

Farmed insects to create a circular bio-economy in the food and feed industry

Andreas Vilcinskas

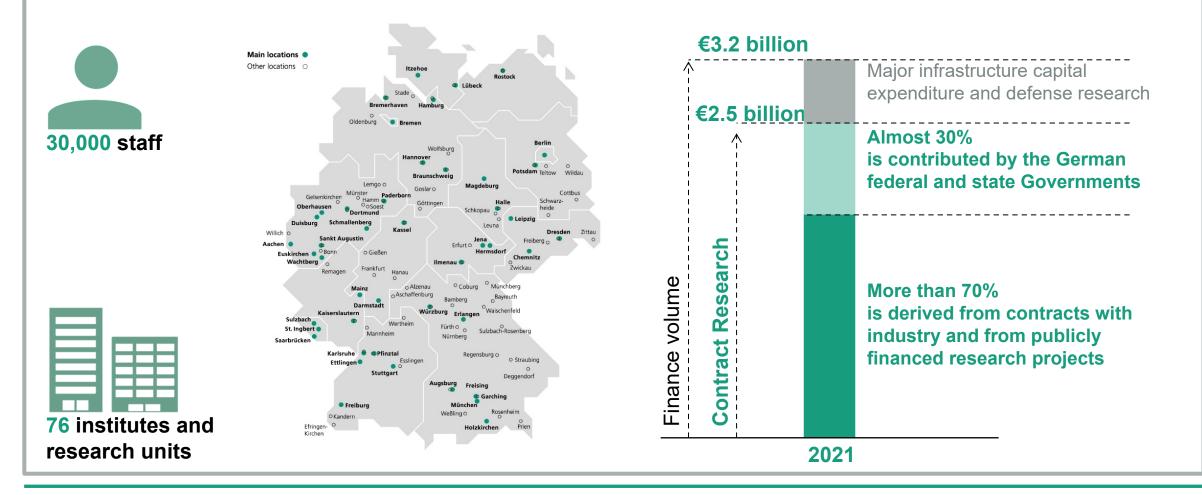
Institute for Insect Biotechnology, Justus-Liebig-University, Giessen, Germany Fraunhofer Institute for Molecular Biology and Applied Ecology, Branch Bioresources, Giessen





The Fraunhofer-Gesellschaft at a glance

The Fraunhofer-Gesellschaft undertakes applied research of direct utility to private and public enterprise and of wide benefit to society.





Intellectual Property Rights of Fraunhofer

Fraunhofer Patents	2015	2016	2017	2018	2019
Active patent families *	6573	6762	6695	6874	7050
Invention disclosures reports p.a.	670	798	756	734	733
Patent applications p.a.	506	608	602	612	623

* Portfolio of active rights (patents and utility models) and patent applications at year end.

2019: Fraunhofer was

Nr. 18 of the most active patent applicants and Nr. 7 of the most active trade mark registrations at the German Patent and Trade Mark Office

2019: Fraunhofer was

No. 32 of the most active patent applicants at the European Patent and Trade Mark Office Deutsches Patent- und Markenamt

2020: Fraunhofer has been awarded as Top 100 Global Innovator for the years 2014-2020 according to Clarivate Analytics / Thomson Reuters

Derwent Top 100 Global Innovator 2020 Clarivate Analytics

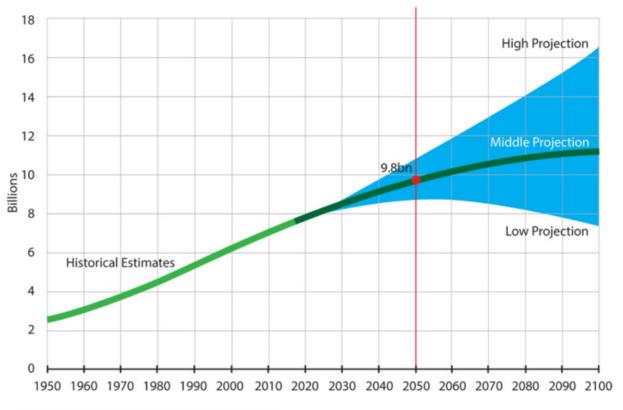


Europäisches Patentamt

Office européen des brevets

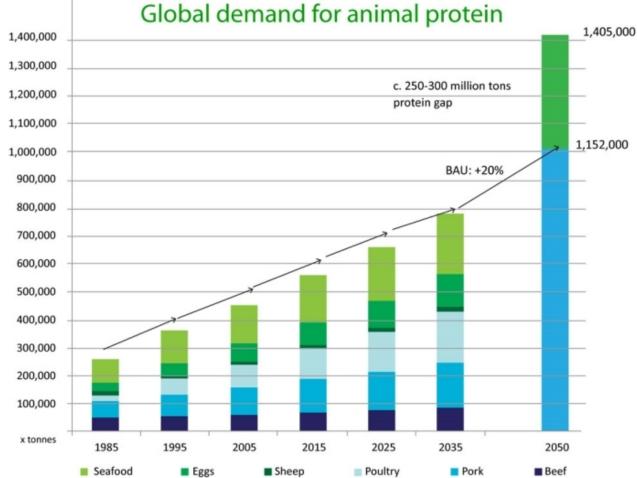
European Patent Office





Projected World Population

Data Source: United Nations, "World Population Prospects: 2015 Revision"



Data Source: Rabobank analysis, FAO, OECD, FAPRI, 2016

Insects such as the Black soldier fly are world-wide used for the

bioconversion of organic side streams into protein, lipids and chitin.

Insect farming is an environmentally sustainable alternative to address the rapidly growing demand for animal-protein.

Resources needed for 1000 kg protein production

	Cattle	Pigs	Broilers	Plant protein*	Insects**
Area needed	7,000 m²	3,000 m²	2,000 m² ja_rau	4,000 m²	< 0 m²
Water needed	17,000 m³	5,500 m ³	3,800 m ³	2,500 m³	< 0 m ³

* Soybean

** Waste as feed

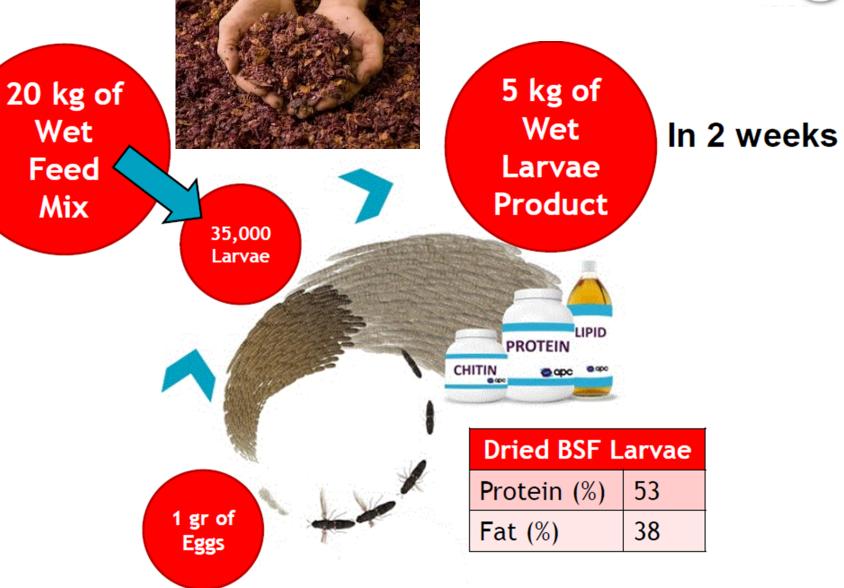




Bioconversion with Black Soldier Fly







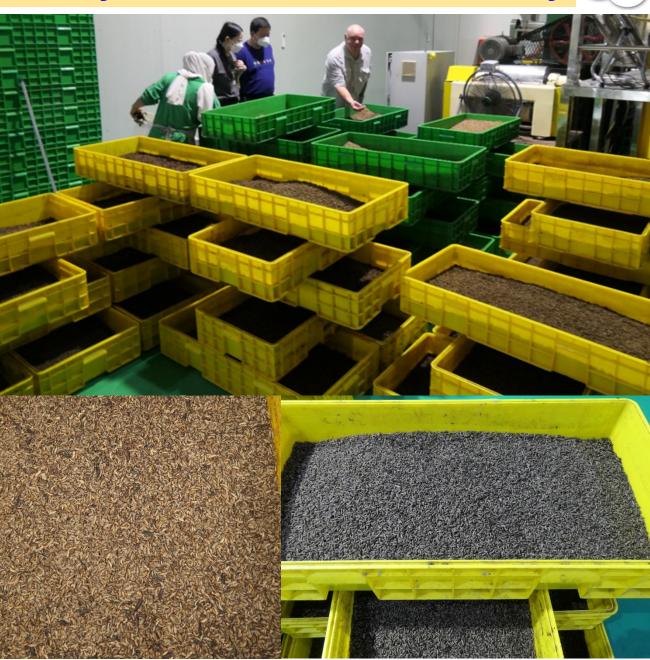




Farmed insects to create a circular bio-economy in the food and feed industry

Challenges in industrial production of insects

- Large-scale availability of insect feed
- > How to prevent the outbreak of diseases?
- Optimization of industrial processes
- Economic competitiveness
- Integration of insect farming in the
- **Controlled Environment Agriculture (CEA)**
- Insect feed in sustainable crustacean aquaculture
- Insect feed in animal nutrition
- Insects as a missing link in the circular bio-economy of the food and feed industry
- Insect farming in Controlled Environment Agriculture



Insects as a source of alternative Proteins: reduce costs

- Utilization of agricultural or industrial sides streams
- Large quantities with defined quality
- Challenge: seasonal avalability
- EU-regulations
- Detoxification of side streams
- Diet mixtures for optimized growth of farmed insects











cocoa bean shell



depectinised apple pomace







Klüber et al. 2022: Sustainability



Empty fruit bunches of the palm oil industry as a diet for BSF

- The palm oil industry of Malaysia produces app. 25 mio. tons and that of Indonesia 40 mio. tons of EFB per year
- This industrial waste, which is burned or used for land filling, causes tremendous environmental pollution
- The decay of empty fruit bunches results in methane production promoting climate change





