Agricultural University of Athens

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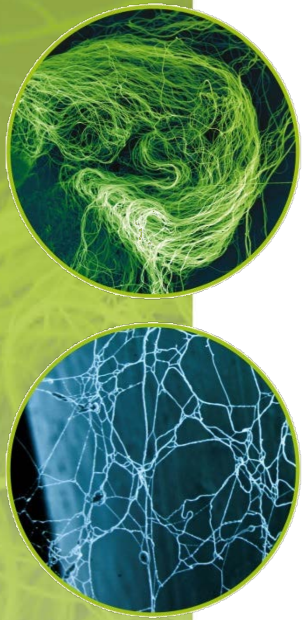

Utilisation of food wastes and biodiesel industry by-products for Bacterial Cellulose production

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Bacterial cellulose $(C_6H_{10}O_5)_n$

❖ Highly functional biopolymer

❖ Produced extracellularly by the Gram-negative bacterial strain *Acetobacter xylinum*

BC properties

High chemical purity, biocompatible

Highly porous material

High degree of polymerization (2000-20000)

High crystallinity index (60-90%)

Young´s modulus (15-35 Gpa)

Tensile strength (200-300 Mpa)

Water holding capacity (>94%)

Due to the uniform, continuous and nanoscalar network of cellulosic fibers , oriented 3-dimensionally.

Determined by the culture conditions, the microorganism and the fermentation media employed.

Metabolic pathway and biosynthesis

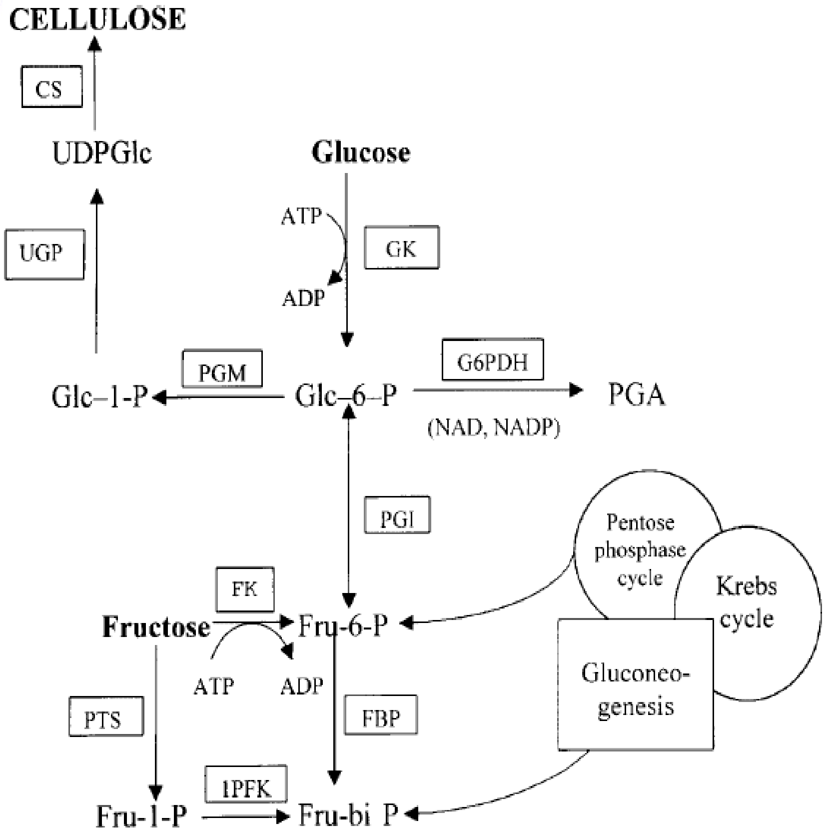


Figure 1. Metabolic pathway of *A. xylinum* for the biosynthesis of BC

Biosynthesis mechanism

(i) Formation of β-1→4 glucan chain with polymerization of glucose units

(ii) Self-assembly and crystallization of cellulose chain

A. Pentose phosphate cycle for oxidation of carbohydrates

B. TCA cycle combined with gluconeogenesis for oxidation of organic acids

Uses

Medical field

Scaffold for tissue engineering applications

Wound healing applications

Microsurgery

Food

Food packaging

Raw material, thickening and stabilizing agent, bulking agent

Commercial products

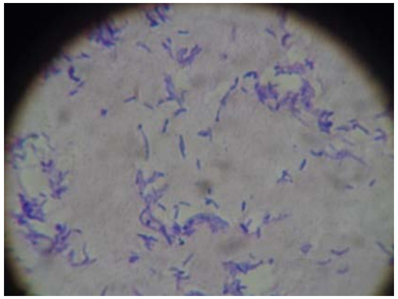
Reinforcing material in transparent/translucent nanocomposites

Cosmetic industry

Broadcasting (sound transducing membrane)

Textile industry

Culture conditions



Type: batch fermentations under microaerophilic conditions

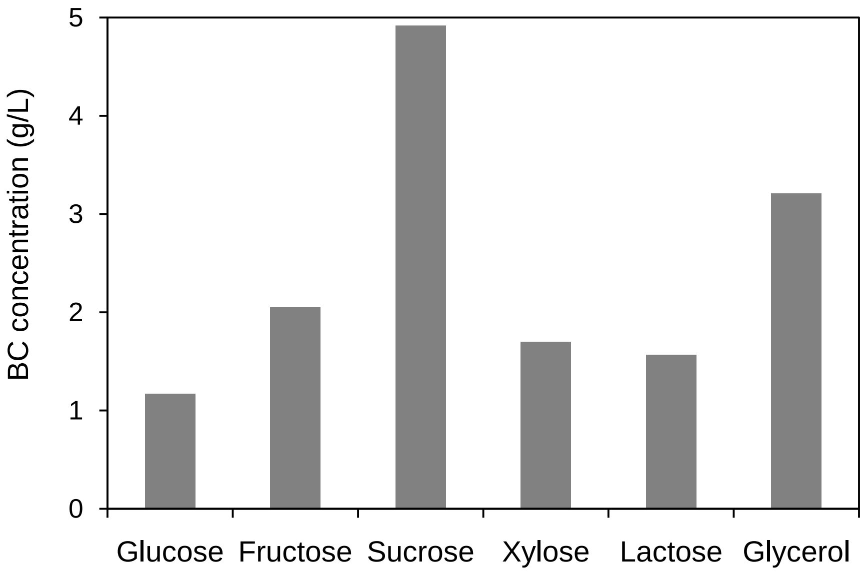
Bacterial strain: *Komagataeibacter sucrofermentans* DSM 15973

Duration: 15 days

pH = 6; T = 30 ^{o}C; $V_{working}$ = 50 mL; $V_{inoculum}$ = 10% v/v

BC ⟶ treated with 2 M NaOH to remove bacterial cells, washed repeatedly until a neutral pH is achieved and air dried at 35 °C until constant weight

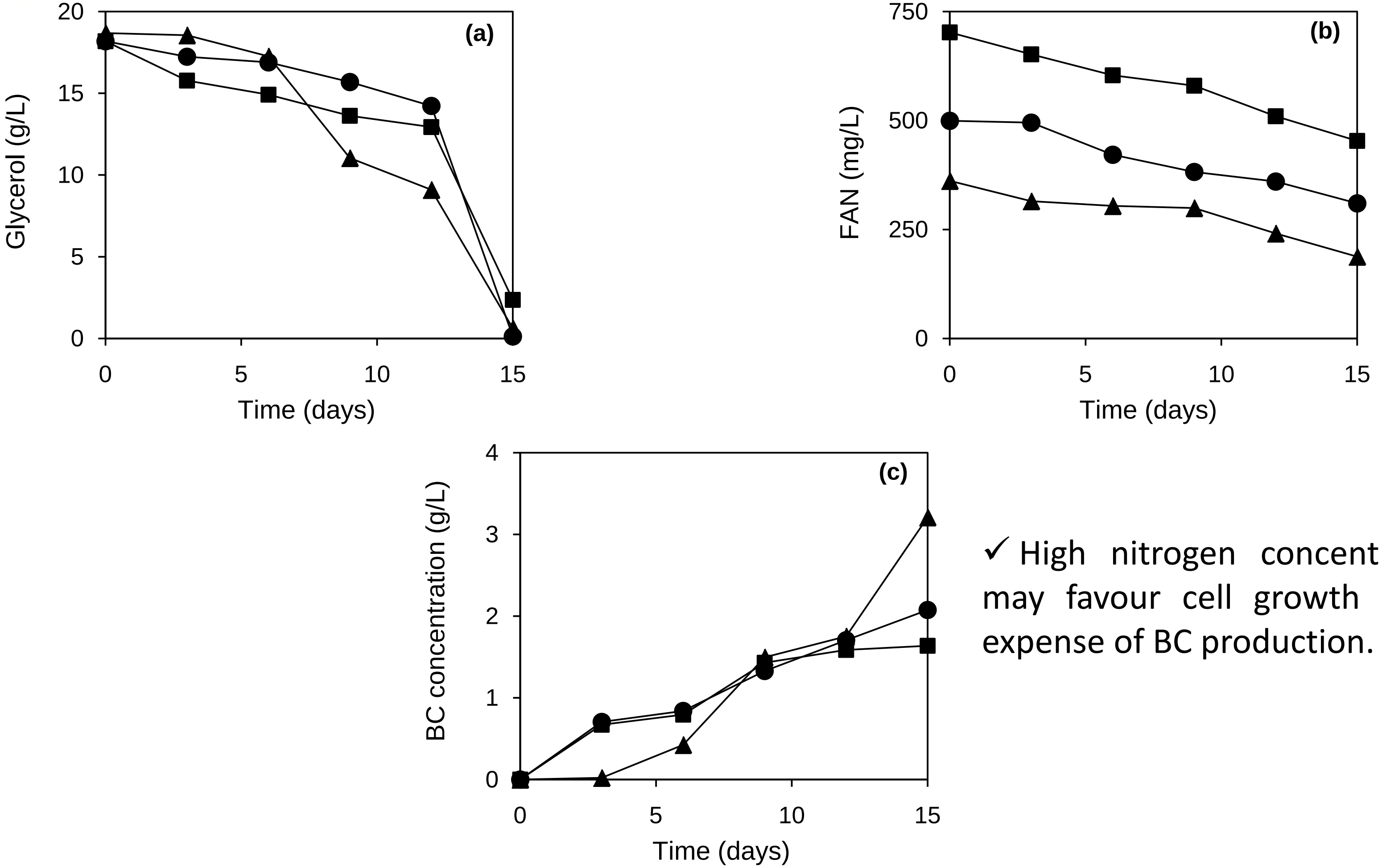
Assessment of carbon sources for BC production



BC production achieved when various commercial sugars and crude glycerol were used in shake flask fermentations.

✓ Crude glycerol and commercial sucrose led to the highest BC concentrations of 3.2 g/L and 4.9 g/L, respectively

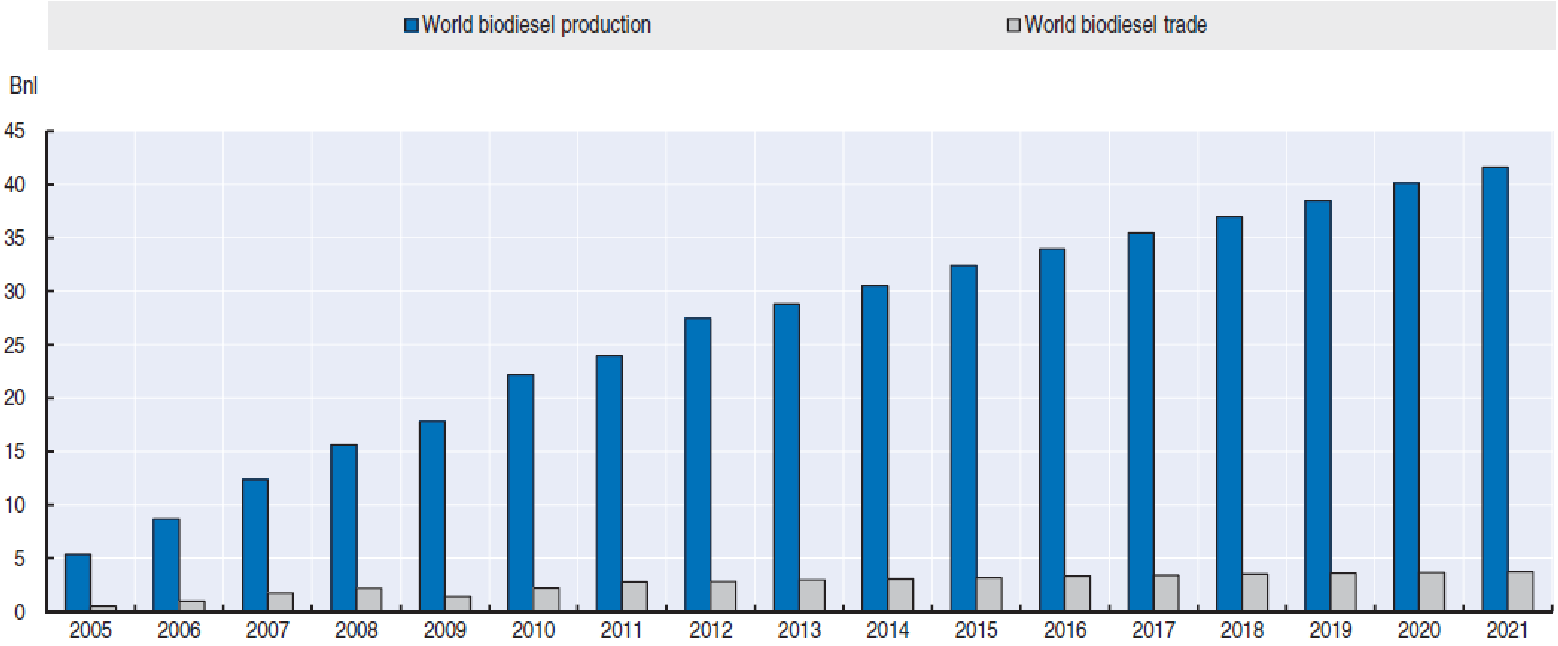
Evaluation of different free amino nitrogen (FAN) concentrations for BC production



✓ High nitrogen concentrations may favour cell growth at the expense of BC production.

Glycerol (a) and Free Amino Nitrogen (FAN) consumption (b) and BC production (c) using crude glycerol as carbon source and yeast extract with peptone as nitrogen sources at different initial FAN concentrations. The fermentation duration was 15 days. (▲), 360 mg/L FAN; (●), 500 mg/L FAN; (■), 700 mg/L FAN

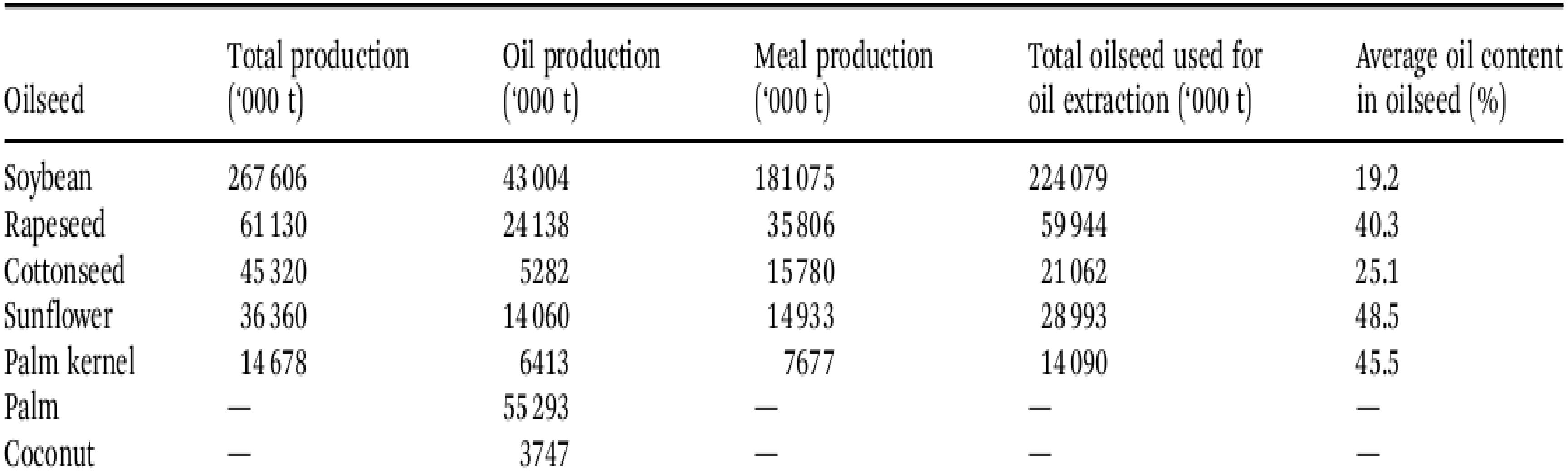
Development of the world biodiesel market



Source: OECD and FAO Secretariats.

StatLink http://dx.doi.org/10.1787/888932639400

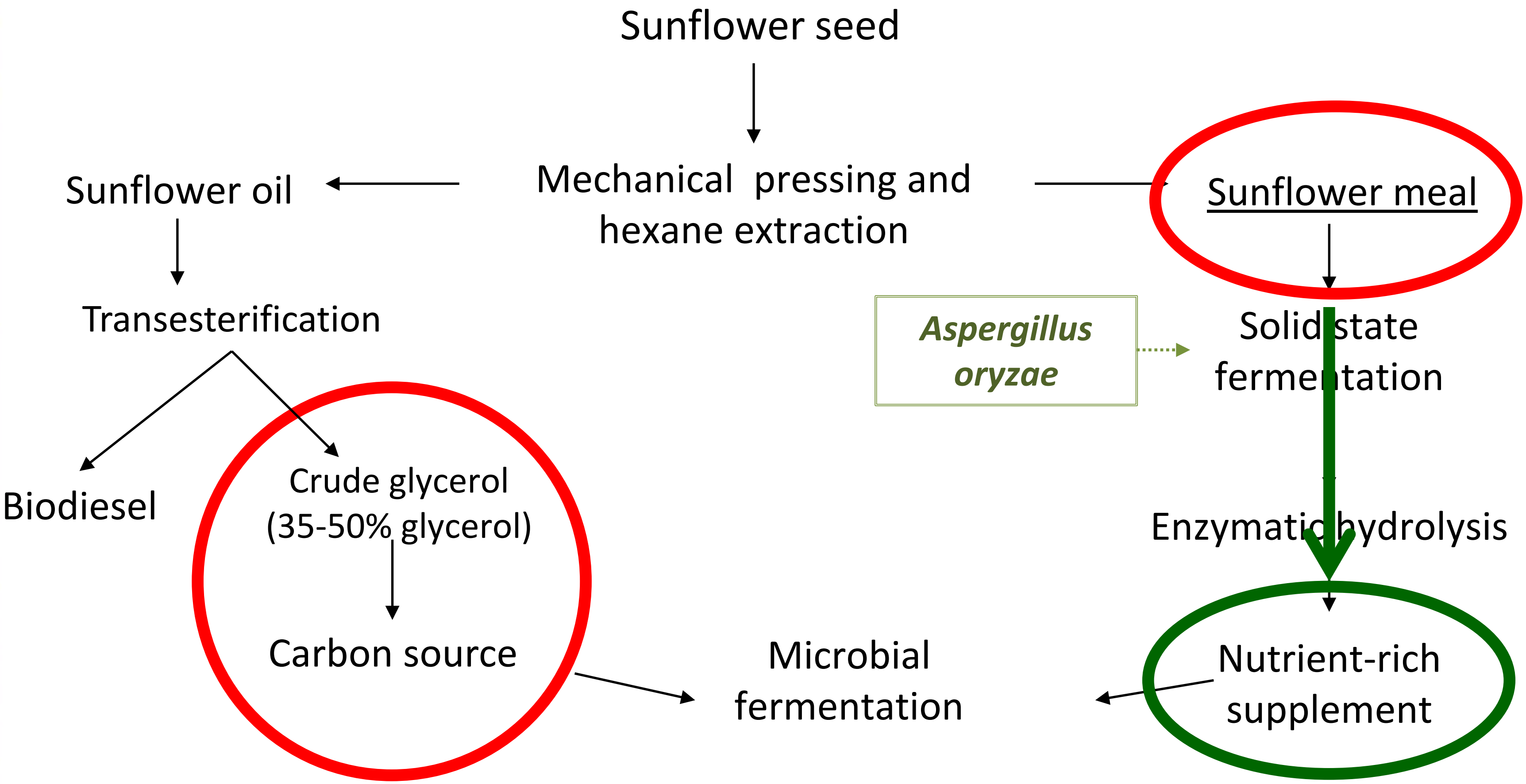
Worldwide production of major oilseeds, oil and meal in 2012/2013



Oilseed	Total production ('000 t)	Oil production ('000 t)	Meal production ('000 t)	Total oilseed used for oil extraction ('000 t)	Average oil content in oilseed (%)
Soybean	267 606	43 004	181 075	224 079	19.2
Rapeseed	61 130	24 138	35 806	59 944	40.3
Cottonseed	45 320	5282	15 780	21 062	25.1
Sunflower	36 360	14 060	14 933	28 993	48.5
Palm kernel	14 678	6413	7677	14 090	45.5
Palm	—	55 293	—	—	—
Coconut	—	3747	—	—	—

