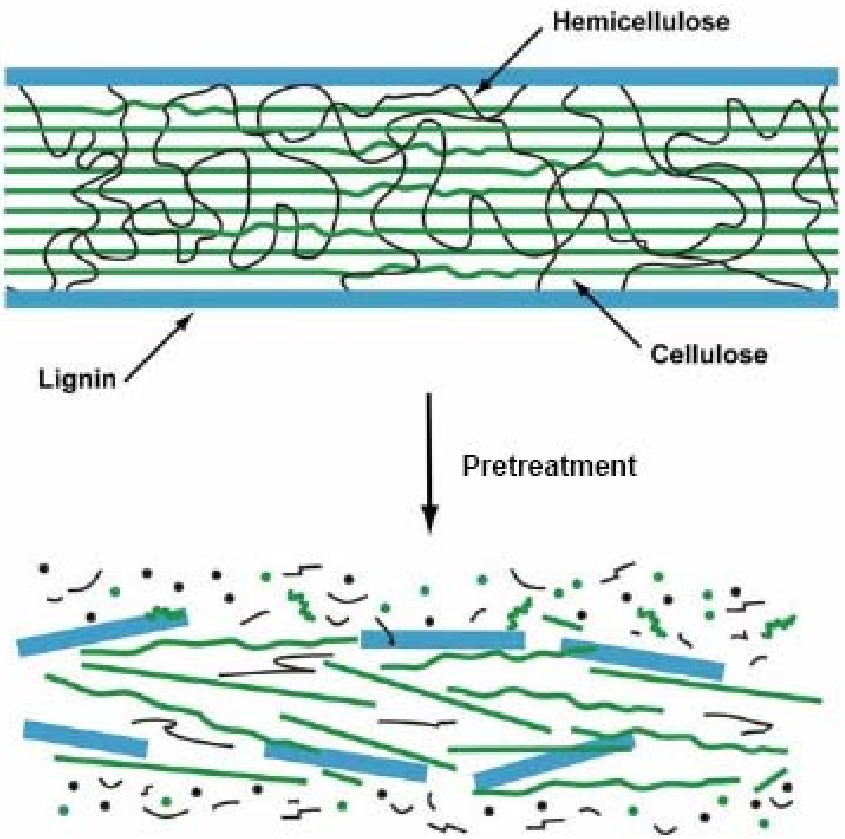


Figure 2.7 Effects of pretreatment on lignocellulose (after Mosier, 2005)

Reference: D. Ernst, A. Neumann and C. Syldatk (in preparation)

Physical and chemical pretreatment of lignocellulose

Table 2.10 Effect of physical and chemical pretreatments on the structure of Lignocellulose (after Hendriks, 2008)

+ positive *- negative* *n.k. = not known*	Increase in the available surface area	Cellulose decrystallization	Hemicellulose solubilization	Lignin solubilization	Formation of furfural and HMF	Change in the lignin structure
Mechanical	+	+				
Steam Explosion	+		+	-	+	+
LHW (batch)	+	n.k.	+	-	-	-
Dilute Acid	+		+	-	+	+
Alkali	+		-	+/-	-	+
Oxidative	+	n.k.		+/-	-	+
AFEX	+	+	-	+	-	+
CO_2 Explosion	+		+			

Reference: D. Ernst, A. Neumann and C. Syldatk (in preparation)

The lignocellulose feedstock biorefinery –
Effect of mechanical pretreatment of straw by grinding

Figure 5. Reduction in straw bulk density through grinding (production trial).

