

Lignocellulosic Biomass and Residues as Potential Substrates for the Industrial Biotechnology

J. Venus



5th Latin American Congress on Biorefineries From laboratory to industrial practice January 7-9, 2019 - Concepción, Chile



History

- 1927 Experimental farm of the Agricultural University Berlin
- 1933 Independent research center on agricultural mechanization
- 1952 Central institute of agricultural engineering of East Germany
- 1992 Reestablished after the reunification of Germany

Today:

Leibniz Institute for Agricultural Engineering and Bioeconomy

- member of the Leibniz Association





Our mission:

Our research is aimed at sustainable intensification. We analyze, model and evaluate bio-economic production systems. We develop and integrate new technologies and management strategies for a knowledge-based, site-specific production of biomass, and its use for food, as biobased materials and fuels - from basic research to application.







Biotechnological

Biomass conversion into high-value chemical products and fuels



Biorefineries in theory would use multiple forms of biomass to produce a flexible mix of products, including fuels, power, heat, chemicals and materials. In a biorefinery, biomass would be converted into high-value chemical products and fuels (both gas and liquid). Byproducts and residues, as well as some portion of the fuels produced, would be used to fuel on-site power generation or cogeneration facilities producing heat and power.



(lignocellulosic) biorefineries in the scientific community#

Biorefineries are classified based on, technological (implementation) status, type of raw materials used or main type of conversion processes applied. A search of the literature revealed a variety of terms describing **Biorefineries**



<u>08.01.19</u>: 2.414 records Title=(bioref*) OR Title=(bio-ref*); 645 AND (lignocell*)

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17	2:2009:01]

27 November 2017

Map of 224 European biorefineries published by BIC and nova-Institute

Biorefineries are the heart of the bioeconomy. Here, different types of biomass are fully utilised and transformed into a large variety of chemicals and materials.

The map distinguishes between "Sugar-/starch based biorefineries", producing bioethanol and other chemicals (63), "Oil-/fat-based biorefineries biodiesel" (64) and "Oil-/fat-based biorefineries oleochemistry" (54), "Wood-based biorefineries" (25) excluding those that produce pulp for paper only, "Lignocellulose other than wood" (5) and finally "Biowaste-based biorefineries" (13).

The prevalence of biorefineries differs considerably between countries. The type of biorefinery is clearly dependent on the locally available biomass. Wood-based biorefineries can be found mainly in Northern Europe and "Sugar-/starch based biorefineries" mainly in France, Belgium, Germany and Hungary, where we see high yields in sugar and starch.

Biorefineries in Europe 2017



The map can be downloaded for free at <u>www.bio-based.eu/graphics</u> or <u>www.biconsortium.eu</u>





10.01.2019

Building blocks that could be produced via fermentation

REVIEW ARTICLE

Cite this: Chem. Soc. Rev., 2014

43 2587

Valorization of industrial waste and by-product streams *via* fermentation for the production of chemicals and biopolymers

Apostolis A. Koutinas,†^a Anestis Vlysidis,†^a Daniel Pleissner,^b Nikolaos Kopsahelis,^a Isabel Lopez Garcia,^c Ioannis K. Kookos,^d Seraphim Papanikolaou,^a Tsz Him Kwan and Carol Sze Ki Lin^{*b}

Numbers next to biochemicals designate the total annual production in thousands of t





Berlin, 5 December 2018 – The results of the European Bioplastics' annual market data update, presented today at the 13thEuropean Bioplastics Conference in Berlin:

The global bioplastics production capacity is set to increase from around 2.1 Mio t in 2018 to 2.6 Mio t in 2023. Innovative biopolymers such as PLA and PHAs are driving this growth.

Biorefinery-concept for (1st, 2nd, 3rd...?) biomass feedstocks



Pleissner, D.; Qi, Q.; Gao, C.; Perez Rivero, C.; Webb, C.; Lin, C.S.K.; Venus, J.: Valorization of organic residues for the production of added value chemicals: A contribution to the bio-based economy. Biochemical Engineering Journal 116 (2016) 3-16

(Different) Composition & Behaviour of (lignocellulosic) Biomass

The contents of cellulose, hemicellulose, and lignin in various types of lignocellulosic biomass (% dry weight).^a

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%
Algae (green)	20-40	20-50	NA ^b
Aspen hardwood	51	29	16
Birch Hardwood	40	39	21
Chemical pulps	60-80	20-30	2-10
Coastal Bermuda grass	25	35,7	6,4
Corn cobs	45	35	15
Cornstalks	39-47	26-31	3-5
Cotton seed hairs	80-95	5-20	0
Cotton, flax, etc.	80-95	5-20	NA ^b
Grasses	25-40	25-50	10-30
Hardwood	45±2	30 ± 5	20 ± 4
Hardwood barks	22-40	20-38	30-55
Hardwood stems	40-55	24-40	18-25
Leaves	15-20	80-85	0
Newspaper	40-55	25-40	18-30
Nut shells	25-30	25-30	30-40
Paper	85-99	0	0-15
Pine softwood	44	26	29
Primary wastewater solids	8-15	NAÞ	24-29
Softwood	42 ± 2	27 ± 2	28 ± 3
Softwood barks	18-38	15-33	30-60
Softwood stems	45-50	25-35	25-35
Solid cattle manure	1.6-4.7	1,4-3,3	2,7-5,7
Sorted refuse	60	20	20
Spruce softwood	43	26	29
Swine waste	6,0	28	NA ^b
Switch grass	45	31,4	12,0
Waste papers from chemical pulps	60-70	10-20	5-10
Wheat straw	37-41	27-32	13-15
Willow Hardwood	37	23	21

M.A. Abdel-Rahman et al. Journal of Biotechnology 156 (2011) 286– 301

10.01.2019

Feedstocks Carbohydrate composition (% dry wt) References Cellulose Hemicellulose Lignin Barley hull 34 19 36 [12]Barley straw 36 - 4324 - 3363-98 [13, 14]Bamboo 49 - 5018 - 2023 [15,16] Banana waste 13 15 14 [17] Corn cob 32.3-45.6 39.8 67 - 139[18,19] Corn stover 35.1-39.5 20.7 - 24.611.0 - 19.1[20] Cotton 85-95 5 - 150 [21] Cotton stalk 31 11 30 [22] 15.6-19.1 Coffee pulp 33.7-36.9 442 - 475[23] 35 - 4820 - 2215 - 21[24]Douglas fir Eucalyptus 45 - 5111 - 1829 [16, 25]Hardwood stems 40-55 24 - 4018 - 2526,27 Rice straw 292 - 34.723 - 25.917 - 1928,29 11.96 - 29.3Rice husk 28.7 - 35.615A - 20[30,31] Wheat straw 35 - 3922 - 3012 - 1629,32] Wheat bran 105 - 14.835.5-392 83-125 [33] 25 - 4025 - 5010 - 30Grasses [34,35] Newspaper 40-55 24 - 3918 - 30[26]25 - 4528 - 3215 - 25[16,36] Sugar cane bag as se 32 Sugarcane tops 35 14 [37] Pine 42 - 4913 - 2523 - 29[25] Poplar wood 45 - 5125 - 2810 - 21[38] Olive tree biomass 25.2 15.8 19.1 [39] 45 - 5321 - 26lute fibres 18 - 21[40] 35 - 4025 - 3015 - 20[26] Switchgrass Grasses 25 - 4025 - 5010 - 30[26,27] Winter rye 29 - 3022 - 2616.1 [41] Oilseed rape 27.3 20.5 14.2 [41]Softwood stem 45 - 5024 - 4018 - 2526.27 Oat straw 31 - 3520 - 2610 - 15[14]25 - 30Nut shells 22 - 2830 - 40[42]Sorghum straw 32 - 3524 - 2715 - 21[43,44] Tamarind kernel 10 - 1555 - 65[45] powder Water hyacinth 182 - 22.148.7-50.1 35 - 54[46,47]

Composition of representative lignocellulosic feedstocks,

V. Menon, M. Rao Progress in Energy and Combustion Science 38 (2012) 522-550

Percent dry weight composition of lignocellulosic feedstocks

Feedstock	Glucan (cellulose)	Xylan (hemicellulose)	Lignin
Corn stover ^a	37.5	22.4	17.6
Corn fiber ^{b,c}	14.28	16.8	8.4
Pine wood ^d	46.4	8.8	29.4
Popular ^d	49.9	17.4	18.1
Wheat straw ^d	38.2	21.2	23.4
Switch grass ^d	31.0	20.4	17.6
Office paper ^d	68.6	12.4	11.3

N. M

N. Mosier et al. / Bioresource Technology 96 (2005) 673-686

Beyond Petrochemicals: The Renewable Chemicals Industry**

P. N. R. Vennestrøm, C. M. Osmundsen, C. H. Christensen, and Esben Taarning*

Chemical	Market type	Market size (Mty ^{–1}) ^[a]	Major player(s)	Feedstock
acetic acid	existing	9.0	-	ethanol
acrylic acid	existing	4.2	Arkema, Cargill/Novozymes	glycerol or glucose
C₄ diacids	emerging	(0.1-0.5)	BASF/Purac/CSM, Myriant	glucose
epichlorohydrin	existing	1.0	Solvay, DOW	glycerol
ethanol	exisiting	60	Cosan, Abengoa Bioenergy, ADM	glucose
ethylene	existing	110	Braskern, DOW/Crystalsev, Borea- lis	ethanol
ethylene glycol	existing	20	India Glycols, Dacheng Industrial	glucose or xylitol
glycerol	existing	1.5	ADM, P&G, Cargill	vegetable oil
5-hydroxymethylfurfu- ral	emerging	-	-	glucose/ fructose
3-hydroxypropionic acid	emerging	(≥0.5)	Novozymes/Cargill	glucose
isoprene	existing /	0.1 (0.1-0.5)	Danisco/Goodvear	glucose
	emerging	(,		0
lactic acid	existing/	0.3 (0.3-0.5)	Cargill, Purac/Arkema, ADM, Ga-	glucose
	emerging		lactic	
levulinic acid	emerging	(≥0.5)	Segetis, Maine Bioproducts, Le Calorie	glucose
oleochemicals	existing	10-15	Emery, Croda, BASF, Vantage	vegetable
	•		Oleochemicals	oil/fat
1,3-propanediol	emerging	(0.1-0.5)	Dupont/Tate & Lyle	glucose
propylene	existing	80	Braskern/Novozymes	glucose
propylene glycol	existing/	1.4 (≥2.0)	ADM, Cargill/Ashland, Senergy,	glycerol or
	emerging	·- ·	Dacheng Industrial	sorbitol
polyhydroxyalkanoate	emerging	(0.1-0.5)	Metabolix/ADM	glucose

[a] Market size of an existing market is given as its current size including production from fossil

resources; for emerging markets the expected market size is reported in parenthesis.



<u>Table 1:</u> Overview of chemicals that are currently produced, or could be produced, from biomass together with their respective market type, size of the market, and potential biomass feedstock. Major players involved are also given.



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The processes for producing lactic acid from biomass/residues include the following 4 main steps:

(1) Pretreatment - breaking down the structure of the feedstock matrix

- (2) Enzymatic hydrolysis depolymerizing biopolymers like starch, cellulose etc. to fermentative sugars, such as glucose (C6) and xylose (C5), by means of hydrolytic enzymes
- (3) Fermentation metabolizing the sugars to lactic acid, generally by LAB
- (4) Separation and purification of lactic acid purification of lactic acid to meet the standards of commercial applications



Pilot plant facility for lactic acid fermentation at Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB Potsdam)





Chemicals from Biomass: A Market Assessment of Bioproducts with Near-Term Potential

Mary J. Biddy, Christopher Scarlata, and Christopher Kinchin - National Renewable Energy Laboratory

Data Gaps

Scale-up of lactic acid production would require clean, cheap sugars from lignocellulosic biomass to compete with commodity sugar and starch substrates. There is a lack of data about lactic acid production and purification from biomass hydrolysates, including issues of C5 sugar utilization, although it appears work has started to address some of these issues.

This report is available at no cost from the National Renewable Energy Laboratory (NREL) at www.nrel.gov/publications Technical Report NREL/TP-5100-65509 - March 2016/Contract No. DE-AC36-08G028308



Example coffee residues: residues from the coffee production



L(+)-lactic acid

Poly(lactic acid)

Pleissner, D.; Neu, A.-K.; Mehlmann, K.; Schneider, R.; Puerta-Quintero, G.I.; Venus, J.: Fermentative lactic acid production from coffee pulp hydrolysate using *Bacillus coagulans* at laboratory and pilot scales. Bioresource Technology 218 (2016) 167–173

Example agro-residues: Sugarcane bagasse



Figura 1 – Fotos de bagaço da cana de açúcar: (a) sem tratamento térmico; (b) 180°C; (c) 200°C e (d) 220°C por 5, 10 e 15 minutos (da esq. para dir.).



Figura 4 – Produção de ácido lático e consumo de açúcares presentes no meio MRS modificado contendo hidrolisado de bagaço (glicose 33 g l^{-1} , xilose 19 g l^{-1} , arabinose 0,4 g 1^{-1} , extrato de levedura 15 g 1^{-1} , K₂HPO₄ 2 $g l^{-1}$, MgSO₄ 0,1 $g l^{-1}$ e MnSO₄ 0,04 $g l^{-1}$).

2011

Hidrólise Térmica de Bagaço da Cana-de-açúcar para Produção Homofermentativa de L-Ácido Lático

XVIII Simpósio Nacional de Bioprocessos Caxias do Sul/RS - 24 a 27 de julho de 2011



10.01.2019

Giselle de Arruda Rodrigues¹, Joachim Venus² e Telma Teixeira Franco¹

Example wheat straw: Sugar uptake & product formation





cereal residues such as straw



- Fermentation ended after 50-60 hours with a yield of nearly 100% and 64 g/L (top left)
- (Total) Sugars (firstly Glucose followed by Arabinose/Xylose with residues of Disaccharides) have been used completely in the same time (bottom left)
- (Max) Lactate productivity (>5 g·L⁻¹·h⁻¹) is much higher than comparable published results [Li/Cui: Microbial Lactic Acid Production from Renewable Resources, pp. 211-228. In O.V. Singh and S.P. Harvey (Eds.), Sustainable Biotechnology - Sources of Renewable Energy. Springer, 2010]



WO 2013164423 A1; WO 2013164425 A1

Pleissner, D.; Venus, J.: Agricultural residues as feedstocks for lactic acid fermentation. - ACS Books "Green Technologies for the Environment" (2014) Chapter 13, pp 247–263

BranLact





Bioeconomy International

Thermophilic lactic acid production utilizing defatted rice bran in continuous cultivation

Funded by PtJ/BMBF (2016 - 2019)

Partner: Beijing University of Chemical Technology



Flow diagram of the followed process



Alexandri, M.; Neu, A.; Schneider, R.; López-Gómez, J.P.; Venus, J.: Evaluation of various B. coagulans isolates for the production of high purity L-lactic acid using defatted rice bran hydrolysates. International Journal of Food Science & Technology, In Press, <u>https://doi.org/10.1111/ijfs.14086</u>

Biosurfactant production by Yeasts using sugarcane bagasse for solid state fermentation





Green Chemistr



UNIVERSITY OF SÃO PAULO; DEPARTMENT OF BIOTECHNOLOGY SCHOOL OF ENGINEERING OF LORENA

Brumano, L.P.; Antunes, F.A.F.; Galeno Souto S.; dos Santos, J.C.; Venus, J.; Schneider, R.; da Silva, S.S.: Biosurfactant production by Aureobasidium pullulans in stirred tank bioreactor: new approach to understand the influence of important variables in the process. Bioresource Technology (2017) 243: 264–272

Metabolic Engineering 47 (2018) 279-293



Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/meteng



UNIVERSITÄT DES SAARLANDES



From lignin to nylon: Cascaded chemical and biochemical conversion using metabolically engineered *Pseudomonas putida*



Michael Kohlstedt^a, Sören Starck^a, Nadja Barton^a, Jessica Stolzenberger^a, Mirjam Selzer^a, Kerstin Mehlmann^c, Roland Schneider^c, Daniel Pleissner^{c,d}, Jan Rinkel^b, Jeroen S. Dickschat^b, Joachim Venus^c, Jozef B.J.H. van Duuren^a, Christoph Wittmann^{a,*}



Demonstration of the value chain from lignin to nylon.

The cascaded process comprised hydrothermal depolymerization of lignin to a mixture of aromatics, containing mainly catechol, phenol and small amounts of cresols; biochemical conversion of the aromatics to cis, cis-muconic acid by the advanced producer Pseudomonas putida KT2440 MA-9; purification of cis, cis-muconic acid; hydrogenation to adipic acid; and final polymerization to nylon 6,6.



CHEMICAL BUILDING BLOCKS FROM VERSATILE MSW BIOREFINERY



PERCAL will exploit Municipal Solid Waste (MSW) as feedstock to develop intermediate chemical products at high yield and low impurity level with huge industrial interest. These will be complementary to the bioethanol, to achieve a cascade valorisation of the MSW components, i.e.:

- Lactic acid (LA) to produce: 1) Eco-friendly ethyl lactate solvents by reactive distillation from lactic acid & bio-ethanol to be used in cleaning products and inks and 2) hot-melt adhesives for cardboard and other non-food applications in combination with maleic anhydride by reactive extrusion.
- Succinic acid (SA) as an intermediate building blocks to production of polyols for the polyurethane industry.
- **Biosurfactants by chemical and/or microbiological** modification of protein and lipid fraction from remaining fraction of MSW fermentation.



Continuous mode fermentation with cell retention by hollow fibre membranes



Pleissner, D.; Qi, Q.; Gao, C.; Perez Rivero, C.; Webb, C.; Lin, C.S.K.; Venus, J.: Valorization of organic residues for the production of added value chemicals: A contribution to the bio-based economy. Biochemical Engineering Journal 116 (2016) 3-16



Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Review

A review on the current developments in continuous lactic acid fermentations and case studies utilising inexpensive raw materials José Pablo López-Gómez, Maria Alexandri, Roland Schneider, Joachim Venus*

In Press, Corrected Proof, https://doi.org/10.1016/j.procbio.2018.12.012

Table 1

Comparison of results for productivity (P), yield (Y) and lactic acid concentration, in batch and continuous mode, from other investigations.

Substrate	Strain		Batch			Continuous		
		P (g1 ⁻¹ h ⁻¹)	Y (gg ⁻¹)	[LA] (g1 ⁻¹)	P (gi ⁻¹ h ⁻¹)	Y (gg ⁻¹)	[LA] (g1 ⁻¹)	
Xylose	E. mundtii	2.08	0.90	44.10	3.14	0.86	21.70	[14]
					6.15	1.01	41.00°	
Cassava starch	L plantarum	0.80	N/S	N/S	3.79	1.08	18.96	[48]
					8.00	1.21	20.00 ^a	
MRS medium	L. delbrueckii	0.52	1.01	86.40	18.00	1.03	20.70	[13]
Defatted rice bran hydrolysate	L. rhamnosus	5.20	0.95	84.00	6.20	0.98	86.00	[25]
Raw sugar (from sugarcane)	S. Laevolacticus	0.25	0.89	55.70	11.20	0.97	67.30	[29]
N/S: Not specified. * Continuous mode with cell r	ecycling.				S			

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Table 4

Compositional analysis of the substrates utilized in the case studies.

Substrate	Ghicose (g1 ⁻¹)	Disaccharide (gl ⁻¹)	Fructose/ Galactose (g4 ⁻¹)	Lactic acid (g1 ⁻¹)	Dry matter (%)	$\stackrel{N_{k \mid \! \! p \mid l}}{(mg l^{-1})}$	PO4 ³⁻ (mg1 ⁻¹)	SO4 ²⁻ (mg·l ⁻¹)	NH4 ⁺ (mg1 ⁻¹)	
Tapioca hydrolysate ^a Acid whey ^c	139.0 n.d.	3.5 ^b 258 ^d	n.d. n.d.	n.d. n.d.	13.7 26.0	113.0 2288.0	6.0 683.0	36.1 2687.0	0.0 159.0	
Molasses	56.6	505.0°	17.0 ^r	29.7	84.8	-	23.1	7177.0	150.0	
Rapeseed meal	5.4	1.7*	4.7*	n.d	4.7	-	291.0	562.1	159.6	
hvdrolysate ^a										



Fig. 1. Schematic representation of hollow fibers membrane-integrated bioreactor system for cell-recycle continuous production of lactic acid.





Alexandri, M.; Schneider, R.; Venus, J.: Membrane Technologies for Lactic Acid Separation from Fermentation Broths Derived from Renewable Resources. Membranes 2018, 8(4), 94, <u>https://doi.org/10.3390/membranes8040094</u>

Effect of several down-streaming steps on the purity of lactic acid



fermentation broth. J. Chem. Technol. Biotechnol. (2017) 92: 504-511

Scale-up of bioprocesses





The Danish Council for Strategic Research



DANISH PRESIDENCY OF THE COUNCIL OF THE EUROPEAN UNION 2013

The Copenhagen Declaration for a Bioeconomy in Action

9 The conference also underlined the need for new pilot and demonstration plants and scaling up facilities, in particularly biorefineries. It was stressed, that the development of these facilities requires smart integration of various funding sources, including the Common Agricultural Policy, the Common Fisheries Policy, the Cohesion Policy, private investments.

Universities, Research **Institutes, SMEs** Applied & basic research artechnik Bornim

Valley

<u>o</u>f

the Renewable Energy Policy, Horizon 2020, and

death 23. August Einweihung Pilotanlage "Milchsäure aus Biomasse" Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. Industry Förderung Industrial application Large-scale production

Copenhagen conference "Bioeconomy in Action" on 26 March - 28 March 2012

> Carus/Carrez/Kaeb/Ravenstijn/Venus: Level Playing Field for Bio-based Chemistry and Materials. - bioplastics MAGAZINE [03/11] Vol. 6, 52-55

2006

Pilot plant facility

- pilot facility for production of lactic acid at the ATB consequently fills a gap in the various phases of bioprocess engineering
- provision of product samples is intended to open up the possibility of interesting partners in industry with specific product requirements in various applications





BIOSTAT[®] Bplus (Sartorius BBI Systems GmbH, Germany) equipped with a digital control unit DCU for the continuous fermentation with cell recycling

Pilot fermentor Type P, 450 L (Bioengineering AG)

Venus, J.; Richter, K.: Development of a Pilot Plant Facility for the Conversion of Renewables in Bio-technological Processes. Eng. Life Sci. 2007, 7, No. 4, 395-402 Pleissner, D.; Dietz, D.; van Duuren, J.B.J.H.; Wittmann, C.; Yang, X.; Lin, C.S.K.; Venus, J.: Biotechnological production of organic acids from renewable resources. Advances in Biochemical Engineer-ing/Biotechnology 166 (2019) pp. 373-410







Mulching film made of poly-lactic acid is biologically building land. © F. Kesselring, FKuR Willich

Bio-plastic and medical technology

Biotechnologically, lactobacilli are used to produce lactic acid on an industrial scale - about 500,000 tons per year in Germany. As a food additive (E 270), lactic acid increases the shelf life of baked goods, sweets and lemonades. Soaps, creams and detergents also contain the disinfecting lactic acid.

Linking several lactic acid molecules produces lactic acid chains called polylactides. The resulting materials are stable but biodegradable, making them bio-films and packaging. Medical technicians use polylactides for sutures and implants that eventually disintegrate in the body.



Informationen, Experten-Kontakte, Bildmaterial: 10.01.2019 Dr. Anja Störiko |Tel. 06192 23605 | info@mikrobe-des-jahres | www.mikrobe-des-jahres.de





Thank you for your attention!



Pilotanlage Milchsäure

DIESES PROJEKT WIRD VOM EUROPÄISCHEN FONDS FÜR REGIONALE ENTWICKLUNG KOFINANZIERT



More information: www.atb-potsdam.de

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