By 2021, the annual production of oilseed meals is expected to increase up to 23% corresponding to approximately 315×10^6 tonnes

Fermentation feedstock production from by-product streams of sunflower-based biodiesel production processes



Sunflower seed

Mechanical pressing and hexane extraction

Sunflower oil

Sunflower meal

Transesterification

Biodiesel

Crude glycerol (35-50% glycerol)

Carbon source

Aspergillus oryzae

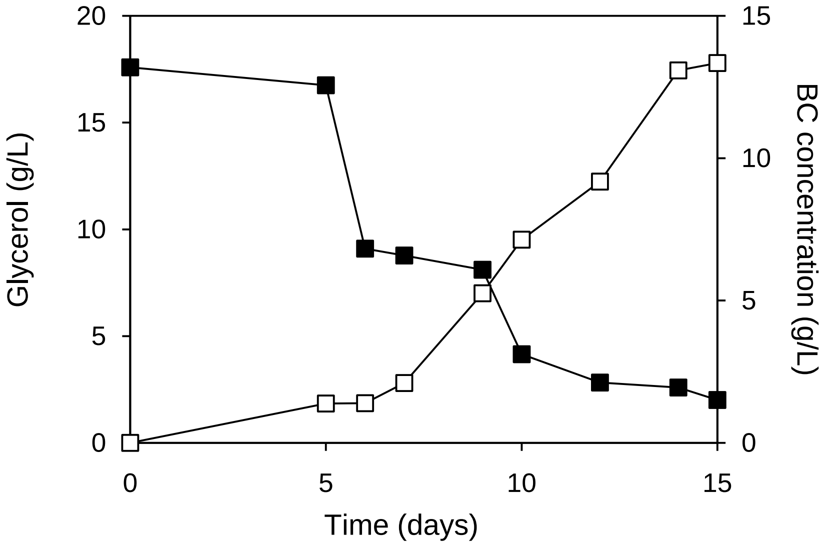
Solid state fermentation

Enzymatic hydrolysis

Nutrient-rich supplement

Microbial fermentation

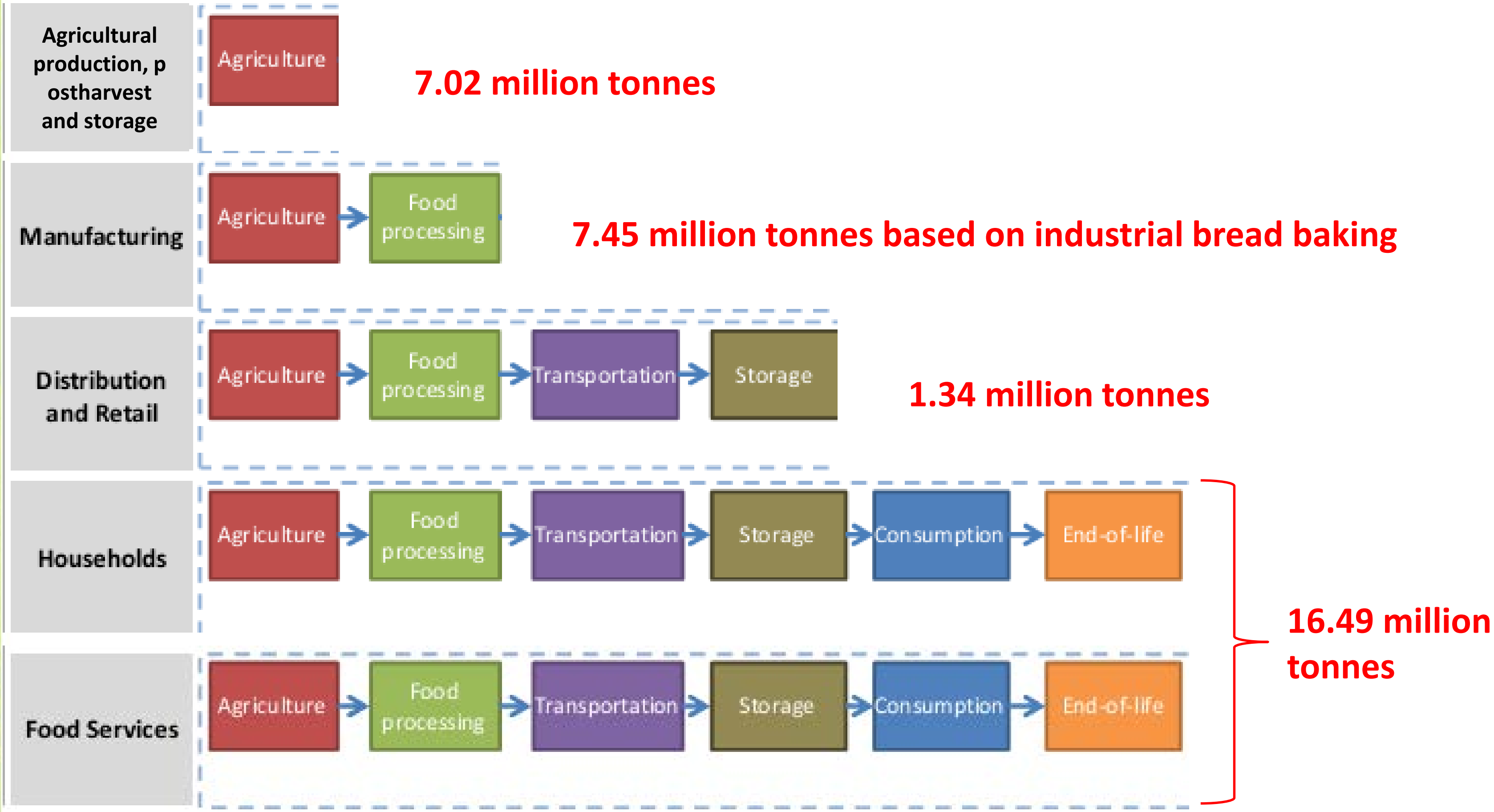
BC production from biodiesel industry by-product streams



Crude glycerol consumption and BC production using sunflower meal hydrolysates and crude glycerol as fermentation media. (■), Glycerol; (□), BC concentration

- ✓ BC production: 4 fold (13.3 g/L) higher in comparison to commercial nutrient supplements and crude glycerol
- ✓ Glycerol to BC conversion yield: 0.8 g BC per g of consumed glycerol
- ✓ Productivity: 0.89 g/L/day

Losses and waste generated annually at different stages of the life cycle of wheat and rye production and processing in Europe



Agricultural production, postharvest and storage
Agriculture
7.02 million tonnes

Manufacturing
Agriculture → Food processing
7.45 million tonnes based on industrial bread baking

Distribution and Retail
Agriculture → Food processing → Transportation → Storage
1.34 million tonnes

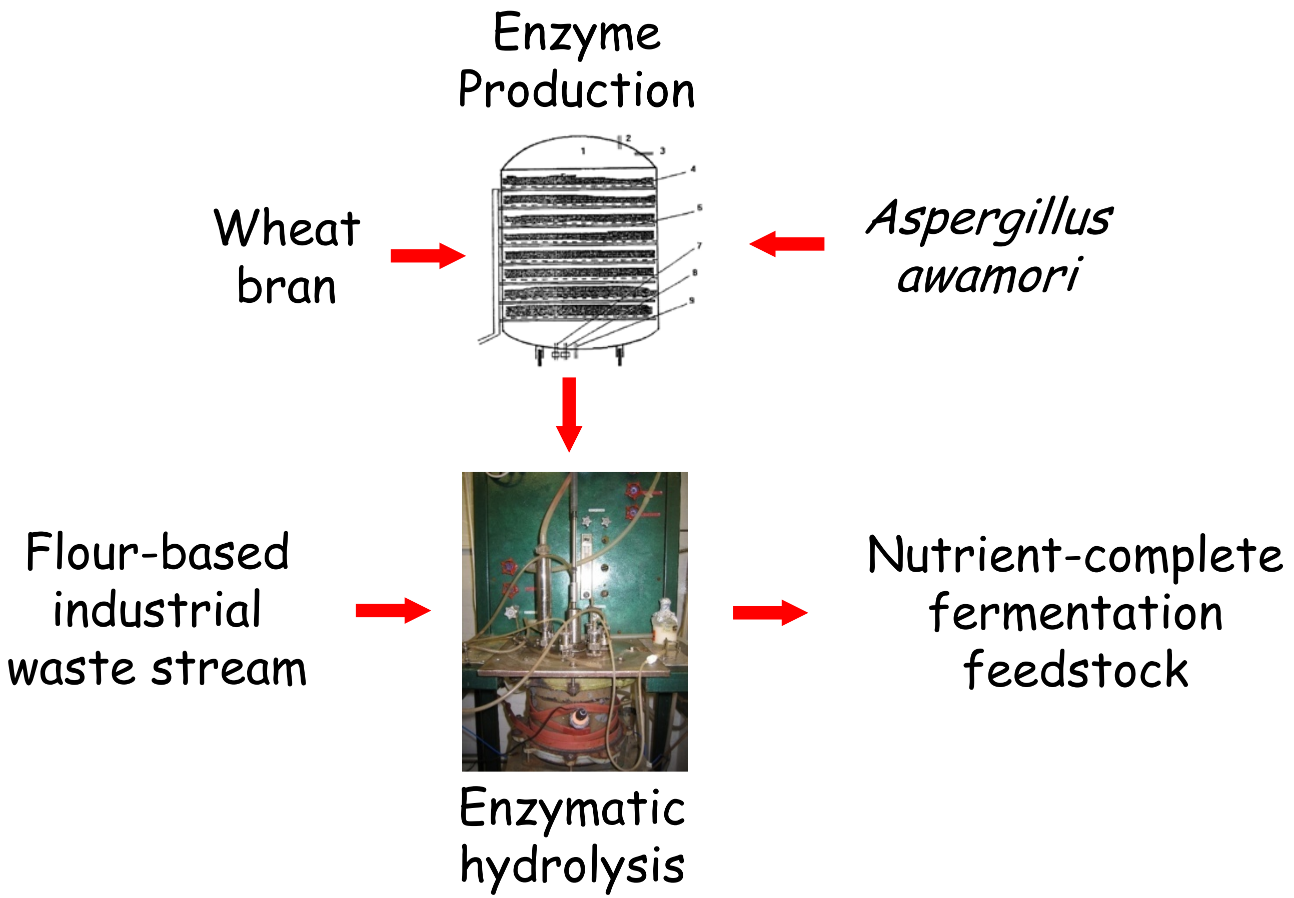
Households
Agriculture → Food processing → Transportation → Storage → Consumption → End-of-life

Food Services
Agriculture → Food processing → Transportation → Storage → Consumption → End-of-life

16.49 million tonnes

Gustavsson J. et al. 2013, The methodology of the FAO study: "Global Food Losses and Food Waste - extent, causes and prevention" - FAO, 2011, The Swedish Institute for Food and Biotechnology

Fermentation feedstock production from flour-rich waste streams



Enzyme Production

Wheat bran

Aspergillus awamori

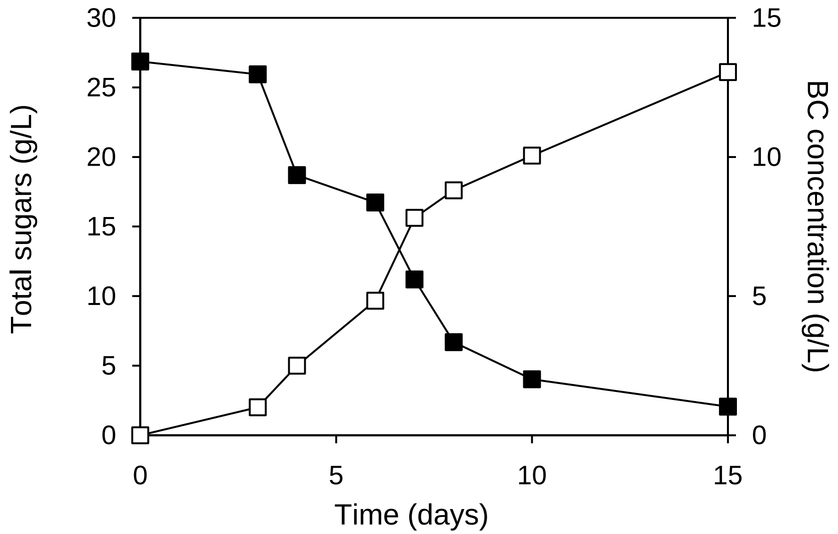
Flour-based industrial waste stream

Nutrient-complete fermentation feedstock

Enzymatic hydrolysis

Crude enzymes production	Solid state fermentation
Starch & protein hydrolysis of flour-based industrial waste streams	Glucose and FAN production

BC production from confectionery industry by-product streams



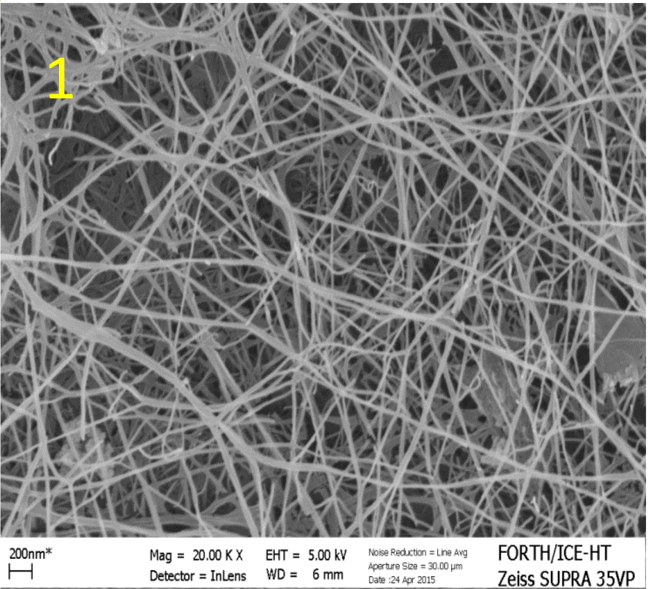
Total sugar consumption and BC production when flour-rich waste hydrolysates were used as fermentation media.

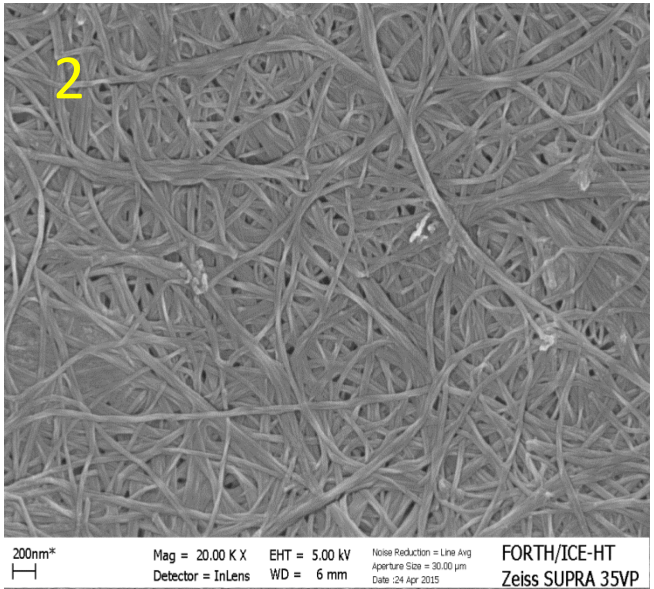
- ✓ BC production: 2.6 fold (13 g/L) higher in comparison to commercial sucrose
- ✓ Sugar to BC conversion yield: 0.53 g BC per g of consumed sugars
- ✓ Productivity: 0.87 g/L/day

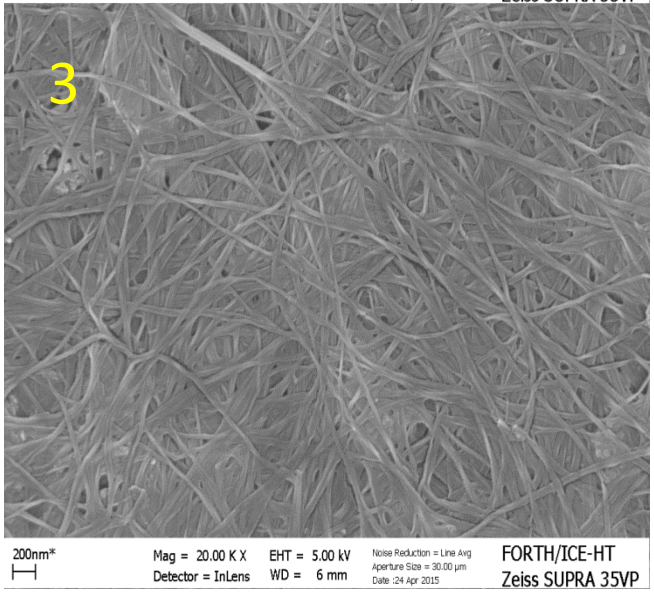
BC production achieved using various natural resources based on literature-cited publications

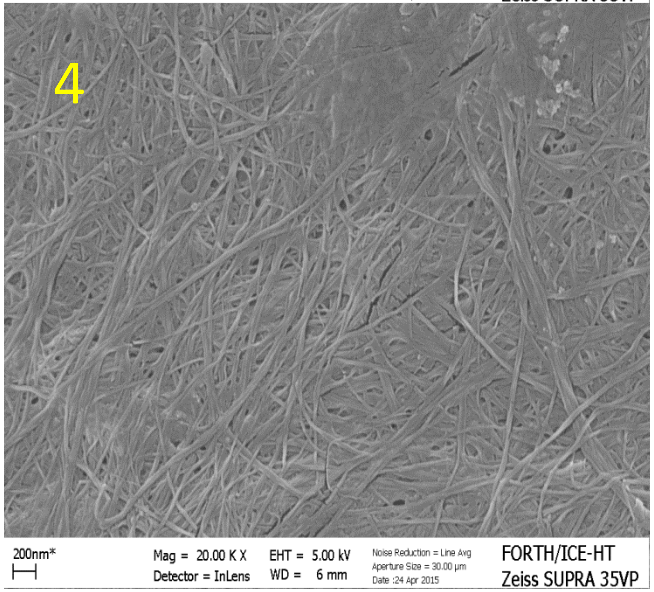
Strain	Carbon source	BC production (g/L)	BC production rate (g/L/d)
A.aceti subsp.*xylinum* ATCC 23770	konjac powder	2.12	0.26
A. xylinum NBRC 13693	fruit juices	5.9	0.42
G. xylinus ATCC 23770	wheat straw hydrolysates	8.3	1.18
G. xylinus ATCC 23770	cotton cloth hydrolysates	10.8	0.77
G. hansenii CGMCC 3917	waste beer yeast hydrolysates	7.02	0.50
A. xylinum KJ1	saccharified food wastes	18	3.60
A. xylinum KJ1	saccharified food wastes	5.6	1.87
G.xylinus CH001	acetone-butanol-ethanol fermentation wastewater	1.34	0.17
A. xylinum BPR 2001 ATCC 700178	maple syrup	1.51	0.07
G.xylinus ATCC23770	spruce hydrolysates	8.2	0.59
G.xylinus CGMCCNo.2955	Wastewater of candied jujube	2.25	0.37
G. xylinus NRRL B-42	glycerol from biodiesel and grape bagasse	10 and 8	0.71 and 0.57
G. xylinus BCRC 12334	thin stillage from rice wine distillery	3.05-10.38	0.44-1.48
G.medellinensis	sugar cane juice and pineapple residues	0.82-3.97	0.12-0.31
G.sacchari	residues from agro-forest industries	0.1-0.6	0.025-0.15

SEM micrographs









✓ BC_1 produced with crude glycerol combined with the Hestrin and Schramm medium

✓ BC_2 produced from biodiesel industry by-products

✓ BC_3 produced with the Hestrin and Schramm medium

✓ BC_4 produced from confectionery industry wastes

BC properties

Properties of bacterial cellulose samples

Properties	**BC_1**	**BC_2**	**BC_3**	**BC_4**
Stress at break (MPa)	139.5 ± 12.6	79.8 ± 7.6	94.5 ± 8.2	72.3 ± 6.0
ε% (ΔL/Lo)	8.5 ± 0.2	7.1 ± 0.0	9.2 ± 0.4	7.05 ±0.02
Young’smodulus (GPa)	1.64 ± 0.2	1.13 ± 0.11	1.02 ± 0.09	0.97± 0.05
CrI (%)	88	74	81	89
CrS (nm)	5.9	6.4	6.1	5.7
$[\eta]$ (dL/g)	9.34	7.47	4.66	6.19
M_w (10^6 $gmol^{-1}$)	0.433	0.387	0.306	0.353
DP	2672.8	2391.2	1889.1	2176.1
WHC (g water/g dry BC)	138 ± 9	124 ± 5	131 ± 4	102 ± 6

Conclusions

✓ The bacterial strain *K. sucrofermentans* DSM 15973 can produce high BC concentrations when it is cultivated in by-product streams from oilseed-based biodiesel industries and waste streams from confectionery industries as the sole sources of nutrients.

✓ The renewable resources employed provided all nutrients required for bacterial growth and BC production. This could lead to improved cost-competitiveness of industrial BC production.

✓ The properties of the BC obtained from the crude renewable resources compared well with literature-cited publications and the properties of BC produced with commercial nutrient supplements.

✓ Future research should focus on the identification of specific applications for the BCs produced from the crude renewable resources employed in this study.

THANK YOU FOR YOUR ATTENTION!!!