Reference and Pictures: F.E.I. Deswarte et al, Bioprod., Biofuels, Bioref. (2007)

Enzymes necessary for hemicellulose degradation

Table 3.12 Typical Hemicellulases (after Shallom, 2003; Saha, 2003)

Hydrolase		
Endo-β-1,4-xylanase	3.2.1.8	Hydrolyzes β-1,4-bonds in xylose polymers to shorter xylo-oligomers
β-Xylosidase	3.2.1.37	Splits xylo-oligomers to simple xylose
β-Mannanase	3.2.1.78	Hydrolyzes mannose polymers to manno-oligomers
β-Mannosidase	3.2.1.25	Split manno-oligomers to simple mannose
α-L-Arabinofuranosidase	3.2.1.55	Hydrolyzes arabinofuranosyl containing hemicelluloses
α-D-Glucuronidase	3.2.1.139	Splits α-1,2-glycosidic bonds in 4-O-methyl-glucuron acid
Esterases		
Feruloylesterase (FAE)	3.1.1.73	Splits ester bonds between arabinose and ferulic acid; not from *Trichoderma reesei*, only from *Aspergillus niger* and works together with xylanases and pectinases (Buranov, 2008)

Reference: D. Ernst, A. Neumann and C. Syldatk (in preparation)

Commercially available „enzyme cocktails“ for lignocellulose degradation

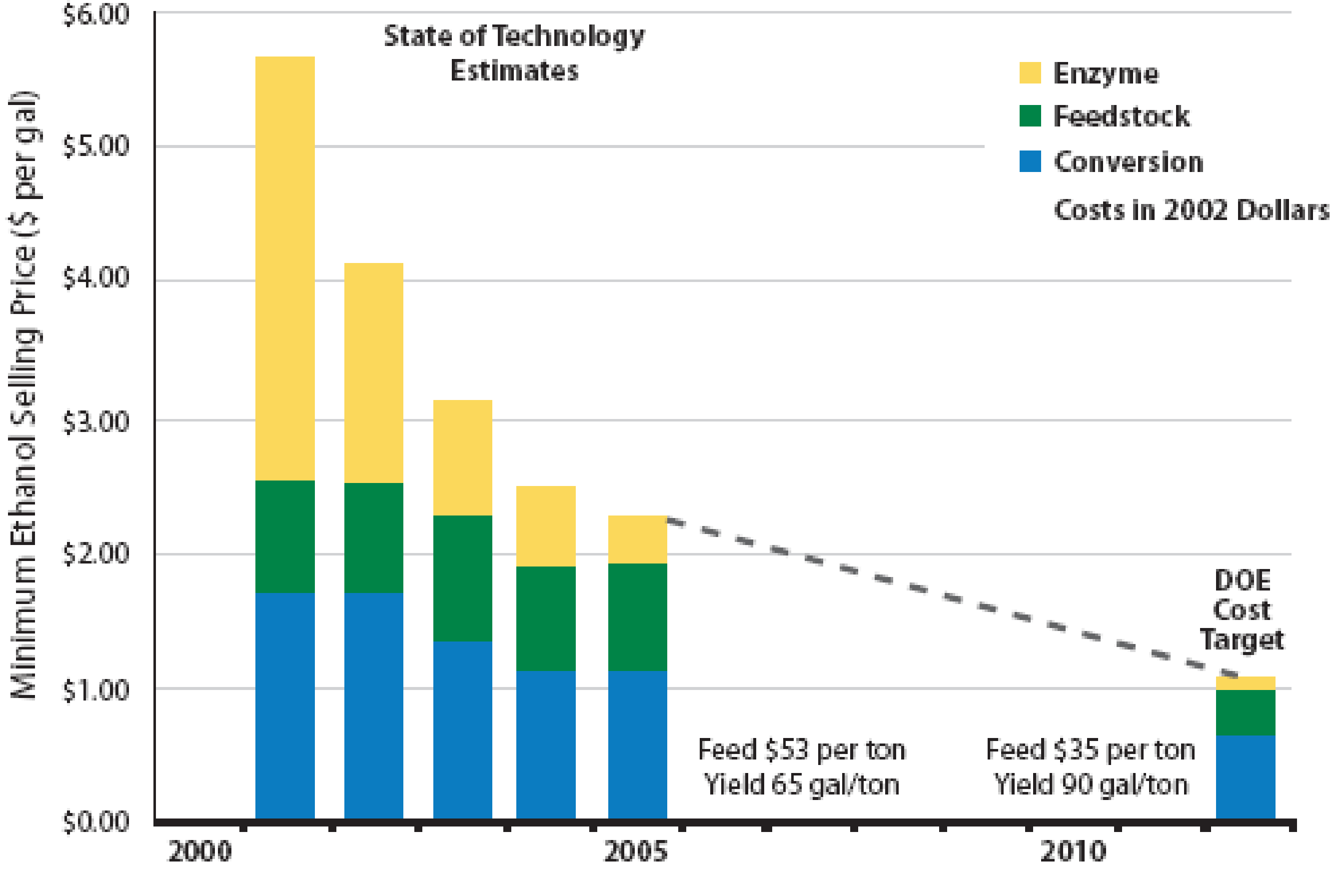
3.3.2 Producers of Cellulolytic Enzymes

Table 3.13 Overview of commercially available lignocellulose degradation enzymes (after Berlin, 2007)

Trade name	Producer	Enzyme	Strain
Celluclast 1,5L	Novozymes	Cellulase mixture	*T.reesei*
Novozym 188	Novozymes	β-glucosidase	*Asp.niger*
Multifect Xylanase	Genencor Int.	Xylanase	*T.reesei* recombinant
Multifect Pectinase	Genencor Int.	Pectinase complex	*Asp.niger*
Accellerase	Genencor Int.	Cellulase mixture	unknown

Reference: D. Ernst, A. Neumann and C. Syldatk (in preparation)

The lignocellulose biorefinery – Enzyme costs are still the limiting economic factor



Reference: Top Value Added Chemicals from Biomass, NREL (2007)

Availabilty of biomass –
Estimated EU biomass potential in millions of tons

Year	2010	2020	2030
Organic Waste	100	100	102
Energy Crops	43 - 46	76 - 94	102 - 142
Forest Products	43	39 - 45	39 - 72
Total	186	215 - 239	243 – 316

Demand of Oil in the EU (2000): 1.660

Reference: J.F. Clarke and F.E.I. Deswarte in: Chemicals from Biomass, Wiley (2008)

The lignocellulose feedstock biorefinery - R&D-potential

Table 4.2 Limitations and R&D Potential in producing bioethanol from lignocellulose