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Bioconversion of empty fruit bunches into Black Soldier fly diet

- Fraunhofer-IME has developed a fungus-based fermentation process for the digestion of empty fruit bunches
- and for the enrichment with nitrogen to provide a suitible diet for insect farming
- Enabling the bioconversion of EFB into insect-derived protein, lipids, chitin and frass at industrial scale





Empty fruit bunches of the palm oil industry as a diet for BSF

- Fermentation of empty fruit bunches using specialized fungi
- Degradation of Cellulose and Lignin

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Enrichment of nitrogen and increase of protein content

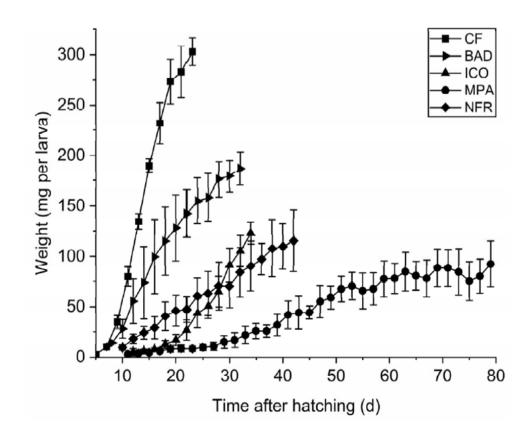
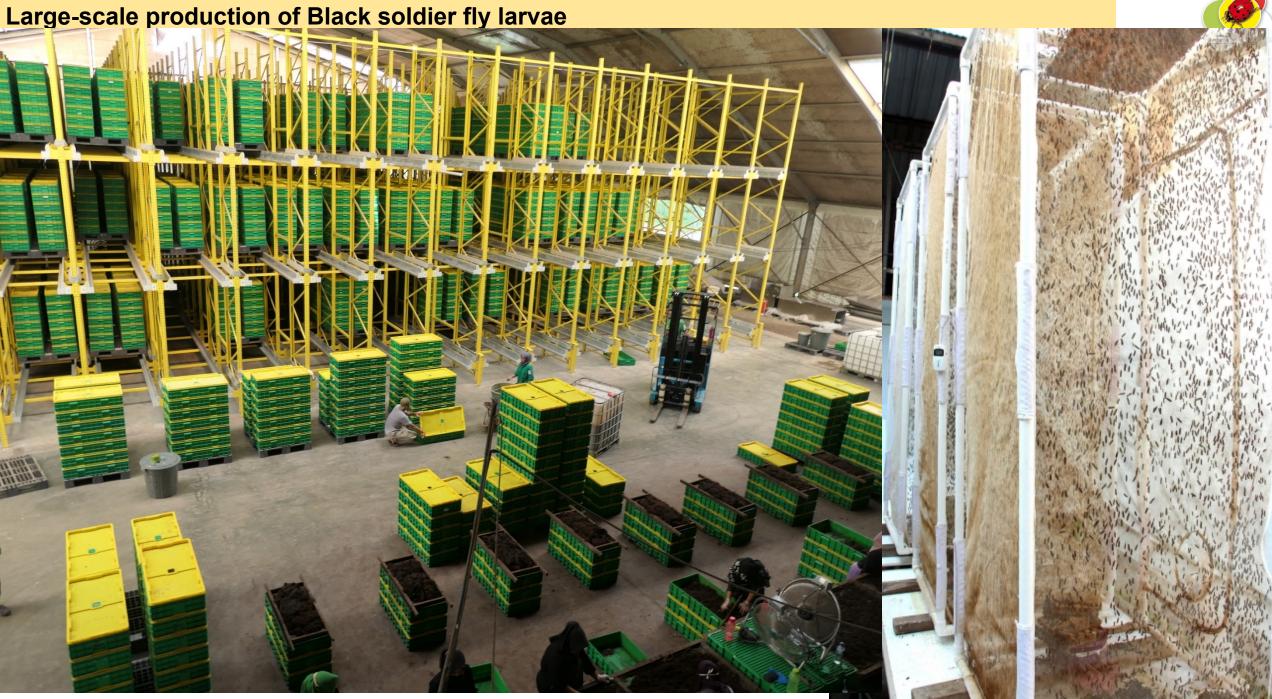


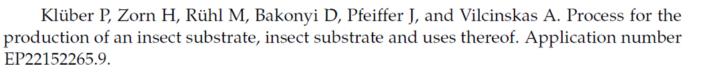
Figure 1. Growth curves of BSF larvae reared on chicken feed (CF), *B. adusta* (BAD), *I. consors* (ICO), or *M. palmivorus* (MPA) fermented EFB + PKM (7:3) mixtures, as well as a corresponding non-fermented reference (NFR). Data are mean larval weights (\pm SD) of three replicate boxes per diet (n = 25).

Klüber et al. 2022: Sustainability



Empty fruit bunches of the palm oil industry as a diet for BSF

- Fermentation of empty fruit bunches using specialized fungi
- Degradation of Cellulose and Lignin
- Enrichment of nitrogen and increase of protein content







Article

Diet Fermentation Leads to Microbial Adaptation in Black Soldier Fly (*Hermetia illucens*; Linnaeus, 1758) Larvae Reared on Palm Oil Side Streams

Patrick Klüber ¹, Dorothee Tegtmeier ¹, Sabine Hurka ², Janin Pfeiffer ¹, Andreas Vilcinskas ^{1,2}, Martin Rühl ^{1,3} and Holger Zorn ^{1,3,*}



Klüber et al. 2022: Sustainability



Insect frass as biofertilizer

- Insect frass (a mixture of excrements, chitin and feed leftovers) represents a valuable biofertilizer
- which can for example double the rice yield
- The benefit of this biofertilizer can be expanded beyond providing nutrients to encompass bacteria that produce plant growth hormones
- Insect frass can replace chemical fertilizers in palm tree agriculture

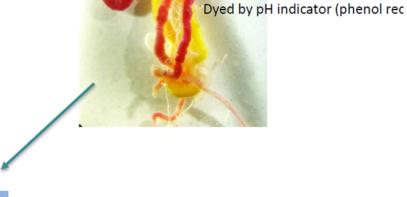




Beneficial microbes from insect frass

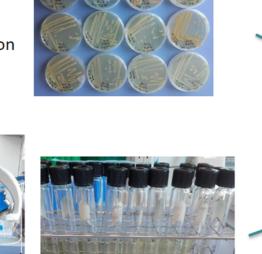
Harvesting the gut microbiota of Hermetia

- Pre-digestion of industrial waste products ٠
- Screening for natural products ٠
- Strain collection (cryo bank) ٠



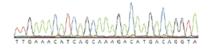
Gut of Hermetia illucens

Aerobic cultivation



Identification: 16S rRNA sequencing







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Dorothee Tegtmeier

Anaerobic cultivation





Beneficial microbes from insect frass



microorganisms

Article

Culture-Independent and Culture-Dependent Characterization of the Black Soldier Fly Gut Microbiome Reveals a Large Proportion of Culturable Bacteria with Potential for **Industrial Applications**

Dorothee Tegtmeier ¹, *¹, Sabine Hurka ², Sanja Mihajlovic ¹, Maren Bodenschatz ¹, Stephan **Frontiers** and Andreas Vilcinskas 1,2,*





Bundesministerium für Bildung und Forschung

> published: 29 March 202 doi: 10.3389/fmicb.2021.63450



Antonie van Leeuwenhoek https://doi.org/10.1007/s10482-022-01735-7

ORIGINAL PAPER

Isolation of *Hermetia illucens* larvae core gut microbiota by two different cultivation strategies

Yina Cifuentes · Andreas Vilcinskas Peter Kämpfer · Stefanie P. Glaeser

Cottonseed Press Cake as a **Potential Diet for Industrially Farmed Black Soldier Fly Larvae Triggers** Adaptations of Their Bacterial and **Fungal Gut Microbiota**

Dorothee Tegtmeier^{1*}, Sabine Hurka², Patrick Klüber¹, Karina Brinkrolf³, Philipp Heise¹ and Andreas Vilcinskas^{1,2}

¹ Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany, ² Institute for Insect Biotechnology, Justus Liebig University Giessen, Giessen, Germany, ³ Department of Bioinformatics and Systems Biology, Justus Liebig University Giessen, Giessen, Germany



in Microbiology

Detoxification of diet substrates: Gossypol in Cottonseed press cake



- Gossypol is a natural phenol derived from the cotton plant.
- Plant defense compound mediating infertility in insects.
- Toxic for humans





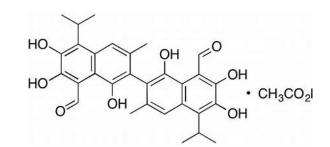


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ORIGINAL RESEARCH published: 29 March 2021 doi: 10.3389/fmicb.2021.634503







Cottonseed Press Cake as a Potential Diet for Industrially Farmed Black Soldier Fly Larvae Triggers Adaptations of Their Bacterial and Fungal Gut Microbiota

Dorothee Tegtmeier^{1*}, Sabine Hurka², Patrick Klüber¹, Karina Brinkrolf³, Philipp Heise¹ and Andreas Vilcinskas^{1,2}

¹ Department of Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany, ² Institute for Insect Biotechnology, Justus Liebig University Giessen, Giessen, Germany, ³ Department of Bioinformatics and Systems Biology, Justus Liebig University Giessen, Giessen, Germany

Molecular pathogen detection in insect farms

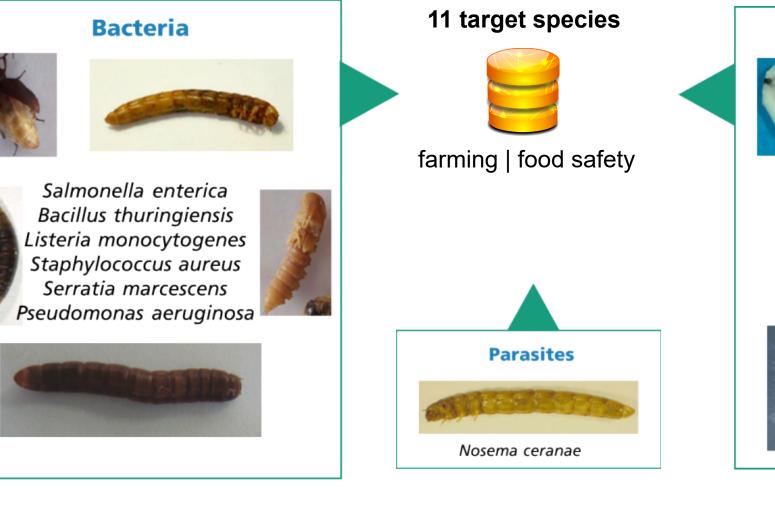
Pathogens responsible for more than half of insect production loss

Eilenberg et al. 2015

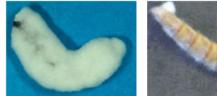
Pathogens detected in > 80% of 300 reviewed edible insects farms >30% potentially pathogenic for humans >35% potentially parasitic for animals Galecki et al., 2019

Expect new diseases to emerge in all insect production systems over time

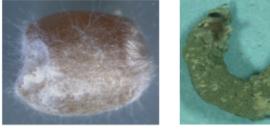
Insect and food borne pathogens

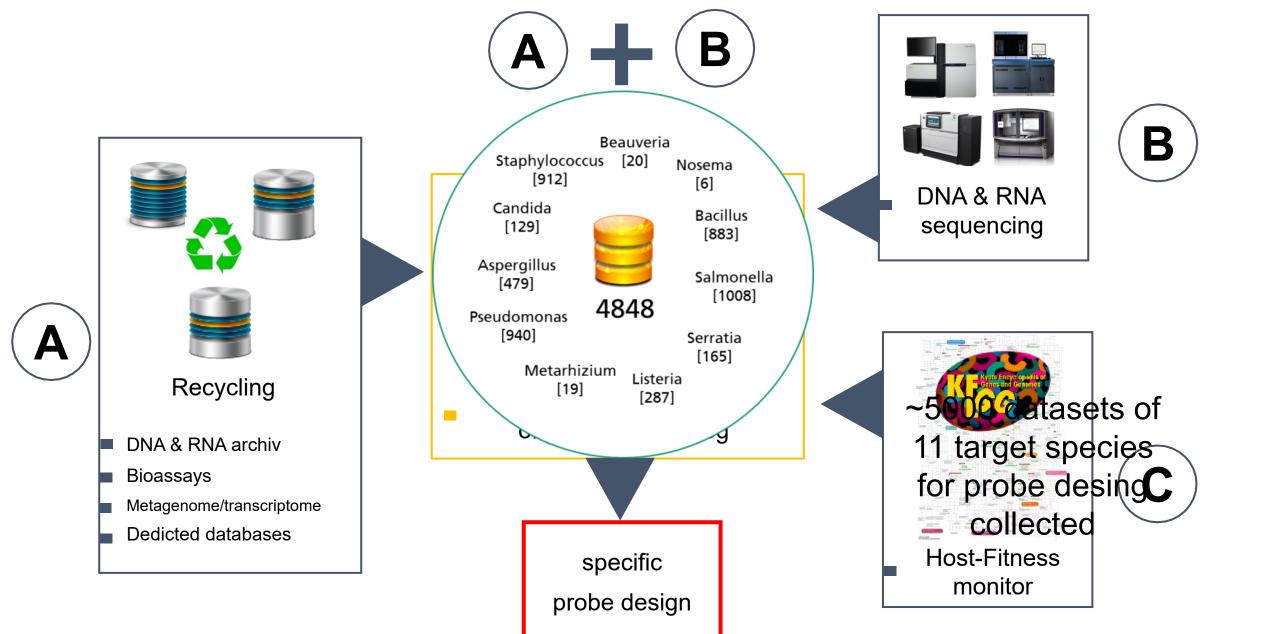


Fungus

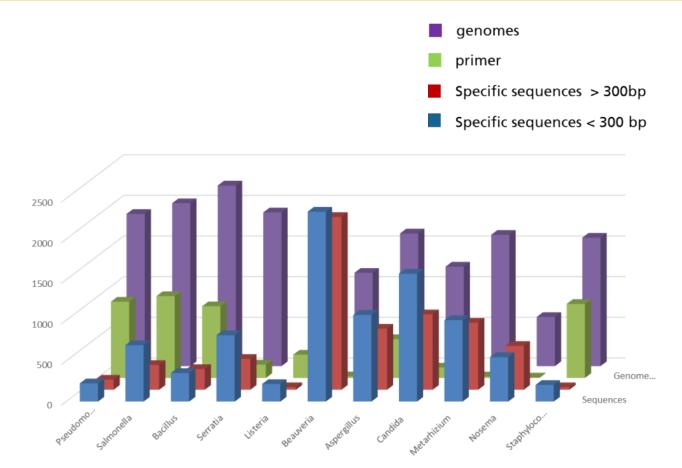


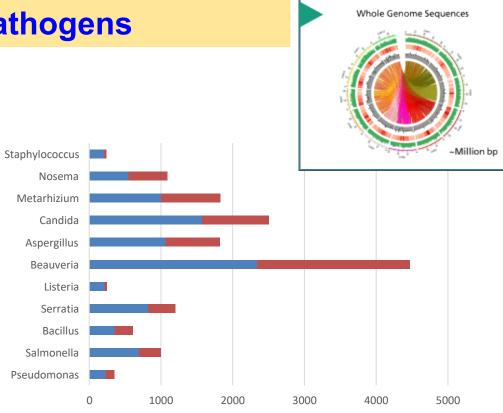
Beauveria bassiana Metarhizium brunneum Candida albicans Aspergillus flavus





Identification of target regions in genomes of pathogens

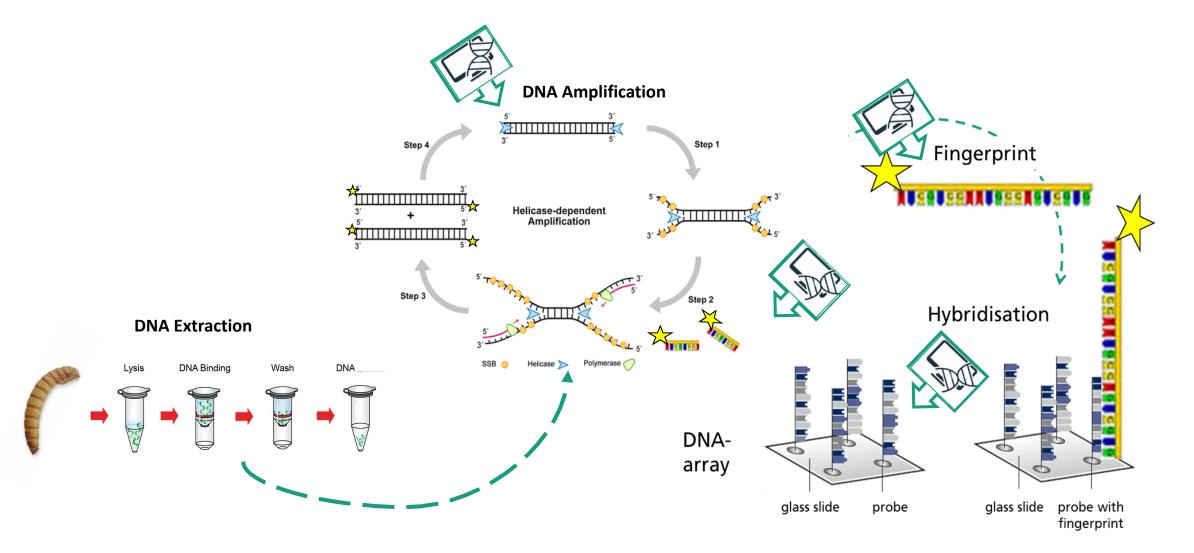




<u>Results</u>

- > 6k specific probes > 300bp overall
- All species sufficient covered for detailed montioring
- Lowest *count on Listera*, less then 350 specific probes

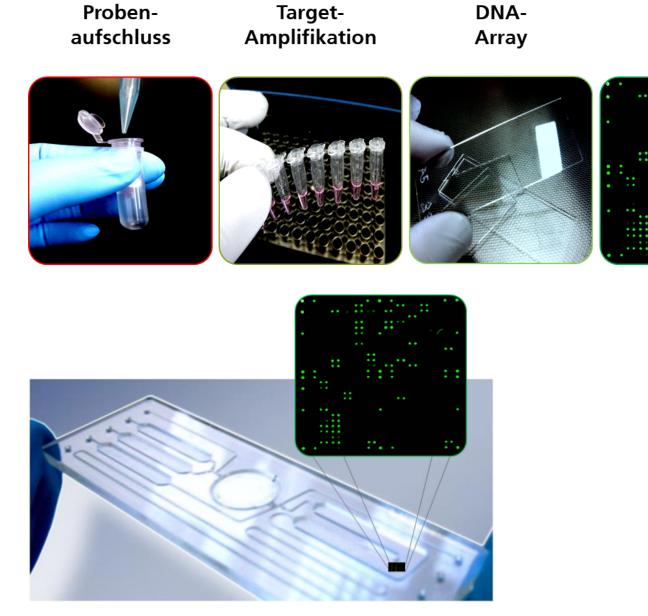
Detection of pathogens in automated insect production systems



Detection of pathogens in automated insect production systems

Readout

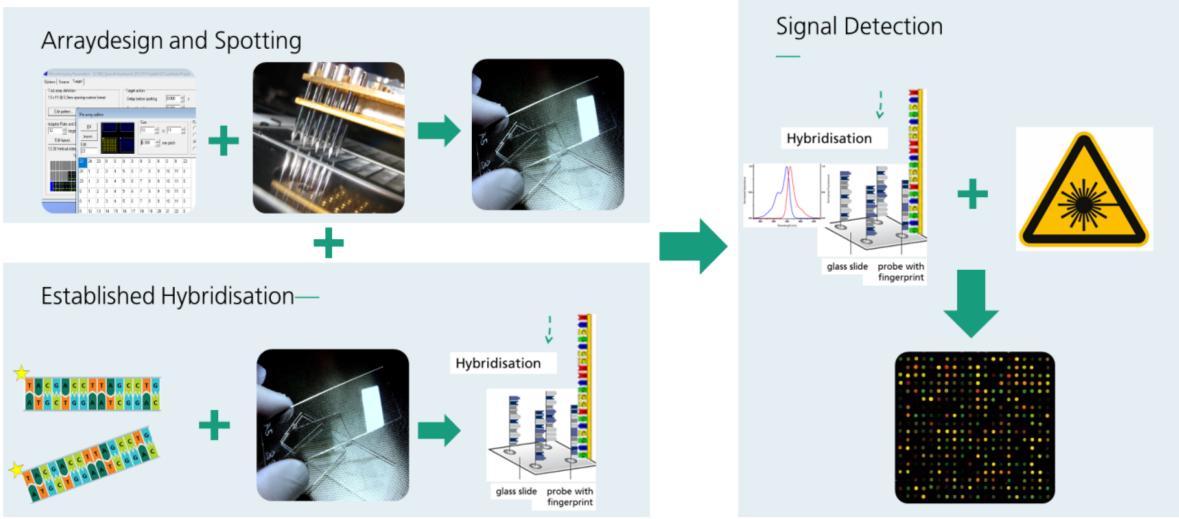
Ergebnis



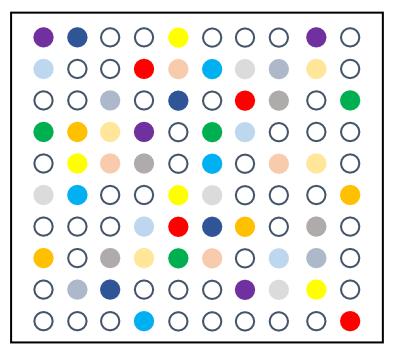


Detection of pathogens in automated insect production systems

Development of DNA µ-Arrays



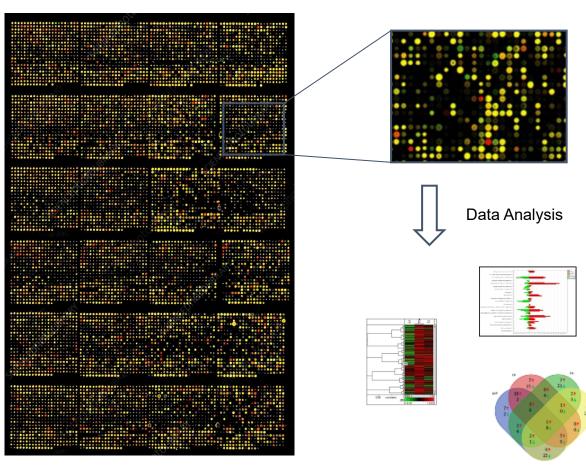
Pathogen diagnostic for insect farms



Salmonella enterica
 Bacillus thuringiensis
 Listeria monocytogenes
 Staphylococcus aureus
 Serratia marcescens
 Pseudomonas aeruginosa

Nosema ceranae

- Beauveria bassiana
 Metarhizium brunneum
 Candida albicans
 Aspergillus flavus
 Hybridization Control
- Host Control
- O Internal Control



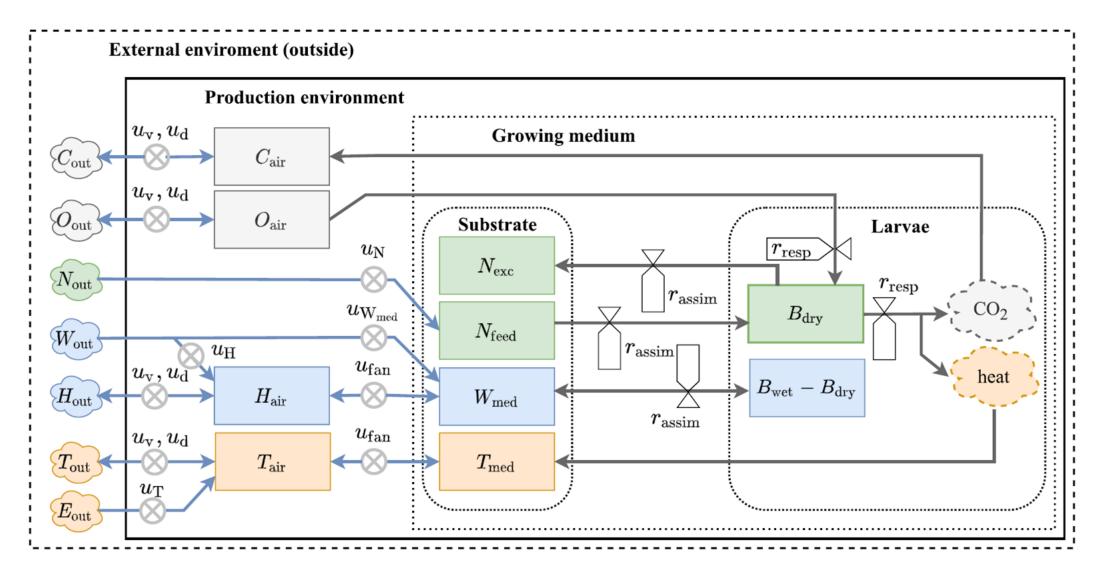


Fig. 1. Overview of the larvae production setup and the internal resource flows. It consists of a growing medium filled with larvae and feed, placed in either a closed or an open production environment. The valves representing the rates labelled as r_{resp} and r_{assim} are internally regulated by the larvae and the substrate states. Circular valves labelled u_{xyz} represent the control signals that control the flow of resources (fluxes).

Modelling and optimization of industrial BSF production

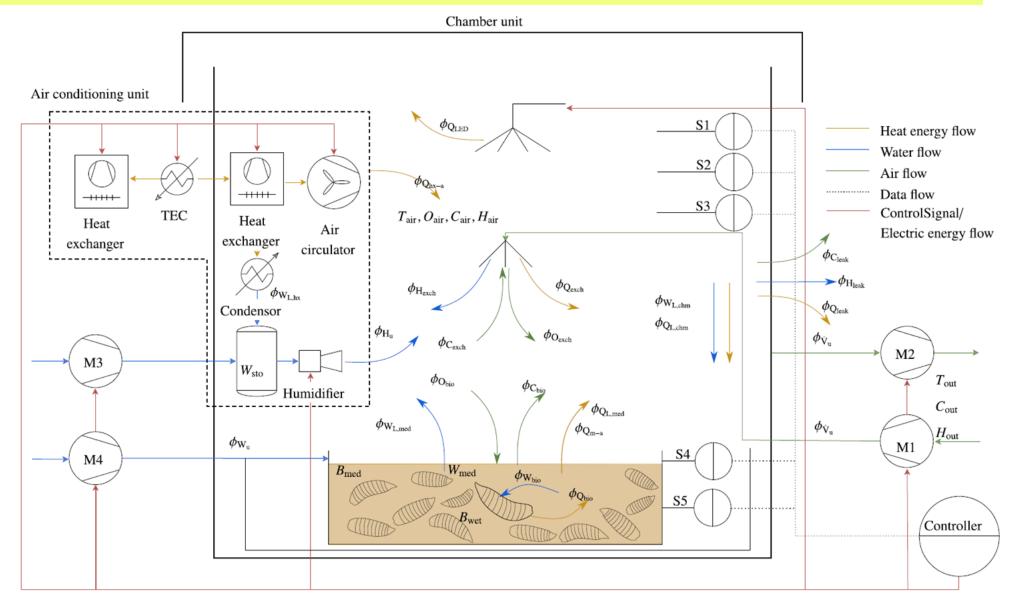


Fig. 2. Production unit components and fluxes in larvae production. Production unit with sensors (S1–S5), air pumps (M1–M2), water pumps (M3–M4), air conditioning unit (based on thermoelectric cooler (TEC) for heating, cooling, and condensation), humidifier, and LED lighting as presented in Padmanabha and Streif (2019).

A comprehensive dynamic growth and

Murali Padmanabha^(b), Alexander Kobelski, Arne-Jens Hempel, Stefan Streif* Automatic Control and System Dynamics Lab, Technische Universität Chemnitz, Chemnitz, Germany

development model of Hermetia illucens larvae



Computers and Electronics in Agriculture 206 (2023) 107649

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Computers and Electronics in Agriculture



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

Original papers



PLOS ONE

Abstract

RESEARCH ARTICLE

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Larvae of Hermetia illucens, also commonly known as black soldier fly (BSF) have gained significant importance in the feed industry, primarily used as feed for aquaculture and other livestock farming. Mathematical models such as the Von Bertalanffy growth model and dynamic energy budget models are available for modelling the growth of various organisms but have their demerits for their application to the growth and development of BSF. Also, such dynamic models were not yet applied to the growth of the BSF larvae despite models proven to be useful for automation of industrial production process (e.g. feeding, heating/ cooling, ventilation, harvesting, etc.). This work primarily focuses on developing a model based on the principles of the afore mentioned models from literature that can provide accurate mathematical description of the dry mass changes throughout the life cycle and the transition of development phases of the larvae. To further improve the accuracy of these models, various factors affecting the growth and development such as temperature, feed quality, feeding rate, moisture content in feed, and airflow rate are developed and integrated into the dynamic growth model. An extensive set of data was aggregated from various literature and used for the model development, parameter estimation and validation. Models describing the environmental factors were individually validated based on the data sets collected. In addition, the dynamic growth model was also validated for dry mass evolution and development stage transition of larvae reared on different substrate feeding rates. The developed models with the estimated parameters performed well, highlighting their potential application in decision-support systems and automation for large scale production.

Modelling and optimal control of growth, energy, and resource dynamics of Hermetia illucens in mass production environment

Murali Padmanabha, Alexander Kobelski, Arne-Jens Hempel, Stefan Streif* Automatic Control and System Dynamics Lab, Technische Universität Chemnitz, Chemnitz, 09107, Germany

ARTICLE INFO

Keywords: Hermetia illucens mass production Mass and energy flux modelling Energy and resource optimization Process design and control Bioreactor optimal control



ABSTRACT

Mass production of Hermetia illucens insect larvae is now being adopted in many countries and is taking an industrial production approach. Despite abundant literature on factors that affect larvae growth and the optimal static parameters identified in laboratory setup, for an industrial production process it is necessary to identify the trajectories such that the growth as well as the production process is optimal. To achieve this in this work, some of the important requirements and challenges involved thereof are identified and objectives of the automation process are formulated within a model based optimal control setup. Mechanistic models necessary for the optimization framework are derived as differential equations that describe the dynamic variation of resources (feed, water, O_2 etc.), energy, and larval biomass. In addition, the elevated metabolic activity of larvae corresponding to the final instar development is identified and also modelled based on the observation from experiments. The mass and energy balance approach used in modelling enables the quantification and distinction of the mass and energy flux components in various levels (e.g. larvae body, growing medium, production environment, and external environment) while holding its applicability for both open and closed/reactor based production setups. Finally, the trajectories generated using the synthesized optimal controller are tested under different scenarios showcasing significant reduction in resource consumption compared to a constant set-point operation of the production setup. Results presented in this work not only showcase the potential of the mechanistic models and their application in identifying the relevant process parameters (e.g. reactor properties such as volume, thermal conductivity, actuator capacities), but most importantly in optimizing the process dynamically and tuning the process objectives as desired (e.g. maximize larvae mass, reduce energy).



OPEN ACCESS

Citation: Padmanabha M, Kobelski A, Hempel A-J, Streif S (2020) A comprehensive dynamic growth and development model of Hermetia illucens larvae, PLoS ONE 15(9); e0239084, https://doi.org 10.1371/journal.pone.0239084

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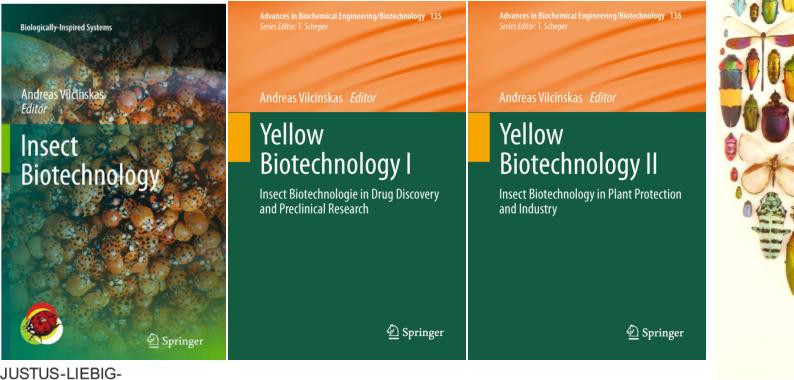
Accepted: August 28, 2020 Published: September 18, 2020

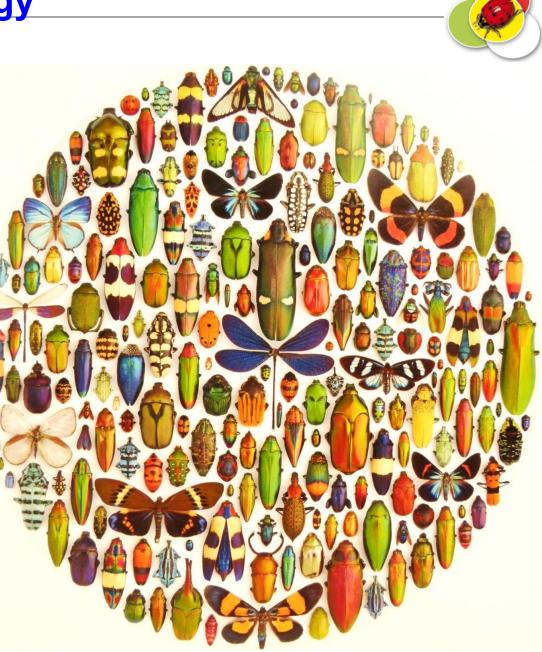
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Yellow Biotechnology

We define Insect Biotechnology or Yellow Biotechnology as:

The development and application of biotechnological methods to translate insects, their molecules, cells, organs or associated microorganism, respectively, into products or services for specific use in medicine, agriculture or industry.





Vilcinskas 2014: Encyclopedia of Biotechnology in Agriculture and Food



Yellow Biotechnology

- Insects are the most diverse and, therefore, the evolutionarily most successfull group of organisms
- Their biodiversity at species level can be expanded to the molecular level
- Insects represent a incredible compound library
- Insect Biotechnology aims to explore and to develop this compound library for human welfare.
- Consequent translational approach for value creation at industrial scale.







Food

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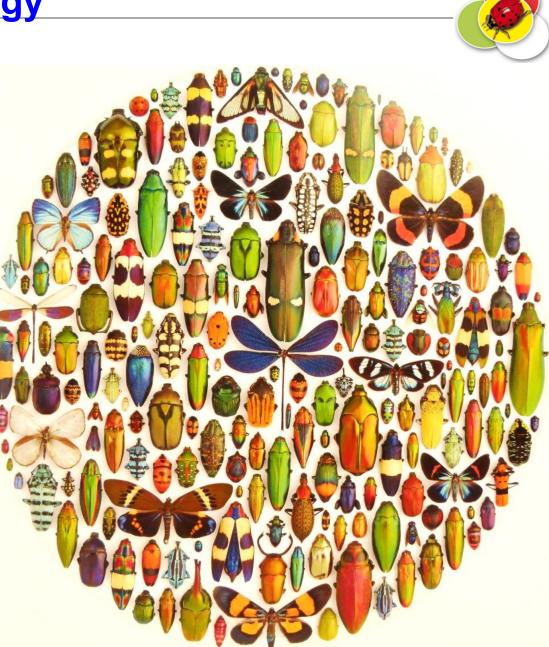
Medicine





Cosmetics















Prof. Klaus Eder

4-week feeding trial

- 36 homozygous obese Zucker rats and 12 heterozygous lean Zucker rats (Crl:ZUC (Orl)-*Lepr^{fa}*)
- housed in groups of two animals each under controlled conditions
- randomly divided into three obese groups (OC, OI50 and OI100) of 12 rats each; and lean rats served as lean control group (LC)
- insect meal from *Tenebrio molitor* L.

group	n	diet (<i>ad libitum</i>)
LC	12	100% casein as protein source
OC	12	100% casein as protein source
OI50	12	50% of casein replaced isonitrogenously by insect meal
OI100	12	100% of casein replaced isonitrogenously by insect meal

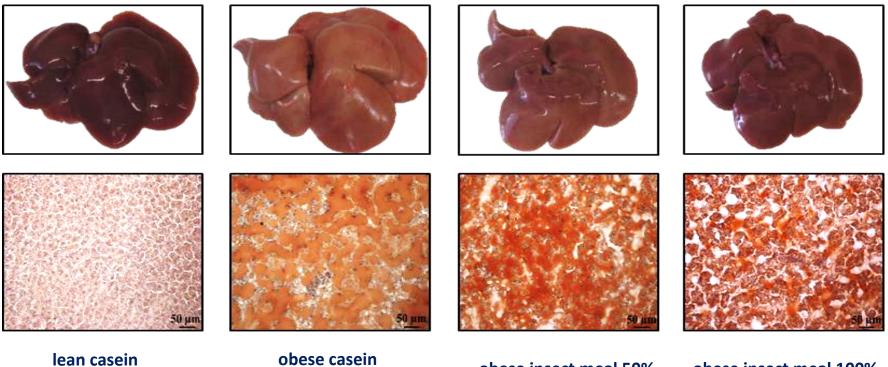


(Ÿnsect)



crude nutrients			
(% DM)			
crude protein	76.6		
crude fat	14.0		
crude fibre	13.2		
cude ash	4.3		
chitin	12.8		

effect of insect meal on lipid metabolism



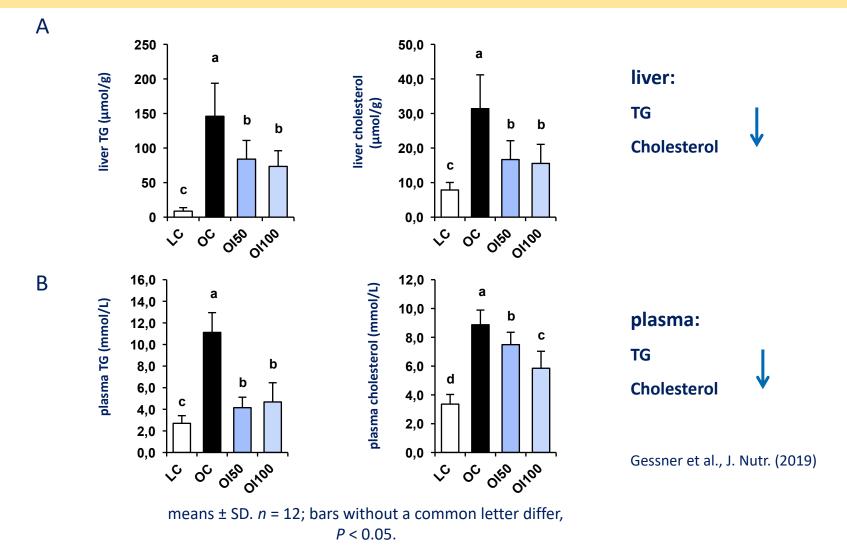
obese insect meal 50%



fold change			lipid synthetic pathway	
gene	OC vs. LC	OI50 vs. OC	OI100 vs. OC	
Scd2	14.11*	-6.21*	-9.59*	fatty acid
G6pd	1.24	-1.62	-2.41*	fatty acid
Aacs	2.28*	-1.50*	-2.28*	cholesterol
Fads1	1.27*	-1.32*	-2.01*	fatty acid
Acacb	1.47*	-1.38*	-1.80*	fatty acid
Me1	1.38	-1.37	-1.79*	fatty acid

Gessner et al., J. Nutr. (2019)

total livers and oil red O-stained liver sections of rats



concentrations of triacylglycerol (TG) and cholesterol in the liver (A) and plasma (B) of rats fed

either casein (OC), casein and insect meal (OI50) or insect meal (OI100)

a ah

The Journal of Nutrition Biochemical, Molecular, and Genetic Mechanisms



Insect Meal as Alternative Protein Source Exerts Pronounced Lipid-Lowering Effects in Hyperlipidemic Obese Zucker Rats

Denise K Gessner,¹ Anne Schwarz,¹ Sandra Meyer,¹ Gaiping Wen,¹ Erika Most,¹ Holger Zorn,² Robert Ringseis,¹ and Klaus Eder¹

¹Institute of Animal Nutrition and Nutrition Physiology and ²Institute of Food Chemistry and Food Biotechnology, Justus-Liebig-University Giessen, Giessen, Germany

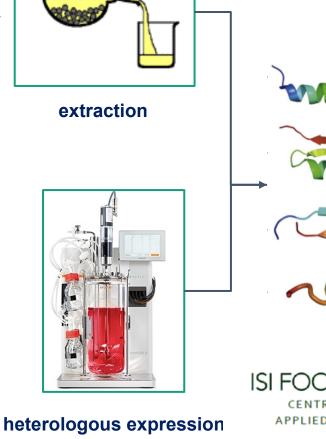
Insect-derived AMPs for food and feed preservation





AMP-Food

- Customer demand nature-like or natural, healthy ingredients
- Biologically degradable
- > Taylor-made activity profiles





Bundesministerium für Bildung und Forschung

- Purification
- Characterization
- application









YellowCare













AMERICAN SOCIETY FOR MICROBIOLOGY





In Vitro Evaluation of Antimicrobial Peptides from the Black Soldier Fly (*Hermetia Illucens*) against a Selection of Human Pathogens

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 ^bDepartment of Microbial and Molecular Systems (M²S), Research Group for Insect Production and Processing, KU Leuven, Geel, Belgium
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 ^dFraunhofer Institute for Molecular Biology and Applied Ecology, Branch for Bioresources, Gießen, Germany
 ^eInstitute for Insect Biotechnology, Justus Liebig University of Gießen, Gießen, Germany
 ^fLOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt am Main, Germany

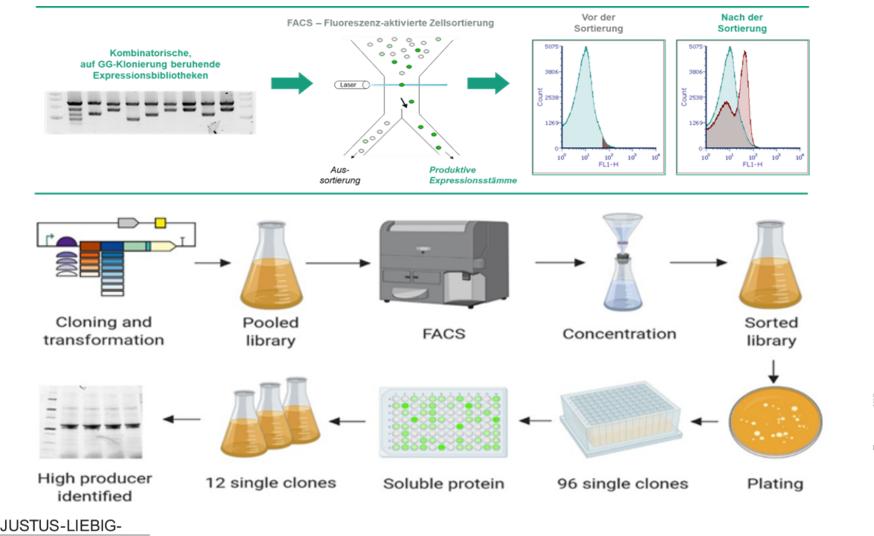


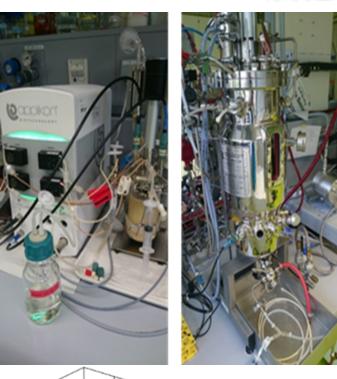


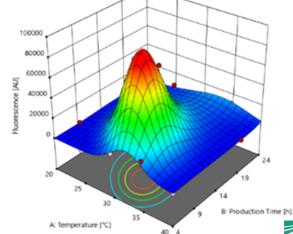
> Develpment of an up-scaling process

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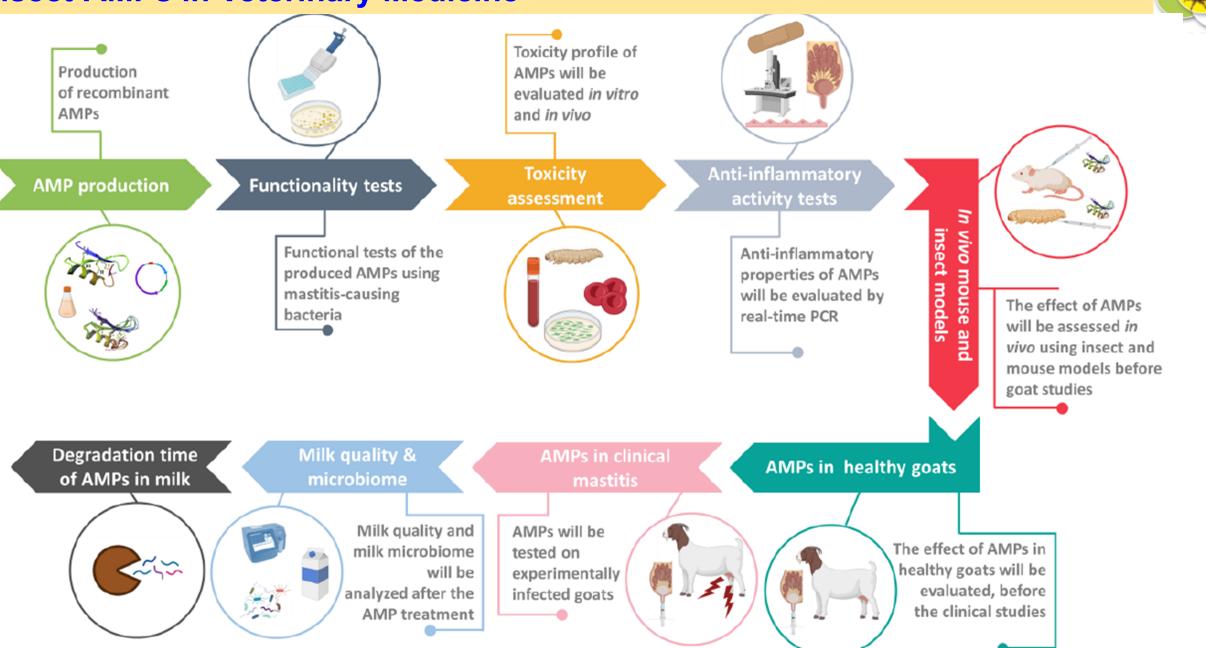






Fraunhofer

Insect-AMPs in Veterinary Medicine









Development of BSF lipids into high quality lubricants

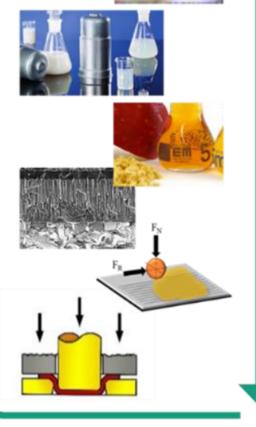




Prozesskette bei der Schmierstoffentwicklung:

Prozesskette





- Optimierung Basiswerkstoffe
- Fluidentwicklung
- > Additiventwicklung
- Anpassung der Oberflächen
- Untersuchung Anwendungseigenschaften
- Untersuchung Recyclingfähigkeit

Development of BSF chitin/chitosan into cosmetics and medical care products







PRODUCT SAMPLE

Chitosan Draf

A STEP-BY-STEP GUIDE TO CONSUME BIOACTIVE CHITOSAN ORAL FOR GUT HEALTH

RECOMMENDED USAGE

Consume once a day. Mix two drops in a quarter cup of drinking water and consume. May add two drops of propolis extract into the mix if desired.

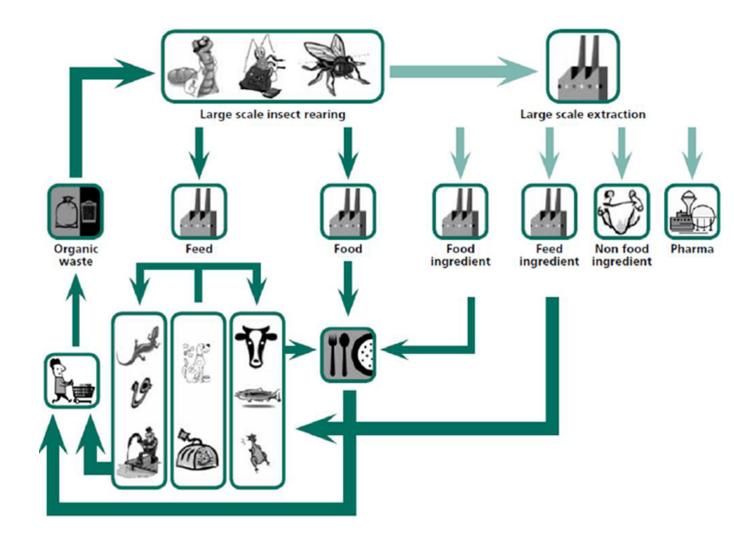
INGREDIENTS

Aqua/water, chitosan, glycerin, weak acid, gelling agent, salt, preservatives (ethanol and disodium EDTA)

Hermetia BIO SCIENCE

Insects as the missing link in the circular economy

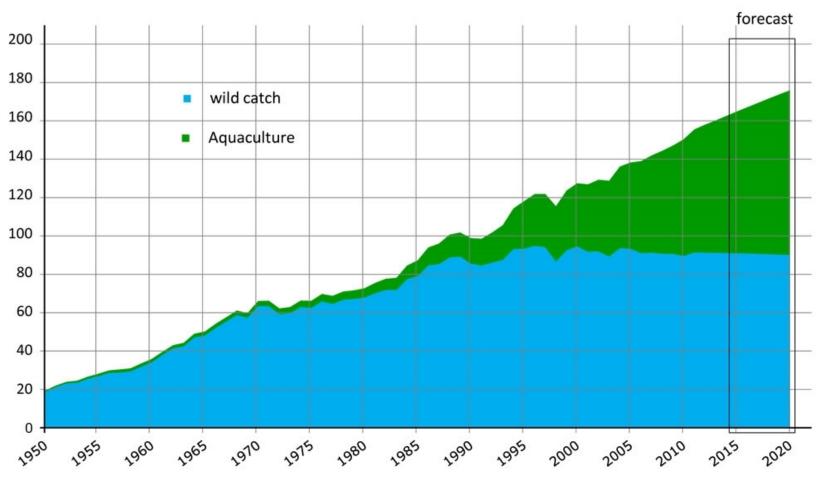
Insects as the missing link: ecology designs a circular economy





Total Seafood supply

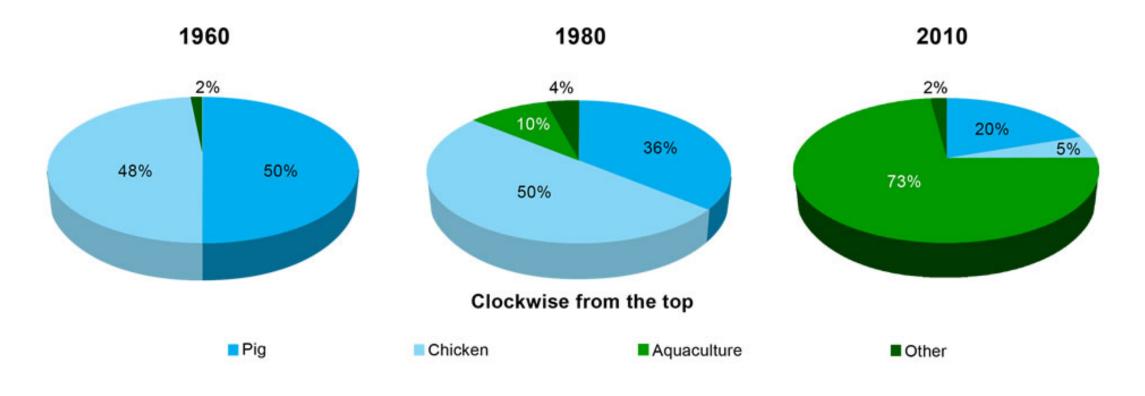
Millions tonnes



Data Source: Rabobank, FAO



Changing uses of fishmeal 1960 to 2010



Insect feed in sustainable crustacean aquaculture

- Optimizing the side-stream based died for BSF
- To enhance nutritive quality for shrimps
- Complete replacement of fish meal
- Decoupling of shrimp farming from the ocean









apple

pomace

cocoa bean shell



pomace

depectinised apple





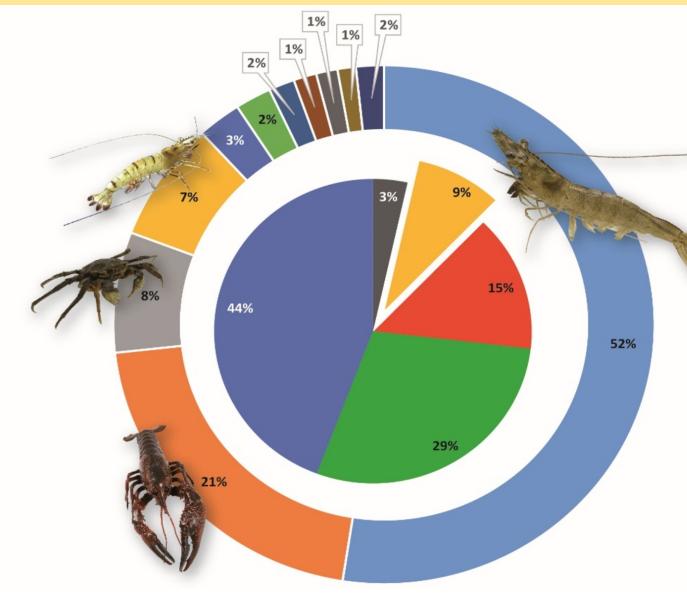
Bundesministerium für Bildung und Forschung



Tailor-made shrimp food (InFeed)

Insect-based sustainable agriculture (InA)

Global aquaculture production and main crustacean species 2019



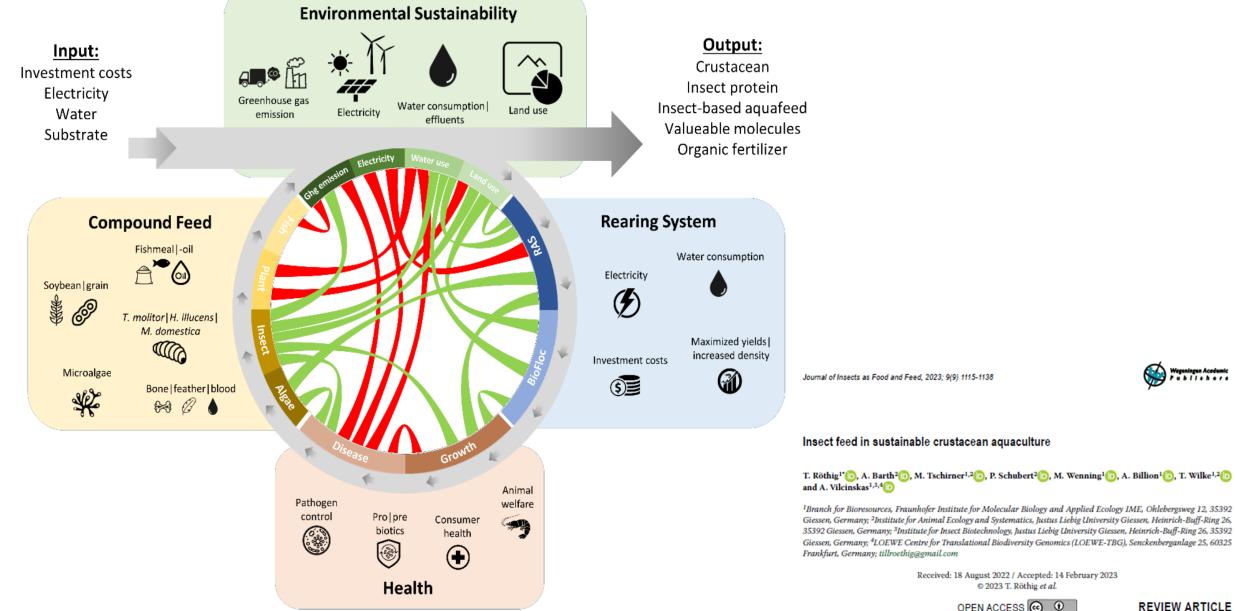
Inner Circle





Conceptual overview on sustainable, insect-based crustacean aquaculture



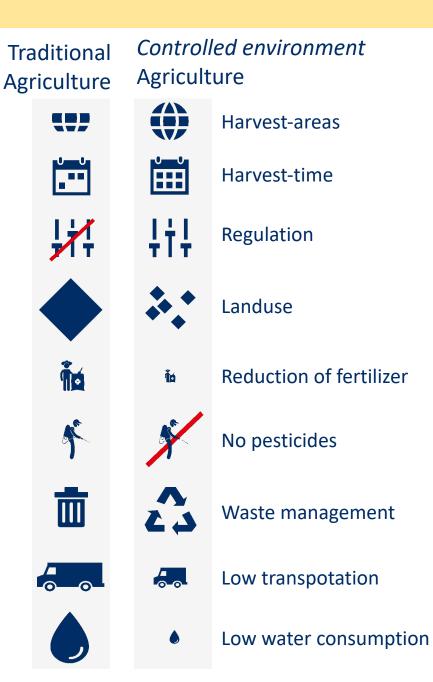


Controlled Environment Agriculture

Conflict among SDGs

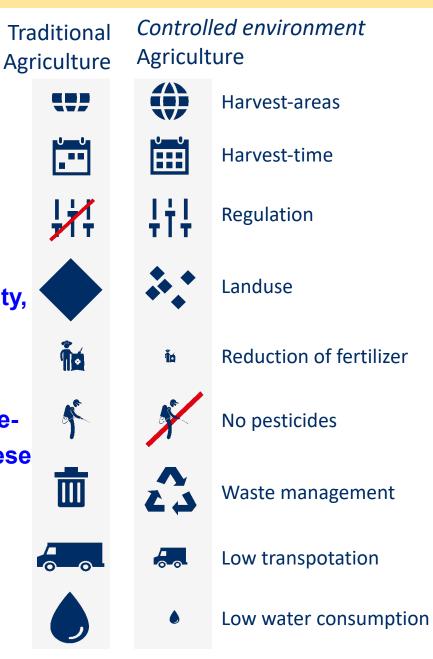






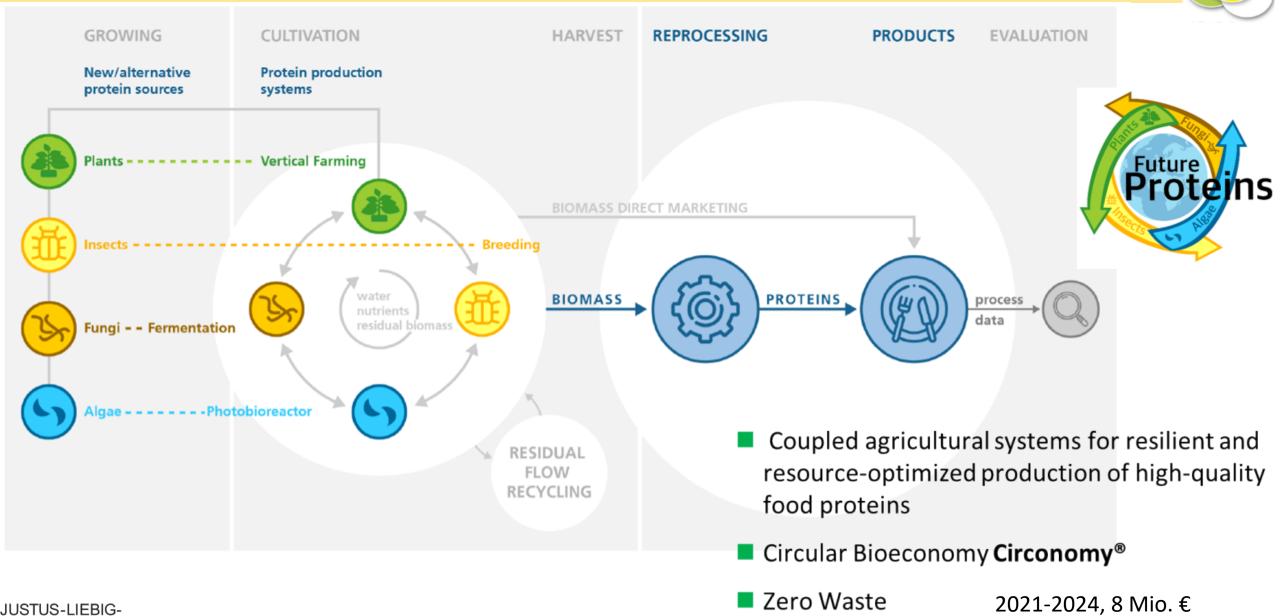
Controlled Environment Agriculture

- More food needs to be produced, more sustainable despite climate change
- Novel food production systems, including crop, algae, mushroom, fish and insect production in controlled environments-agriculture offer substantially higher productivity
- with no pesticides, low water use, towards full circularity, minimal externalities, close to the consumer and independent of climate, weather and region.
- Improving efficiencies, particularly in energy use and reuse in controlled-environment agriculture will make these new technologies a component of our future food systems.





Fraunhofer Lead Project: Future Proteins

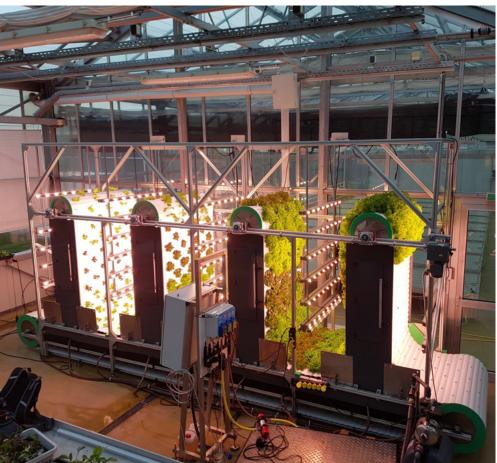




Fraunhofer Lead project: Future Proteins



















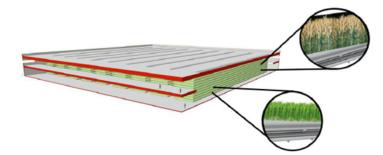








- Vertical farming of wheat (Asseng et al. 2020 PNAS)
 - 10 levels
 - 700 ± 20 t/ha
 - 1.940 ± 230 t/ha wheat per year
 - = 220-600 more than wheat production in the field





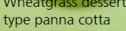


Fraunhofer Lead project: Future Proteins

First food products from *FutureProteins*







JUSTUS-LIEBIG-

UNIVERSITÄT GIESSEN





Crackers with mealworm flour





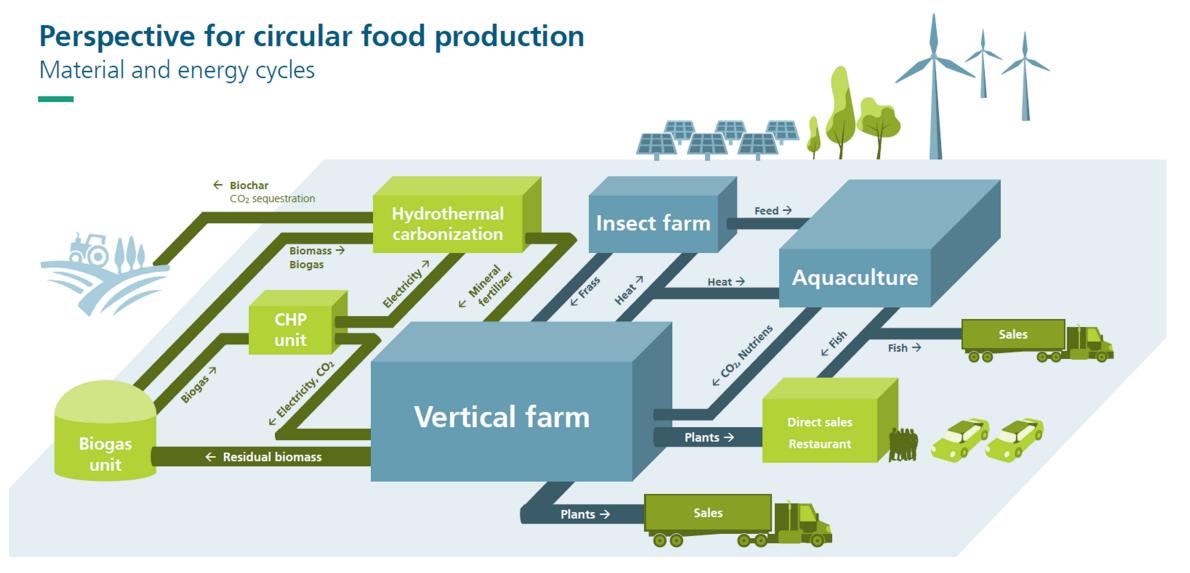
Vegan balls:left with pea protein, right with mushroom mycelium





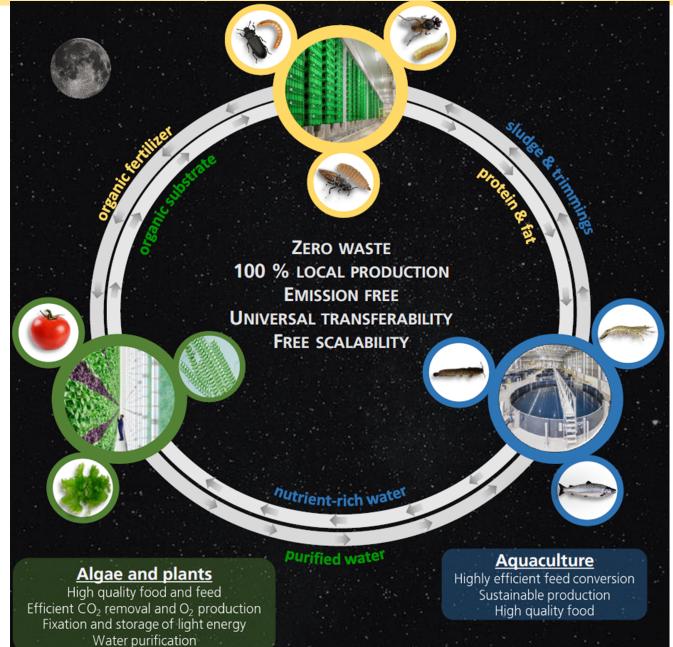








Insect Farming in Controlled Environment Agriculture





First international master program



Core Modules

- Bioprocess Engineering I (6 CP)
- Biostatistics and Experimental Design (6 CP)
- Entomology I (6 CP)
- Entomology II (6 CP)
- Food Technology (6 CP)
- Integrated Pest Management (6 CP)
- Natural Product Discovery Platforms (6 CP)
- Natural Product Chemistry (6 CP)

Profile Modules

- Students tailor their individual profile by selecting 8 modules from the entire profile module catalogue of the faculty. Selection of recommended English modules for this study program:
- Antibiotics: present, past, and future
- Bioinformatics
- Bioprocess Engineering II Advanced
- Insect Biotechnology
- Insects for food and feed production systems
- Instrumental, biochemical and trace analytical methods in food analysis
- Laboratory Course I
- Laboratory Course II
- Method development in food analysis and food biotechnology
- Milestones of Insect Biotechnology & Bioresources
- Molecular Techniques
- Pharmaceutical Basics
- Selected Chapters of Pharmaceutical & Industrial Biotechnology
- Trends and Advances in Natural Product Research
- Quality Management

https://www.uni-giessen.de/stu</mark>dy/courses/master/ibb?set_language=en



Exzellente Forschung für Hessens Zukunft LOEWE Zentrum für Insektenbiotechnologie & Bioressourcen







Thank you for your attention

raunhofer