	Limitation	Research and Development Potential
Substrate	i) Substrate costs ii) Substrate availability from cellulose, hemicellulose and lignin iii) Substrate composition	- Improvement of the pretreatments - Changes in the plant material (reduced lignin content or separation from the lignin) - Formation of inhibitors avoiding/improved detoxification processes
Enzymatic Hydrolysis	i) Enzyme costs ii) Enzyme inactivation through binding on lignin.	- Addition of auxiliary agents, e.g., surfactants, proteins, solvents to avoid lignin adsorption. - Improved enzyme properties via protein engineering and screening methods - Cheaper enzyme production through improved process controls (avoiding adsorption on lignin)
Ethanol Formation	i) Use of C5 sugars ii) Product inhibition	- Use of alternative strains. Metabolic engineering, or use of mixed cultures - Altered process controls (e.g, vacuum fermentation, In-situ Product removal (ISPR), integrated processes such as SSF, SSCF, CBP)

Reference: D. Ernst, A. Neumann and C. Syldatk (in preparation)

Conclusions – Chemicals from waste biomass and by-products

- Only the development of integrated processes (chemistry, biology, agriculture, forest and process engineering) will enable an economic production of chemicals from biomass in future.
- The aim should be to develop new integrated processes at the same time meeting the demands of food, animal feed and energy production.
- The use of cellulose, hemicellulose and lignin as well as of plant and microalgae „green juices“ and „press cakes“ is promising but still demanding strong R&D input.

What are we doing at the moment?

- **ERA-SME-project „Bi-Cycle“**: An integrated microalgae & yeast approach for production of single cell oils, biofuels and fine chemicals.

  Companies are still welcome to join our project!
- **BMBF-project „BioSysPro“** (Network of 3 FhG institutes and 3 universities): New routes to chemical synthons
- **FNR-project „Microbial biosurfactants from renewable resources“** together with industrial and academic partners

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- Thank you for your attention!

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- For further reading: