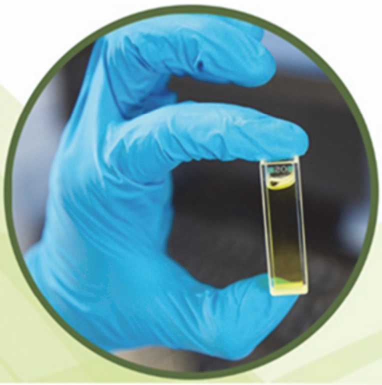
Biowaste derived volatile fatty acid platform for biopolymers, bioactive compounds and chemical building blocks

Towards a Circular Bioeconomy

VOLATILE FATTY ACID PLATFORM FOR BIOWASTE RECYCLING



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Notice

This e-book was prepared in the context of the EU funded project VOLATILE in accordance with the grant agreement No 720777 (European Union's Horizon 2020 research and innovation programme).

It must be stressed that the statements and views expressed in the chapters are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission.

No responsibility is accepted for the accuracy of information contained in the published chapters and the authors do not accept any liability for any direct or indirect damage resulting from the use of this e-book, its content or parts of it. The results achieved, conclusions made, and recommendations given by the authors should not be interpreted as a political or legal signal that the European Commission or any other political or legal institution intends to take a given action.

e-book (pdf) ISBN 978-84-88734-12-9

CONTENT

CONTRIBUTORS…………………………………………………………………...……..…4

INTRODUCTION………………………………………………………………..………..5

VOLATILE –TOWARDS BIOWASTE UPCYCLING……………………….…….……….7

FEEDSTOCKS FOR 3rd GENERATION BIOREFINERY……………………...………19

BIOECONOMY – STIMULI AND BARRIERS FROM THE MARKET AND LEGAL PERSPECTIVE……………………………………………………….….………25

DEVELOPMENT OF A VFA PLATFORM…………………………….…..………44

PRODUCTION OF BIOPOLYESTERS FROM WASTE…………………………..………55

CULTIVATION OF OLEAGINOUS YEASTS FOR SINGLE CELL OIL PRODUCTION……………………………………………………………….………63

MICROBIAL PRODUCTION OF ω-3 FATTY ACIDS: THE ROLE OF MICROALGAE IN THE VOLATILE PROJECT…………………………….……….70

EN ROUTE TO STANDARDISATION – THE CEN WORKSHOP AGREEMENT ‘evaVolatile’………………………………………………….....………77

ENVIRONMENTAL IMPACT OF BIOECONOMY (Life Cycle Assessment) - VOLATILE CASE STUDY……………………………………………………….………81

A MULTILEVEL APPROACH IN ASSESSING CIRCULAR GREEN TECHNOLOGY ADOPTION: Deriving plausible business case scenarios through business modelling and agent-based modelling……………………………….....………89

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

CONTRIBUTORS

Jochen Michels - Tijs Mocking - Ido Sellis - Francis Meerburg - Csaba Bauer - Celia Pais - Peter Magielse - Vasco Ferreira - Elisabeth Gonzalez - Marina Stramarkou - Ana Carolas - Alexandru Rusu - Sebastian Hiessl - Thomas Dietrich - Filip Velghe - Bianca Pop - Carlota Peral - Fabian De Wilde - Gerben Spit - Bruno Sommer Ferreira - Adelheid Wiedemann - Markus Neureiter - Marie-Aline Pierrard - Lukas Burgstaller - Paula Sampaio - Laura Oliver – Evangelos Topakas - Angelina Chalima - Laura Fernandez de Castro - Leire San Vicente - Vasiliki Oikonomopoulou - Christos Boukouvalas - Konstantina Laina - Siavash Farahbakhsh - Amaia Santamaria - Javier San José - Jef Van Meensel - Stien Snellinx - Edward Belderbos - Anouk Mertens - Maria Carmen Villaran

We would like to thank all members of the VOLATILE team for their hard work to achieve all project objectives, also if not mentioned above.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

INTRODUCTION

Following the principle of a circular bioeconomy, by-products and biowaste from industry or human consumption must be considered as feedstock for industrial processes. However, currently most of these side and waste streams are used for low-value applications only, such as for energy generation in incineration facilities or anaerobic digestion plants (AD), as fodder in livestock industries or as fertilisers in agriculture.

Therefore, responding to the need to improve resource efficiency, economically viable links between biowaste generation and possible pathways for upcycling back into the industry must be identified and respective technologies must be developed to convert biowaste into higher added value products.

In this context, the main objective of the EU funded research project VOLATILE was to develop a biotechnological approach to convert different types of solid and sludgy biowaste (municipal/industrial) into precursors for other applications and processes. In the VOLATILE process, a mixture of different bacteria and microorganisms is using the biowaste as food and converting it into so called “volatile fatty acids” (VFA). The volatile fatty acids and the microorganisms are then separated using membrane filtration allowing only the volatile fatty acids to pass. These pure and cleaned volatile fatty acids, can then in turn be used as carbon source for different bioprocesses and can be converted into higher added-value bio-based products. Specialized bacteria can transform volatile fatty acids into PHA (Polyhydroxyalkanoates), a biodegradable polymer that can be transformed into bioplastics and used for packaging materials as alternative for fossil-based plastics. Some yeasts are able to convert the volatile fatty acids into single cell oil which can be used in oleochemical applications such as soaps. Finally, versatile heterotrophic microalgae turn volatile fatty acids into health-promoting Omega-3 fatty acids.

Beside the technical developments, the project assessed the current state-of-the-art related to biowaste valorisation to establish a benchmark where the innovative volatile fatty acid platform could be integrated in existing value chains. This was supported by sophisticated agent-based modelling of different test cases related to wastewater and municipal biowaste treatment as well as comprehensive assessment and analysis of legislation at national or European level to identify hurdles and bottlenecks for the integration of the VOLATILE approach.

As standardised rules for biowaste upcycling will strongly support the exploitation of VOLATILE results, the partners initiated a CEN workshop which could be successfully finalized with a CEN workshop agreement providing support for interested stakeholders to assess their test case.

Also, circular bioeconomy approaches favour regional implementation as biomass dependent, Europe-wide solutions are needed. The different aspects of the VOLATILE value chain were at the forefront of the selection of the 21 project partners, from nine European countries. This cooperation was only possible due to the funding provided by the European Horizon 2020 research programme. Therefore, the partners of the VOLATILE project would like to thank for the support received by the programme.

Figure: VOLATILE Consortium at wastewater treatment plant

Thomas Dietrich

Coordinator VOLATILE

Filip Velghe

Project Technical Manager

Bianca Pop

VOLATILE Innovation Manager

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

VOLATILE –TOWARDS BIOWASTE UPCYCLING

T. Dietrich [a], F. Velghe [b], B. Pop [c], M. C. Villaran [a]

[a] *TECNALIA, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Álava, Leonardo Da Vinci 1, 01510 Miñano, Álava, Spain*

[b] *OWS NV, Dok Noord 5, 9000 Gent, Belgium*

[c] *TRITECC SRL, STR COSMINULUI 8, 400642 Cluj Napoca, Romania*

Corresponding author: thomas.dietrich@tecnalia.com

Abstract

Resources in general are not infinitely available, and also renewable resources if consumed outside their normal replacement cycles become scarce. Therefore, the establishment of a circular bioeconomy must respect natural systems and replacement cycles of organic carbon thereby reducing environmental pressure of human consumption. Upcycling of side and biowaste streams towards added value compounds represents hereby a critical aspect reducing land system change and fertilizer use for biomass supply for the bioeconomy. The development of a Volatile Fatty Acids Platform (VFAP) represents an important cornerstone for the upcycling of heterogenous municipal biowaste streams.

Keywords: Volatile Fatty Acids, Circular Bioeconomy, Municipal biowaste

1. INTRODUCTION

Current economic behaviour follows a linear approach from taking a resource, making a product, using and disposing it. Thereby one could assume that resources are infinite available and not considering medium- or long-term environmental impacts or sustainability criteria beyond economic performance. However, all resources are finite, non-renewable petrochemical feedstocks as well as renewable biomass resources if used outside of their biological limits. Furthermore, environmental burden should not be outsourced to following generations – to our children and grandchildren. Therefore, resource efficiency, considering natural balances and replacement cycles, are crucial to establish sustainable societies reaching ecological and economic stability. Already in 1972, the Club of Rome established that resources are limited, and a transition of the economy from growth (linear) to global equilibrium (circular economy) is needed keeping in mind the basic material needs of each person to ensure equal opportunities of each individual to develop its full human potential (Meadows, et al. 1972). However, it needed three decades more before this was taken more seriously and was more broadly accepted by policymakers leading for example to the implementation of a strategy for life sciences and biotechnology (COM(2002) 27 final), a biomass action plan (COM(2005) 628 final, 2005) the thematic priority in FP7 (2007-2013) on Food, agriculture and biotechnology to strengthen the knowledge base and to deliver the innovations and provide policy support for building and developing a European Knowledge Based Bio-Economy (KBBE) as well as the European Green Deal (COM(2019) 640 final).

Despite these efforts, sustainable use of biomass could be substantially enhanced if also biowaste would be properly upcycled beyond energy and compost. Landfill directive 1999/31/EC aimed mainly at the reduction of emissions at landfill sites avoiding decomposing of biowaste. Also, the directive does not prescribe specific treatment options, biogas and compost production are preferred in the context of proper biowaste management, forgetting that

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

agricultural biomass production requires land use, fertilisers and energy. According to the agriculture, forestry and fishery statistics, European farms used in 2016 around 39% of the total European Union land area for agricultural production (EC, 2018). However, agricultural expansion and intensification lead to land-system change, thereby contributing to global environmental changes. This undermines long-term human well-being and sustainability. Therefore, Rockström et al (2009) propose as planetary boundary that no more than 15% of the global ice-free land surface should be converted into cropland. Furthermore, Ahmed et al (2020) calculate that over a 20-year time frame, agriculture accounts for around 20% of global greenhouse gas emissions. Especially the use of fertilisers produces 80% of global nitrous oxide (N_2O) emission, a 264 times more powerful greenhouse gas than carbon dioxide (Ahmed et al, 2020).

Therefore biomass / biowaste management must be improved significantly with the aim to keep biomass in the economy following a circular approach. The main hurdle on current developments is the focus on homogenous biomass conversion. Heterogenous biomass streams are mainly used for bioenergy and compost production which should be only the last option if no other strategy is feasible under sustainability aspects (environmental, social, economic). Therefore, new and innovative conversion options for heterogenous biomass / biowaste sources are needed to be transformed into added value compounds under circularity aspects.

2. VOLATILE strategy

Anaerobic digestion is an established and proven technology for the treatment of different kind of solid (heterogenous) biowaste from multiple sources and consists of several consecutive steps, breaking down complex organic matter into biogas (methane).

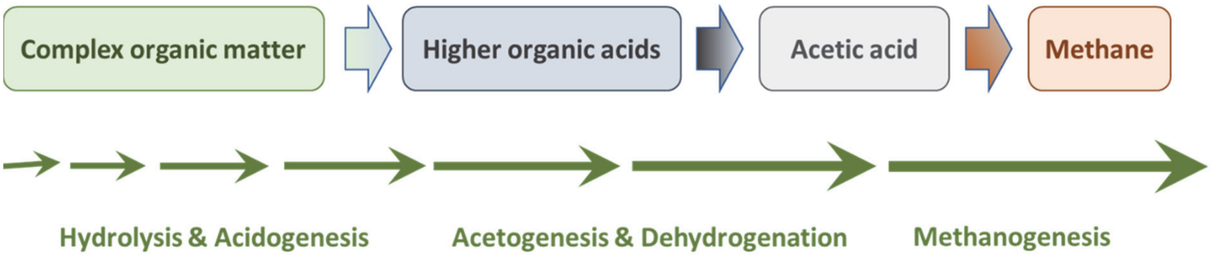


Figure 1: Stages of anaerobic digestion
(modified from McCarty, 1982; Miyamoto, 1997; Bauer et al, 2018)

Based on anaerobic digestion, the European funded H2020 research project VOLATILE (grant agreement Nº 720777) aimed successfully at the re-direction of anaerobic digestion towards the recovery of volatile fatty acids, thereby implementing a Volatile Fatty Acid Platform technology. The approach was successfully tested with different kind of municipal biowaste streams from Belgium, Greece, the Netherlands, Portugal and Spain.

As transport of biomass/biowaste from distant places affects significantly the economic viability of biomass valorisation facilities, regional circular bioeconomy clusters are favoured for the transformation into added value compounds (Dietrich et al, 2016). Therefore, the implementation of circular bioeconomy clusters will help to create new local jobs and income opportunities for municipalities.

Figure 2 is showing a scheme of the biowaste up-cycling perspective using different technologies. Landfilling must be avoided as the biowaste will produce greenhouse gases and will negatively affect the environment. Composting is a controlled biological decomposition of organic matter / biowaste under aerobic conditions. Easily decomposable carbon sources such as sugars and starch are used by microorganisms as an energy source, transformed into carbon dioxide and released into the environment. This carbon is lost and cannot be upcycled. Compounds resistant to degradation represent the major part of final compost/fertiliser. A preliminary anaerobic step recovers the easy degradable carbon in the form of methane. The methane can be burned to obtain bioenergy and the carbon is again released as carbon dioxide and thereby lost. The resistant compounds to AD can be recovered as fertiliser via composting of AD sludge. However, the most appropriate way to resource/carbon efficiency would be the direct up-cycling of the carbon present in the biowaste. Therefore, the VFAP technology offers the opportunity to recover the carbon in the form of volatile fatty acids which can be used for diverse application. The remaining and less degradable material can be still be treated via anaerobic digestion or composting, increasing thereby the resource efficiency of the VFAP.

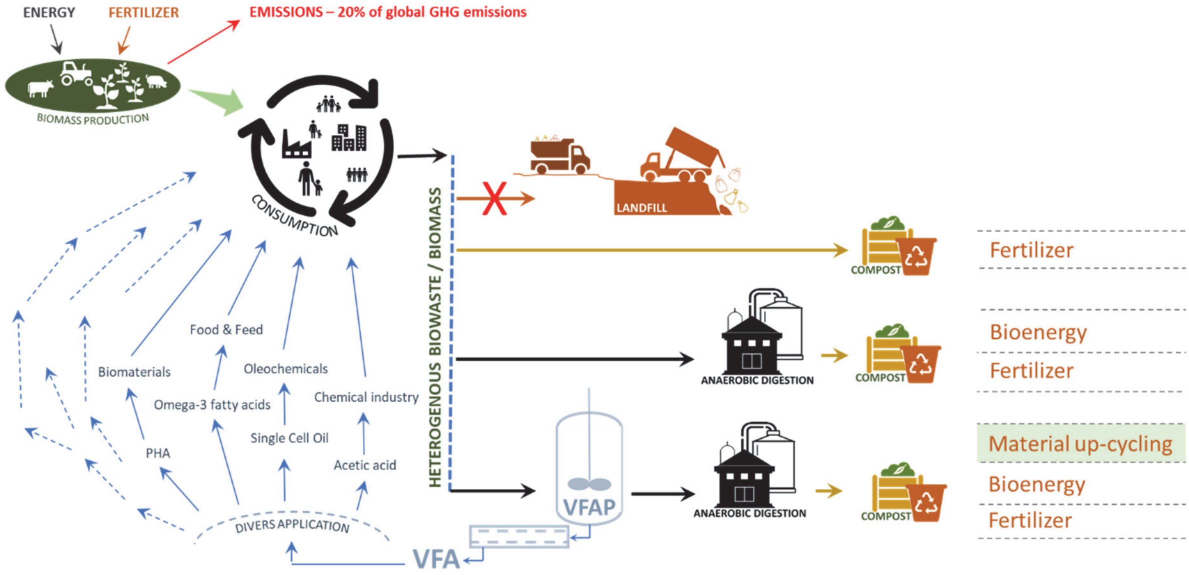


Figure 2: Landfilling, Composting Biogas, VFAP

In order to prove the application potential of VFA's, the project VOLATILE implemented the following strategy to convert municipal solid and sludgy biowaste into added value compounds. The first step was related to the establishment of the volatile fatty acid platform technology, validated in lab and industrial relevant environment. The platform provided volatile fatty acids to be used as carbon source for added value fermentation approaches. The project successfully demonstrated the potential use of volatile fatty acids (VFA) to produce single cell oil (SCO) for oleochemical applications, the transformation of VFA into polyhydroxyalkanoates (PHA) for material use as well as the conversion of VFA into long-chain polyunsaturated Omega-3 fatty acids.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

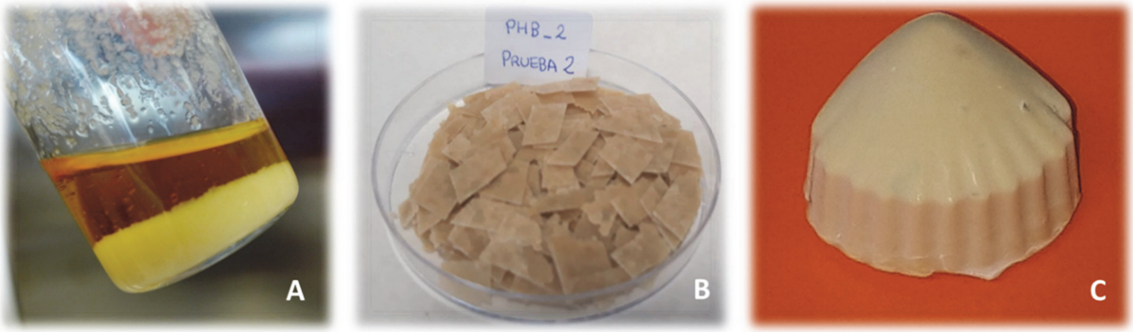


Figure 3: Municipal biowaste derived added value compounds produced in VOLATILE
A: VFA derived Omega-3 rich algae oil, B: VFA derived PHA films, C: SCO derived soap

Beside optimisation and up-scaling of fermentation protocols using VFAs as carbon source, down-stream processing and application development was successfully implemented. The technical developments were accompanied by sophisticated agent-based modelling to assess aspects such as stakeholder behaviour, separate biowaste collection and attitude towards VFAP as treatment option. The developed agent-based model was integrated into a web-based decision support tool accessible via the project website. The tool allows interested stakeholders to analyse the potential of a VFAP at a specific test case.

Furthermore, to facilitate later VFAP implementation and to support standardisation in the area of sustainable biomass / biowaste use, the project initiated a CEN workshop to develop a procedure for evaluating if the use of a Volatile Fatty Acid Platform technology for a given type of biowaste at a given location is economically and ecologically reasonable (EvaVOLATILE). The workshop could be successfully finalized with the CEN Workshop Agreement (CWA 17484: 2020). The CWA is openly accessible via the CEN/CENELEC CWA download area (https://www.cencenelec.eu/research/cwa/pages/default.aspx).

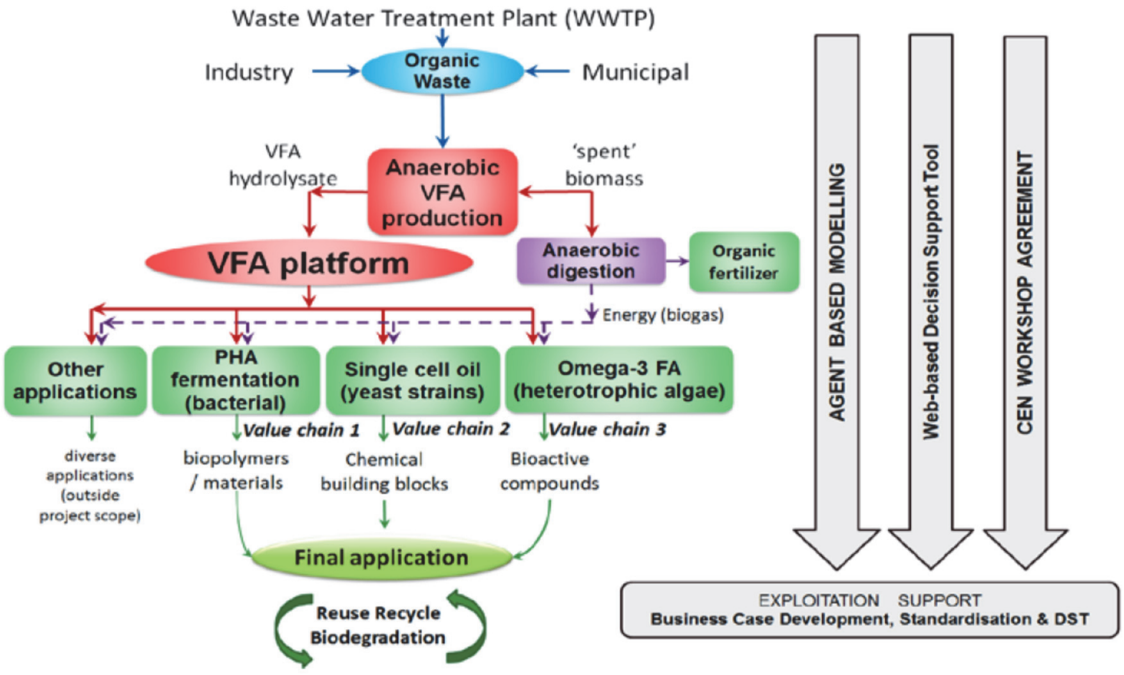


Figure 4: General VOLATILE approach

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

As legal aspects strongly affect the implementation of biowaste treatment / transformation technology, the project assessed EU related legislative barriers and stimuli to VFA based value chains (VOLATILE – D2.2, 2017) and prepared an outlook on future legislation which could affect biowaste derived volatile fatty acid value chains (VOLATILE – D2.6, 2020).

Finally, based on the VOLATILE developments, policy recommendations towards sustainable bioconversion of municipal solid and sludgy biowaste were prepared (VOLATILE – D 7.9, 2020).

3. VALUE CHAIN & VOLATILE CONSORTIUM

3.1 Municipal biowaste & VFAP

The implementation of the innovative and multidisciplinary VOLATILE concept to transform biowaste into a feedstock for other industries required the cooperation of different partners with complementary expertise in various fields of research and technology. Based on a first assessment in VOLATILE, it was calculated that more than 100 Mio tonnes of different type of solid and sludgy biowaste is available in the EU to be transformed / upcycled via the VFAP (VOLATILE – D1.1, 2017). Therefore, the participation of waste treatment facilities combined with a technology provider for anaerobic digestion were crucial to assess the potential of different type of municipal biowaste streams and to implement and validate the volatile fatty acid platform (VFAP). Table 1 summarizes the VOLATILE partners actively involved in biowaste treatment, anaerobic digestion or as technology provider.

Table 1: Biowaste Treatment partners & Technology provider

Biowaste Treatment	**Type of Biowaste**	**Technology provider**	
STAMOU (Greece)	Sludgy waste food production	OWS (Belgium)	Anaerobic digestion (AD) Volatile Fatty Acid Platform (VFAP)
AQUAFIN (Belgium)	WWTP sludge		
TWENCE (Netherlands)	Municipal solid biowaste		
FERROVIAL (Spain)	Municipal solid biowaste		
IGEAN (Belgium)	Municipal solid biowaste		
RESIDEL (Portugal)	Municipal solid biowaste		
AQUASERV (Romania)	WWTP sludge		
AIVE / IDELUX (Belgium)	WWTP sludge, Municipal solid biowaste		

The validation of the VFAP in the relevant environment offers the opportunity to provide new carbon sources for fermentation in form of volatile fatty acids. Current heterotrophic fermentation approaches for added value compounds rely mainly on agricultural products such as sugar, starch or vegetable oil as carbon source. Therefore, sustainability aspects for fermentative produced products such as bioethanol or biobased polymers must be carefully assessed in terms of agricultural land use for carbon source production as it represents a critical aspect in terms of planetary boundaries as established by Rockström et al (2009). Currently around 12% of ice-free land is used as cropland and should not exceed more than 15%. Therefore, further expansion of agricultural land must be critically assessed to avoid negative

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

impacts on regional or on world level. Taking into account that in 2016 around 39% of the total European Union land area was used for agricultural production (EC, 2018) and that the EU depends strongly on agricultural imports (Fuchs et al, 2020) it is clear that new innovative circular approaches are needed to reduce agricultural land use for the production of biobased compounds. Environmental burden should not be outsourced on global scale or to next generations.

Therefore, indigenous sources such as biowaste derived VFA could be used to establish a sustainable circular bioeconomy taking also into account the “finite” nature of renewable resources favouring up-cycling of biowaste derived carbon into added value compounds.

3.2 Omega-3 Fatty Acids

One example for finite renewable resources are fishery products. Overfishing brought several marine species near to collapse. Therefore, current production volumes cannot be increased. In order to feed the growing world population, aquaculture is considered as alternative. Nevertheless, fish aquaculture strongly depends on marine feedstocks coming from capture fishery converted in fish meal and fish oil. These feedstocks are needed for feedstuff production to produce aquaculture fish products with similar nutritional characteristics as fish from capture fishery. As fish meal and fish oil production are directly linked to capture fishery, the production volumes remained more or less constant in the last 30 years as shown in figure 5 (A), resulting to an enormous price increase due to rising demand by aquaculture industry (figure 5, B).

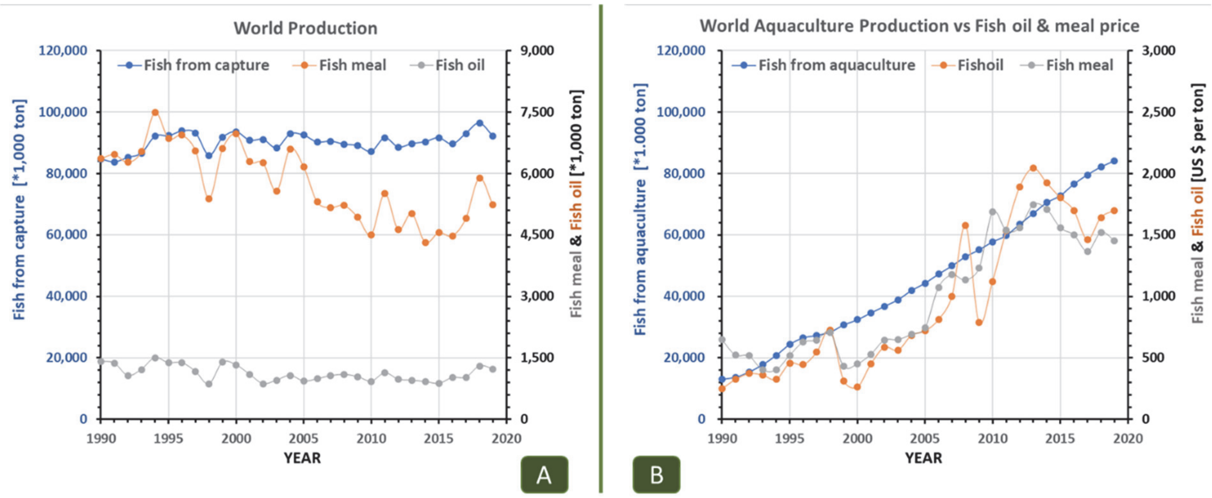


Figure 5: World production of fish and World prices for fish meal and fish oil
A: World production of fish from capture versus fish meal and fish oil production
B: World production of fish from aquaculture versus fish meal and fish oil prices
Dataset: OECD-FAO Agricultural Outlook 2020-2029

This demand led to the replacement of fish oil with alternative terrestrial sources thereby affecting the nutritional value of the resulting fish products. Sprague et al (2016) could show that health promoting Omega-3 fatty acids in Scottish aquaculture salmon reduced significantly from 2006 to 2015 requiring double portion sizes, as compared to 2006, in order to satisfy recommended EPA and DHA intake levels.

However, Omega-3 fatty acids are essential compounds in human nutrition. Stark et al. (2016) concluded that blood levels of EPA+DHA vary across the globe, with most countries having levels that are considered low to very low. These low Omega-3 blood levels are associated with an increased risk in cardiovascular related mortality. In 2019, Tocher et al, reported that a significant gap between supply and demand of Omega-3 fatty acids exists affecting health and well-being of growing world population. Therefore, new Omega-3 sources are needed to supply the growing demand. Production with microalgae, the primary producers of Omega-3, represents an interesting alternative. Especially heterotrophic production must be considered due to higher volumetric yield and not requiring huge land area for photobioreactors. However, current production strategies use primary agricultural products contributing to the before mentioned negative environmental impacts. Volatile fatty acids are considered as a promising carbon source for the production of long chain Omega-3 fatty acids with heterotrophic microalgae (Chalima et al, 2017, Chalima et al, 2019) thereby closing the carbon cycle from primary food consumption to heterogenous bio-residues to health promoting food and feed additives. The VOLATILE project successfully transformed municipal biowaste into Omega-3 rich microalgae oil (figure 6).

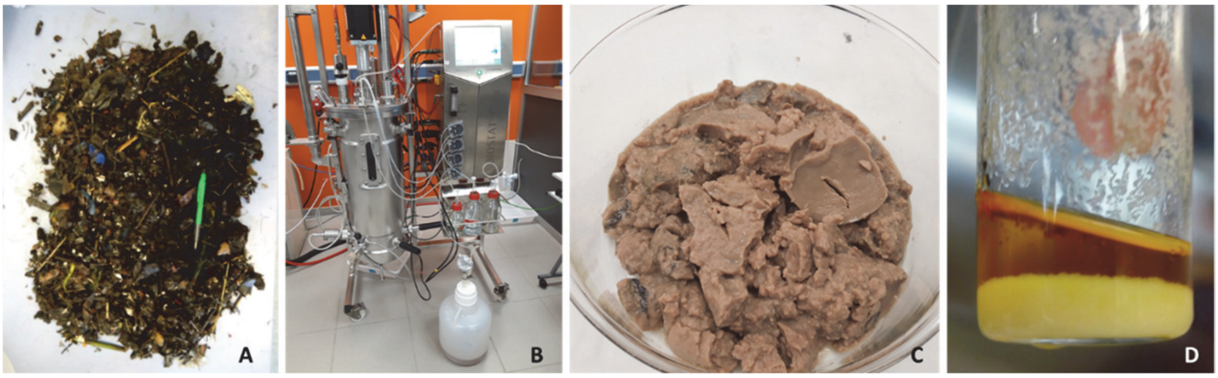


Figure 6: Transformation of VGF waste into Omega-3 rich microalgae oil
A: VGF waste, **B:** Microalgae fermentation using VFA rich effluents of VFAP as carbon source
C: Microalgae biomass, **D:** Microalgae oil extraction

3.3 *Polyhydroxyalkanoates*

Almost in all sectors of our life, plastics have been playing an increasingly important role in the last decades. In 2019, the European plastic industry provided more than 1.56 million direct jobs and produced around 57.9 million tonnes of plastics (PlasticsEurope, 2020). Taking into account that the majority of plastics used today are derived from non-renewable petrochemical base materials and are not biodegradable shows the urgent need for the supply of new innovative bioplastics. Polyhydroxyalkanoates (PHA) are biologically synthetized polyesters (biobased) that are fully biodegradable (VOLATILE – D4.6, 2020). However, at present food grade agricultural products such as refined sugars are used as carbon source for PHA production accounting for as much as 40-50% of total production costs and contributing to negative effects and emission of agriculture due to land use and fertiliser application.

In 2020, biobased plastics accounted for around 1.74 Mio tonnes globally requiring 0.7 Mio ha of land (European Bioplastics Association). However, biobased plastics are intended to take

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

over market shares from fossil-based plastics. In 2019, global plastic production volume was estimated at 368 Mio tonnes. The increasing share of biobased plastics on total plastic market will most likely increase also pressure on agricultural production systems and competition between food production, biofuels and biomaterials. Considering that growing world population will require also more food production, the use of agricultural area must be carefully assessed. Rockström et al (2009) established as planetary boundary for land use that not more than 15% of planetary ice-free land should be used as cropland. In 2017, around 12% of global total land was already used for permanent crops and arable land (UN data). The land system boundary was further developed by Steffen et al. (2015) defining that not more than 75% of originally forested land cover should undergo land-system change. Unfortunately, this boundary reached already the zone of uncertainty with 62% and further deforestation should be avoided. Furthermore, the perturbation of the nitrogen and phosphorous cycle mainly arising from fertiliser application in agriculture is already beyond the zone of uncertainty and represents a high risk for safe operation mode of our planet (Steffen et al., 2015).

Therefore, circular approaches are favoured to produce biobased materials, reducing thereby negative effects of agricultural production (fertiliser use, land-system change). Bioplastic production relying on side and waste streams as feedstock must therefore be favoured. Up-cycling of for example municipal biowaste towards bioplastics offers a huge potential. The implementation of the Volatile Fatty Acids Platform will transform municipal biowaste into a resource. The VOLATILE project successfully developed an innovative fermentation approach, transforming biowaste derived VFA into biodegradable biopolymer PHA (VOLATILE – D4.6, 2020). The exploitation of the VOLATILE concept will affect positively social aspects by creating regional bioeconomy clusters (employment), environmental protection by decoupling agriculture from biomaterial production as well as economic performance by creating new income opportunities for municipalities.

3.4 Single Cell Oil

Oleochemical industry requires a constant supply of agricultural feedstocks such as vegetable oil to produce many versatile products for our daily life. This led to a significant increase of agricultural area used for oil crop production. In 1990 around 185 Mio ha were used, reaching nearly 325 Mio ha in 2019. This is mainly related to increased food use but also to competition for biofuels and other applications. Food use increased its amount 7 times from 1990 to 2020 whereas 45 times more vegetable oil is used for biofuels or other uses. In 1990 more than 92% of vegetable oil was used for food applications which was reduced to around 65% in 2020 (OECD-FAO Agricultural Outlook).

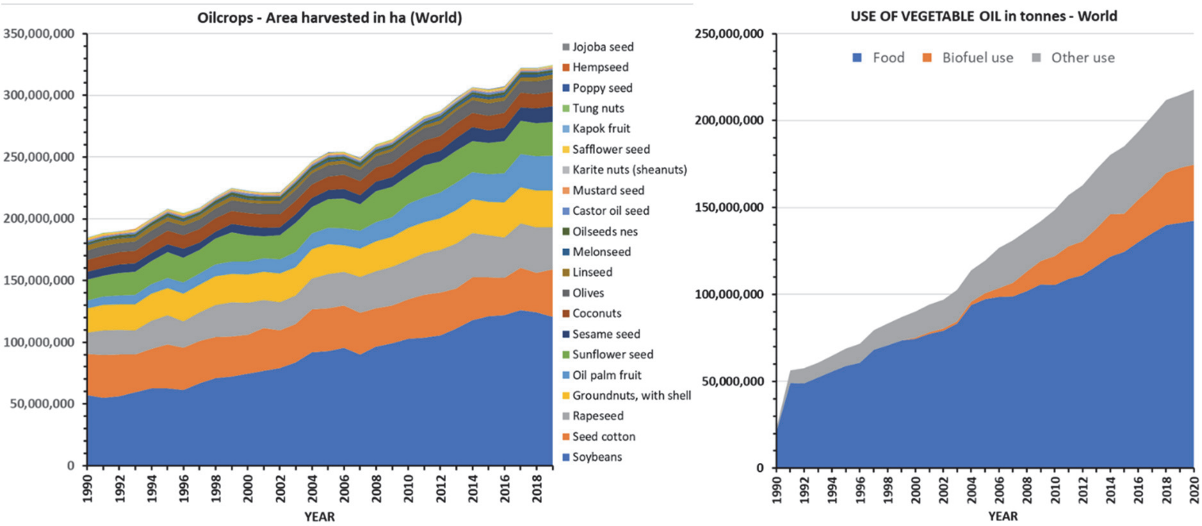


Figure 7: Oil crops harvested area over time (left) – Use of Vegetable oil (right)
Dataset: FAOSTAT – Oil crops harvested area
Dataset: OECD-FAO Agricultural Outlook 2020-2029 on vegetable oil

As further increase of agricultural area is not an option due to negative effects of agricultural emissions and land-system change to feed the industry with oleochemical feedstocks, new supply chains must be established using indigenous resources with low use of agricultural area in order not to compete with the food chain and to improve environmental sustainability. Single cell oil from oleaginous species (e.g. yeasts) offer an interesting alternative as it is known that they can grow on dark fermentation effluents such as volatile fatty acids as carbon source. During the VOLATILE project, the partners started with an extensive screening of potential oleaginous yeast strains (Miranda et al., 2020). Afterwards fermentation strategies were implemented (Bettencourt et al., 2020) and single cell oil obtained. The application potential in for example soaps could be successfully proven.

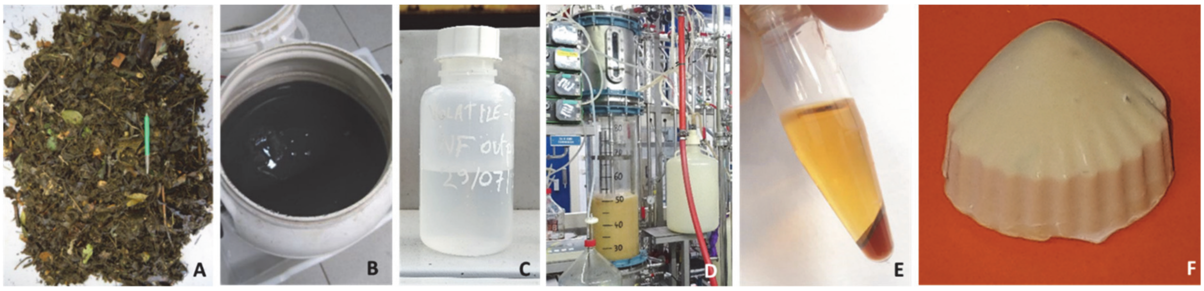


Figure 8: Transformation of VGF waste into SCO and soap
A: VGF waste, **B:** VFA rich effluent of VFAP, **C:** Cleaned VFA effluent, **D:** Yeast fermentation using VFA rich effluents as carbon source, **E:** SCO extraction from yeast biomass, **E:** biowaste derived soap

4. CONCLUSIONS

Circular economy concepts are aiming at the re-definition of growth beyond economic performance. One key aspect is the decoupling of economic activity from the consumption of finite resources by closing cycles between production and consumption. Side and waste stream must be upcycled and fed back in the economy in order to reduce environmental pressure.

The H2020 funded research project VOLATILE successfully showed that municipal sludgy and solid biowaste streams can be transformed into feedstocks (VFA) for different industrial sectors such as oleochemicals, biomaterials or food and feed ingredients.

Therefore, the project contributes to the establishment of a circular bioeconomy, designing out waste and keeping organic carbon in use. The implementation of the VOLATILE concept at industrial level will help to regenerate also natural systems as it reduces the pressure for primary agricultural raw materials and agricultural area.

5. REFERENCES

Ahmed J., Almeida E., Aminetzah D., Denis N., Henderson K., Katz J., Kitchel H., Mannion P. (2020). Agriculture and climate change – Reducing emissions through improved farming practices. McKinsey & Company, April 2020.

Bauer, C.L., Velghe, F., Pop, B., Peral, C., Dietrich, T. (2018). Utilisation of Volatile Fatty Acids resulting from anaerobic fermentation of the municipal WWTP sludge for the production of high added value compounds. Conference Proceedings of DANUBE – Eastern Europe Regional Water Forum – Scientific and Technical Conference on Efficient Use and Management of Water 2018, 198-210.

Bettencourt, S., Miranda, C., Pozdniakova, T.A., Sampaio, P., Franco-Duarte, R., Pais, C. (2020). Single Cell Oil Production by Oleaginous Yeasts Grown in Synthetic and Waste-Derived Volatile Fatty Acids. Microorganisms, 8: 1809; doi:10.3390/microorganisms8111809.

COM(2002)27 final (2002). Communication from the Commission to the European Parliament, the Council, the Economic and Social Committee and the Committee of the Regions. Life sciences and biotechnology - A Strategy for Europe.

COM(2005) 628 final (2005). Commission of the European Communities. Communication from the Commission - Biomass action plan (07.12.2005).

COM(2019) 640 final (2019). Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions - The European Green Deal (11.12.2019).

CWA 17484: 2020 - Anaerobic digestion plants - Feasibility assessment methodology for integrating a Volatile Fatty Acid Platform Technology (VOLATILE)

Chalima, A., Oliver, L., Fernández de Castro, L., Karnaouri, A., Dietrich, T., Topakas, E. (2017). Review - Utilization of Volatile Fatty Acids from Microalgae for the Production of High Added Value Compounds. Fermentation, 3: 54; doi:10.3390/fermentation3040054

Chalima, A., Hatzidaki, A., Karnaouria, A., Topakas, E. (2019). Integration of a dark fermentation effluent in a microalgal-based biorefinery for the production of high-added value omega-3 fatty acids. Applied Energy 241 (2019) 130–138. https://doi.org/10.1016/j.apenergy.2019.03.058

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Dietrich, T., Villaran Velasco, M.C., Echeverría, P.J., Pop, B., Rusu, A. (2016). Crop and Plant Biomass as Valuable Material for BBB. Alternatives for Valorization of Green Wastes. IN: Biotransformation of Agricultural Waste and By-Products - The Food, Feed, Fibre, Fuel (4F) Economy, Eds.: Palmiro Poltronieri & Oscar Fernando D'Urso, ISBN: 978-0-12-803622-8. 1-19.

EC (2018). 1. Agriculture, forestry and fisheries in the EU. IN: Agriculture, forestry and fishery statistics. 2018 edition, 11-13.

Fuchs, R., Brown, C., Rounsevell, M. (2020). Europe's Green Deal offshores environmental damage to other nations. Nature, 586: 671-673.

McCarty, P.L. (1982) One hundred years of anaerobic treatment. In: Hughes PE, Stafford DA, Wheatley B.I., Baader W., Lettinga G., Nyns E.J., Verstraete, W., Wentworth RL (eds) Anaerobic digestion. Elsevier Biomedical Press BV, Amsterdam, 3–22.

Meadows, D.H., Meadows, D.L. Randers, J., Behrens, W.W. (1972). The Limits to Growth – A report for the Club of Rome´s project on the Predicament of Mankind. Universe Books – New York. ISBN 0-87663-165-0.

Miranda, C., Bettencourt, S., Pozdniakova, T., Pereira, J., Sampaio, P., Franco-Duarte, R., País, C. (2020). Modified high-throughput Nile red fluorescence assay for the rapid screening of oleaginous yeasts using acetic acid as carbon source. BMC Microbiology, 20: 60. https://doi.org/10.1186/s12866-020-01742-6

Miyamoto, K. (1997). Chapter 4 - Methane production. IN: Renewable biological systems for alternative sustainable energy production. FAO agricultural services bulletin – 128.

PlasticsEurope (2020). Plastics – the Facts 2020: An analysis of European plastics production, demand and waste data

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F. S., Lambin, E., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H., Nykvist, B., De Wit, C. A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P. K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R. W., Fabry, V. J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley, J. (2009). Planetary boundaries: exploring the safe operating space for humanity. Ecology and Society 14(2): 32.

Sprague, M., Dick, J.R., Tocher, D.R. (2016). Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006–2015. Scientific Reports, 6: 21892. DOI: 10.1038/srep21892

Stark, K.D., Van Elswyk, M.E., Higgins, M.R., Weatherford, C.A., Salem Jr., N. (2016). Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults, Progress in Lipid Research 63: 132–152.

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., de Vries, W., de Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sörlin, S. (2015). Planetary boundaries: Guiding human development on a changing planet. Sciencexpress, 1-17.

Tocher, D.R., Betancor, M.B., Sprague, M., Olsen, R.E., Napier, J.A. (2019). Review: Omega-3 Long-Chain Polyunsaturated Fatty Acids, EPA and DHA: Bridging the Gap between Supply and Demand. Nutrients, 11: 89; doi:10.3390/nu11010089

VOLATILE – D1.1 (2017), Deliverable D1.1 – Report on State-of-the-Art of bio waste valorisation in the test regions and beyond, May 2017

VOLATILE – D2.2 (2017). Deliverable D2.2 – Report on EU legislative barriers/stimuli to VFA based value chains, September 2017.

VOLATILE – D2.6 (2020). Deliverable D2.6 – Outlook on Future Legislation affecting VFA based Value chains, October 2020.

VOLATILE – D4.6 (2020), Deliverable D4.6 – Report on PHA based biomaterial biodegradation performance. November 2020.

VOLATILE – D7.9 (2020). Deliverable D7.9 – Policy recommendations towards sustainable bioconversion of municipal solid and sludgy biowaste, November 2020.

FEEDSTOCKS FOR 3rd GENERATION BIOREFINERY

I. Sellis [a]*; T. Mocking_* [a]*; F. Meerburg* [b]*; C. Bauer* [c]*; M.-A. Pierrard* [d]*; P. Magielse* [e]*; V. Ferreira* [f]*; E. Gonzalez* [g]*; M. Stramarkou* [h]
[a] *Twence BV, Boldershoekweg 51, Hengelo OV 7554 RT, Netherlands*
[b] *AQUAFIN N.V., Dijkstraat 8, 2630 Aartselaar, Belgium*
[c] *COMPANIA AQUASERV SA, Strada kos Karoly 1, 540297 Targu Mures, Romania*
[d] *IDELUX Environnement, Schoppach Dreve de l Arc en Ciel 98, 6700 Arlon, Belgium*
[e] *IGEAN Milieu en Veiligheid, Doornaardstraat 60, 2160 Wommelgem, Belgium*
[f] *PA Residel, Optimizacao Energetica de Residuos SA, Rua Calouste Gulbenkian 169 7 H1, 4050 145 Porto, Portugal,*
[g] *FERROVIAL Servicios SA, Calle Principe de Vergara 135, 28002 Madrid, Spain*
[h] *STAMOU, Leoforos Karamanli 206, 13678 Acharnai. Greece*
Corresponding author: *i.sellis@twence.nl*

Abstract

The project VOLATILE aimed at the development of an innovative Volatile Fatty Acid Platform for the bioconversion of municipal solid biowaste fraction and sludgy biowaste from other industries to be integrated in anaerobic digestion facilities. Key information needed to successfully exploit and implement the Volatile Fatty acid Platform is the availability of feedstocks for 3rd generation biorefineries. Therefore, the partners made an in-depth study on municipal solid biowaste and sludgy biowaste from wastewater treatment plants in the European Union.

Keywords: Municipal biowaste, Circular Bioeconomy, Volatile Fatty Acid Platform

1. INTRODUCTION

The successful implementation of circular biorefinery concepts relies on technologies able to transform versatile biomass sources into building blocks or added value compounds as well as on the availability of feedstocks. The amount and quality of biomass available defines the scale of possible operations as well as economic feasibility.

As feedstock and transport costs represent a large part of operating costs of a biorefinery, the availability of suitable feedstocks in a certain distance to the biomass transformation facility is crucial to implement economic viable strategies. The use of 1st generation feedstocks for biorefinery purposes such as starch or sugar cane may create conflicts with the use for food or feed applications. Therefore, biorefineries using 2nd generation feedstocks were developed relying on biomass feedstocks that are more widely available but that are not in direct competition with food uses. However, also the use of these kind of biomass has to be carefully assessed as land-system change to produce this kind of biomass may have negative effects on the environment. Therefore, 3rd generation biomass sources are attracting attention, including organic side and biowaste streams of our economy.

One interesting feedstock for the volatile fatty acid platform is represented by municipal solid and sludgy biowaste which can be treated by anaerobic digestion. In 2018, EU28 generated more than 251 Mio tonnes of municipal waste and around 98.4% were treated (figure 1) (Eurostat, 2021). Around 17.3% were recycled by composting and digestion representing around 42.8 Mio ton of biowaste.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

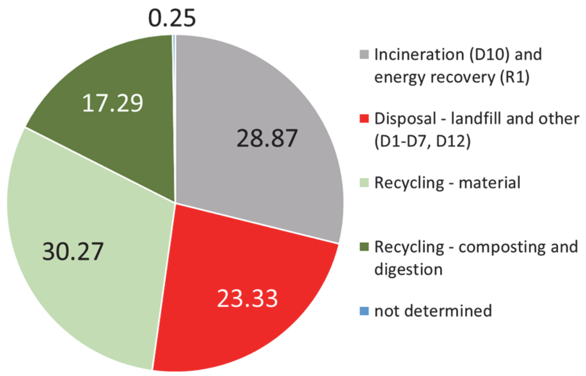


Figure 1: Municipal waste treatment in percent of treated waste

Despite all efforts to reduce this, the majority of EU countries still landfilled approximately 23% of their municipal waste in 2018 (Eurostat, 2020).

The European Environmental Agency considers that around 34% of municipal waste represents biowaste (EEA, 2020). This represents an abundant resource with an unexploited economic potential. The European Compost Network concludes that up to 50% of municipal solid waste is organic and should be considered as feedstock for the circular bioeconomy (Siebert et al, 2019). Based on these assumptions the following amount of municipal biowaste can be estimated:

	MSW generated in EU28	34% Biowaste (EEA, 2020)	50% Biowaste (ECN, 2019)
2018	251,879,000 tonnes	85,638,860 tonnes -	125,939,500 tonnes

In order to analyse the potential of the innovative volatile platform (feedstock supply) in more detail, the VOLATILE partners performed an in-depth desk study for organic waste amounts in the individual EU-28 countries and the EU in total. In the country reports the most important information on total production of organic waste fractions (such as OF-MSW, VGF waste or sludge) is presented. Furthermore, the collection methods and treatment techniques are discussed using a SWOT-analysis (VOLATILE – D1.1, 2017).

2. BIOWASTE POTENTIAL OF MUNICIPAL SOLID WASTE

Municipal solid waste consists of everyday items that are discarded by the public including kitchen and garden waste. In 2018, 491 kg per capita were discarded in EU28 (Eurostat). Considering that 34% of municipal solid waste represent biowaste, the VOLATILE partners assessed the biowaste potential of EU-28 for each country individually (VOLATILE – D1.1, 2017). Looking at the collected data (summary in table 1), there is a total available organic waste potential of approx. 97 million tonnes annually. Through different types of collecting systems three types of organic waste are registered; subdividing the total potential as follows:

• Vegetable and Green Fraction (VGF waste)	~ 17 million tonnes
• Green waste	~ 19 million tonnes
• Organic Fraction Municipal Solid Waste	~ 61 million tonnes

Currently there are six mature technologies/strategies on the market to treat this kind of municipal waste. These are landfilling, incineration, mechanical-biological treatment (MBT), industrial composting, home composting and anaerobic digestion.

Generally organic municipal waste is treated by incineration or MBT in the north/west of Europe. In the other regions of Europe, a large fraction of waste is still landfilled, which presents a major opportunity for improvement. As landfilling of biowaste has a high negative environmental impact, it is important to collect and treat municipal bio-waste separately. In landfills, biowaste produces the powerful greenhouse gas methane during decomposition and degradation.

The separately collected organic waste is generally composted or anaerobically digested. In countries with a well-developed collection system for separate food waste and garden waste, AD is the preferred option for treating food and kitchen waste, while composting is preferably used for garden and other green waste.

Table 1: Overview of country data of annual potential municipal biowaste (VOLATILE – D1.1, 2017)
(data are only given if available)

COUNTRIES	Separate collected Organics [kt]		OF waste [kt]	TOTAL [kt]
	VGF waste	Green waste		
Austria	508	428	253	1,189
Belgium	311	578	966	1,855
Bulgaria			775	775
Croatia	28	91	326	445
Cyprus	58		179	237
Czechia	141		891	1,032
Denmark	50		643	693
Estonia	17		96	113
Finland	341			341
France	4,900		12,700	17,600
Germany	4,569	5,095	5,000	14,664
Greece	209		1,937	2,146
Hungary	500		880	1,380
Ireland	194	25	122	341
Italy	3,900	2,100	9,000	15,000
Latvia	47		376	423
Lithuania	132		576	708
Luxembourg	70		134	204
Malta			128	128
Netherlands	1,346	1,962	1,300	4,608
Poland	1,750		5,940	7,690
Portugal			2,364	2,364
Romania			2,879	2,879
Slovakia	212			212
Slovenia	109		90	199
Spain	540	242	7,937	8,719
Sweden	729			729
UK	640	4,459	5,137	10,236
	36,281		**> 60,629**	**> 96,911**

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Furthermore, Siebert et al (2019) conclude that besides the huge amount of municipal biowaste, more than 40 million tons of commercial and industrial bio-waste is available with similar characteristics to be treated in anaerobic digestion plants and could be therefore be used as feedstock for a Volatile Fatty Acid Platform.

3. WASTEWATER TREATMENT SLUDGE

Besides the analysis of the municipal biowaste potential, the VOLATILE partners studied also the availability of wastewater treatment sludge as feedstock for 3rd generation biorefineries transforming biowaste into volatile fatty acids. Wastewater treatment is a complex process and includes four main process steps as can be seen in Figure 2.

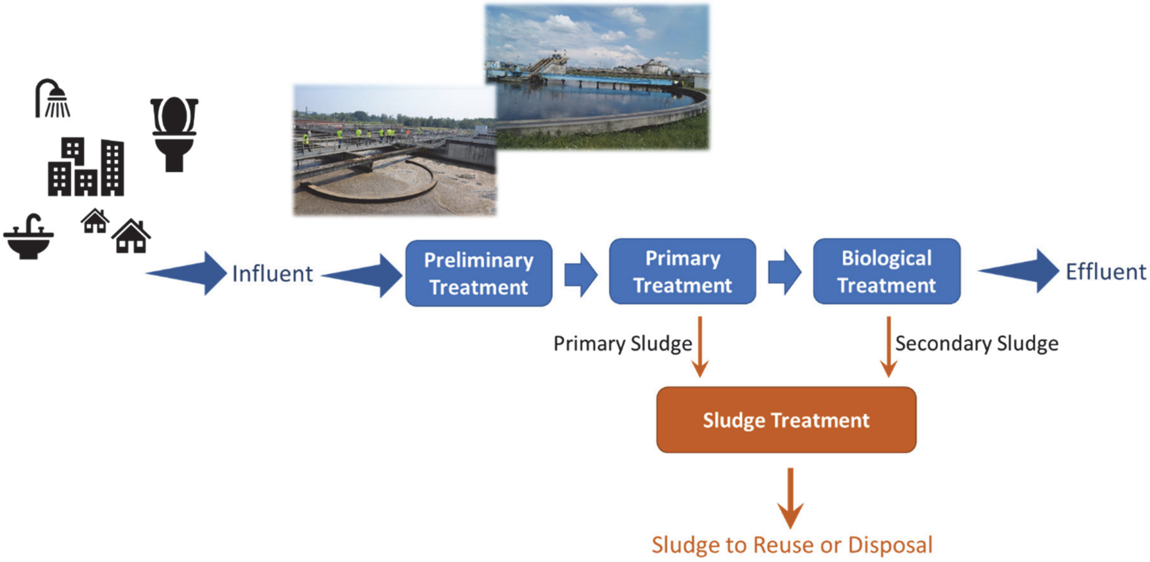


Figure 2: General scheme of wastewater treatment (Bauer et al, 2018).

During the primary treatment suspended solids in the raw wastewater are settled creating the primary sludge. Furthermore, the excess biomass from the biological treatment (activated sludge treatment) contributes as secondary sludge to the total sludge volume created during wastewater treatment.

During the test study performed in VOLATILE, EU-28 country analysis was also performed for sewage sludge. Looking at the collected data, there is a total of approximately 9 million tonnes of sewage sludge dry matter available per year. Given sufficient quality and biodegradability of the sludge biomass, this resource constitutes a second organic resource with an unexploited economic potential.

Table 2 Overview of the country reports collected data for sludge *data only given if available or applicable (VOLATILE – D1.1, 2017)

COUNTRIES	Sewage sludge [tonnes $_{\text{dry matter}}$]	Anaerobically digested *
Austria	235,000	N/A
Belgium	175,000	35%
Bulgaria	60,000	0%
Croatia	32,000	N/A
Cyprus	20,000	0%
Czechia	13,000	0%
Denmark	130,000	4%
Estonia	19,000	87%
Finland	141,000	0%
France	1,100,000	N/A
Germany	1,803,000	N/A
Greece	174,000	0%
Hungary	225,000	N/A
Ireland	65,000	0%
Italy	1,000,000	38%
Latvia	23,000	4%
Lithuania	45,000	7%
Luxembourg	8,000	0%
Malta	10,000	0%
Netherlands	317,000	45%
Poland	540,000	6%
Portugal	N/A	N/A
Romania	173,000	N/A
Slovakia	57,000	N/A
Slovenia	N/A	N/A
Spain	1,131,000	N/A
Sweden	270,000	70%
UK	1,500,000	66%
	9,266,000	

The deliverable D1.1 (VOLATILE – D1.1, 2017) provides further information on country level.

4. CONCLUSIONS

Based on the feedstock analysis it can be concluded that more than 100 Mio ton of municipal solid and sludgy biowaste is available to serve as raw material for the circular bioeconomy using a volatile fatty acid platform as transformation technology. Considering that in 2018 (EU28) only approximately 42.8 Mio ton were treated by composting or digestion, this illustrates an untapped potential to create new regional jobs in biowaste transformation by setting up new treatment routes and facilities. The European Compost Network concludes that between 2.2 (urban) to 7.2 (rural) jobs could be created by treating 10,000 ton of currently not exploited biowaste (ECN, 2016).

5. REFERENCES

Bauer, C.-L., Velghe, F., Pop, B., Peral, C. Dietrich, T. (2018). Utilisation of Volatile Fatty Acids resulting from anaerobic fermentation of the municipal WWTP sludge for the production of high added value compounds. Danube – Eastern Europe Regional Water Forum – International Conference – Efficient Use and Management of Water 2018. 198-210.

ECN (2016). FACTSHEET - BIOWASTE GENERATES JOBS https://www.compostnetwork.info/download/biowaste-generates-jobs/

EEA (2020). Bio-waste in Europe — turning challenges into opportunities. EEA Report No 04/2020

Eurostat (2021). Dataset: Municipal waste by waste management operations [ENV_WASMUN__custom_666228]. Data extracted on 10/03/2021.

Eurostat (2021). Dataset: Municipal waste by waste management operations [ENV_WASMUN$DEFAULTVIEW]. Data extracted on 17/03/2021.

Siebert, S., Gilbert, J., Ricci-Jürgensen, M. (2019). Compost Production in Europe. European Compost Network.

VOLATILE – D1.1 (2017), Deliverable D1.1 – Report on State-of-the-Art of biowaste valorisation in the test regions and beyond, May 2017

BIOECONOMY – STIMULI AND BARRIERS FROM THE MARKET AND LEGAL PERSPECTIVE

A. Wiedemann [a]; A. Rusu [b]; S. Hiessl [c]
[a] *WIEDEMANN GmbH, Rieblinger Strasse 25, 86479 Aichen, Germany*
[b] *BIOZOON GmbH, Fischkai 1, 27572 Bremerhaven, Germany*
[c] *DECHEMA Gesellschaft für Chemische Technik und Biotechnologie e.V., Theodor Heuss Allee 25, 60486 Frankfurt, Germany*
Corresponding author: wiedemann-gmbh@t-online.de

Abstract

This paper gives an overview on the important markets: food, feed, bio-based products and energy directly addressed by the EU Bioeconomy Strategy and considers the situation of biomass production and organic waste. Drivers and obstacles are pointed out with regard to growing markets and sustainability matters**.** It discusses core issues of the EU legislative frameworks in pursuing the route from waste to final applications and refers briefly to the historical and global development of the bioeconomy.

Keywords: resources, products, law

1. INTRODUCTION

Efforts to pave the way for a bioeconomy at the international scale were already made almost three decades ago. The bioeconomy and the replacement of fossil carbon resources in the context of the Agenda 21 which resulted from the World Earth Summit in Rio de Janeiro in 1992 and its update at the World Summit in Johannesburg in 2002 were pointed out by Kamm & Kamm (Kamm B. & Kamm M., (2004)). A great impact has been accomplished, and in the meantime, as shown in Figure 1, more than 50 countries distributed all over the Earth's continents are reported to deal with the bioeconomy or similar approaches (Bioökonomierat, 2020) (EC, 2020e).

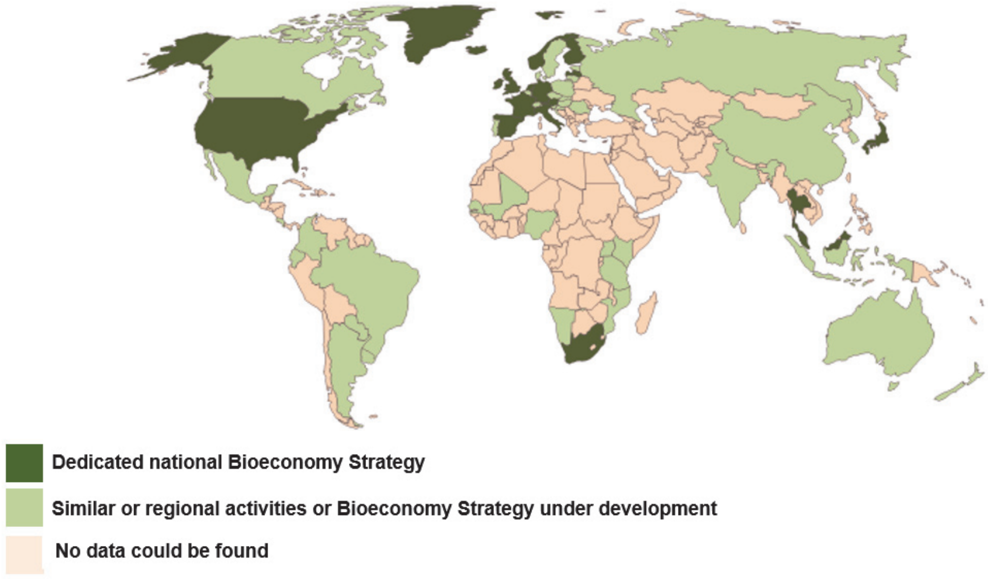


Figure 1: Bioeconomy activities from a global perspective
Source: European Commission (EC, 2020e) and Bioökonomierat (Bioökonomierat, 2020) (modified)
Map: www.freeworldmaps.net (modified)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

To foster the Bioeconomy has a long tradition in the EU, and EU Framework Programmes in Biotechnology and Life Sciences are established by the European Commission (EC) since 1982 (Patermann, C. & Aguilar, A., (2018)). A Life Sciences and Biotechnology Strategy was adopted in 2002 (EC, 2002), and the concept of the Knowledge-Based Bioeconomy (KBBE) was presented in 2007 (Patermann, C. & Aguilar, A., (2018)).

In 2012, a definition has been introduced for the bioeconomy in the EU and its markets: “the production of renewable biological resources and the conversion of these resources and waste streams into value added products, such as food, feed, bio-based products and bioenergy” by a first dedicated strategy at the EU level (EC, 2012). Concerning the spectrum of markets addressed by the update of the strategy at 2018, it is slightly modified and supplemented to “food, feed, bio-based products, energy and services (except biomedicines and health)”. On the input side, it is now explicitly referred to the term organic waste (EC, 2018a). According to the EC and the Green Deal (EC, 2019a), the bioeconomy is contemplated as a strategic economic orientation (Ronzon, et al., 2020).

Various approaches are documented to estimate and calculate the availability of biomass from a global perspective and concerning the EU (Scarlat, et al., 2015), (Camia, et al., 2018) (Ronzon, et al., 2015), (EC, 2019b), and in pursuing sustainability, waste resources gain more and more importance for the bioeconomy. In this respect, it is referred to key data of biowaste, as it is legally defined in the EU (EU, 2018a) and its role also in the context of municipal waste.

Prior to analysing the market sectors, the added value of the bioeconomy and ten of its most important sectors is depicted (Ronzon, et al., 2018). For this work, markets of food, feed, bio-based products and energy will be included. With regard to bio-based products, an emphasis is placed on chemicals, plastics, packaging and fertilisers while analysis on energy focuses on bioenergy.

Even though there are strong policy endeavours on deployment of the bioeconomy, and for example, an evolvement of a regulatory framework for bio-based and biodegradable plastics is announced by the Green Deal (EC, 2019a), there are manifold legal aspects which are either supportive or would need to be harmonised (VOLATILE D2.6, (2020)). In particular, novel value chains starting from organic waste to be transformed into substances, materials and products towards a circular bioeconomy are of specific interest from the legal perspective. Thus, they are highlighted in this paper.

2. METHODOLOGY

The methodology used to prepare this paper comprises a review of literature, including not only peer-reviewed documents but also “grey” literature, such as books and reports on projects and conferences. Furthermore, citations were directly made from legally binding and non-binding sources. This paper focuses on drivers and obstacles concerning the markets of the *bioeconomy* and those derived from policy and legislation. While section three reflects an approach on the

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 720777

availability of biomass, section four deals with specific *bioeconomy* markets, and section five is devoted to policy and legislation affecting the *bioeconomy*. Taking into account global, European and national activities towards a transition into a sustainable and circular bioeconomy, this work, which mainly addresses the European point of view, can be just a spotlight on boosting or hindering factors relating thereto. Considering the specific issues of material and energy use of a certain feedstock, conclusions are made in section six.

3. TOWARDS SUSTAINABLE CARBON SOURCES

Today, the resource fundament of the global economy is mainly built by crude oil, natural gas and coal to meet the demand of energy and carbon for industrial (Kircher M., 2019) and other sectors, such as private consumption. With regard to a reported global crude oil production of 98 million barrels in 2019 (EIA, 2020) and a current low price phase (Worldbank, 2020), in principle, there are no accelerators on the search for alternatives. Nonetheless, due to the significant environmental impact (Kircher M., 2019) and growing awareness on the finiteness of the "fossils", the pressure to prepare phasing-out scenarios increases. Consequently, light is shed on novel solutions, such as biological resources. To substitute the traditional feedstock with these resources, the availability of biomass is a key prerequisite. At the global level, Scarlat et al. (Scarlat, et al., 2015) reported around 14.98 billion tonnes. In the EU, the annual production is estimated on around 2 billion tonnes, (Ronzon, et al., 2015), (Scarlat, et al., 2015) and the following types and quantities of biomass were produced from 2012-2015 (Table 1) (EC, 2020b).

Table 1: Biomass production in the EU-28 by type from 2012 and 2015 (1000 tonnes)

Biomass type	2012	2013	2014	2015
Agri biomass – economic[1]	456 407	471 928	515 269	477 580
Agri biomass – residual [1]	394 859	436 856	452 697	434 728
Fisheries biomass [2]	1 350	1 459	1 527	948
Algae biomass[2]	79	143	n/a	n/a
Forest biomass[1]	n/a	511 544	n/a	n/a
Waste biomass[1]	91 435	n/a	n/a	n/a

1 dry matter 2 fresh matter

Source: Own depiction based on European Commission - Knowledge for Policy, Topic Biomass (EC, 2020b)

Agricultural biomass dominated the scene with around 850 to 950 million tonnes annually. An amount of about 91 million tonnes of waste biomass containing around 12% food waste, 25% green waste from gardens and parks, and almost 63% of biodegradable waste other than "green waste" is reported for 2012 (EC, 2020b).

Commonly, there is a clear distinction between biomass from agriculture and forestry and the legally defined term biowaste (EU, 2018a) while the latter does not cover the former (EUBIA, 2020). Regarding biowaste, the annual quantity generated in the EU is estimated to be in the area from 118 to 138 million tonnes (EC, 2010), and 83 kg biowaste were recycled per capita in 2017 (Eurostat, 2020). Municipal waste is composed of around 50% biowaste (ECN, 2019)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

and from around 249 million tonnes in 2017, just 17% were composted (Figure 6) (Eurostat, 2019). Contemplating the data, there will be a huge potential for further biowaste valorisation in the EU.

4. SEIZING AND ANALYSING MARKETS

Following the route from resources to food, feed, biobased products (chemicals, plastics, packaging, and fertilisers) and energy, data are analysed to define markets and their relevance for the bioeconomy.

4.1 Added value of the ten most important bioeconomy sectors in the EU

By including the estimations of the tertiary sector, a total added value of €1.5 trillion is published for the EU-27 (2020) (Fritsche, et al., 2020) while €614 billion have been achieved by the primary and secondary sector employing 17.5 million people in 2017. From ten bioeconomy sectors, the manufacturing of food, beverages and tobacco as the first, agriculture as the second and bio-based chemicals, pharmaceuticals and plastics belonging to the third, are identified as being the most relevant by representing about 75% of the total added value of the €614 billion (Figure 2) (Ronzon, et al., 2018), (EC, 2020f).

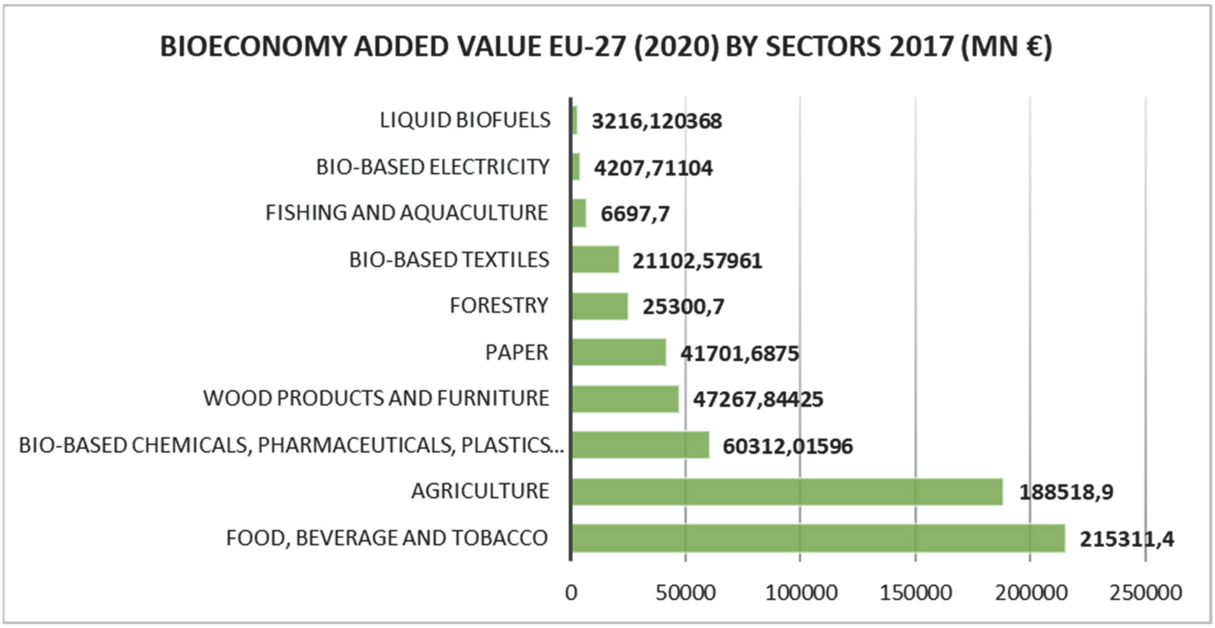


Figure 2: EU-27 (2020) - Bioeconomy added value by sectors 2017 (Mn €)
Source: European Commission (EC, 2020f) and Ronzon, et al. 2018 (Ronzon, et al., 2018) (modified)

4.2 Food and beverages – How to respond to a growing market?

The food and beverage sector is the biggest manufacturing sector in the EU not only in terms of €1.2 trillion turnover and added value (around €236 billion in 2019) (FoodDrink Europe, 2020) but also with its workforce encompassing 4.4 million people (Ronzon, et al., 2018) and 4.72 million in 2019 (FoodDrink Europe, 2020). For the upcoming years (2020-2025), a set of alterations covering, inter alia, exogenous and vertical factors has been detected by the ECSIP Consortium (EC, 2016), which will influence the perspectives of the bioeconomy. From the

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

factors affecting the food industry from outside, the growth of population at the international level will bring additional need, particularly outside of Europe. An increasing income in the developing countries can lead to demand on high quality products (EC, 2016) and more protein intake (Kircher M., 2019). Both could result in additional export (EC, 2016), which is estimated from €90 billion to €110 billion (EC, 2020c), (FoodDrink Europe, 2020) and could be further addressed by the Bioeconomy and its research. Costs for inputs, such as raw materials, affecting the supply chain (EC, 2016) can be seen as an important opportunity for bioeconomy research as well.

In addition, dietary food supplements containing substances, such as minerals, vitamins and poly-unsaturated fatty acids are of interest for the biotechnological processes based on waste resources. The dietary food supplements market which provides also options for Novel Food is valued on $140 billion (globally in 2018) (CISION, 2020) and around $15 billion in Europe in 2019 (Fortune, 2020).

4.3 Feed – the main biomass uses and how to address compound feed?

To generate a value of annually €130 billion from animal production, an amount of 450 million tonnes of feed is required by approximately 5 million farmers in the EU. In addition, ten million tonnes of feed are purchased by 70 million EU households for pet nutrition (EC, 2020d).

Feed and animal bedding take the lion's share (43%) in biomass consumption in the EU which was estimated on a total 1.2 billion tonnes in 2019 while around 1 billion was sourced from the primary sector (EC, 2019b).

The compound feed market has an estimated global value of $528 billion in 2020 (Market Data Forecast, 2020) and a production volume of around 164 million tonnes in the EU in 2019 (FEFAC, 2020a). Since using nutrients and additives, such as volatile fatty acids, this market is of specific interest for biotechnological approaches using secondary resources. Encouragements for the bioeconomy in the feed and feed compound areas are inter alia the Feed Sustainability Charter of the FEFAC (Federation of European Feed Manufacturer's Federation) (FEFAC, 2020b), dietary habits like proteins from meat and new products helping to improve feed conversion ratios (Global Market Insights, 2020).

4.4 Chemicals and the sustainability aspect

From a global sales of chemicals of €3,347 billion, the EU takes the second position achieving €565 billion after China (€1,198) in 2018, and with €158.3 billion in 2017, it takes the main part as regards exports to other areas in the world including intra-EU trade (CEFIC, 2020). Concerning bio-based chemicals, Spekreijse et al. (Spekreijse, et al., 2019) have conducted a study with details of the segment "chemicals" of the EU bio-based products sector. Ten chemical product categories and their applications which are reflected in Figure 3, are presented in the report.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

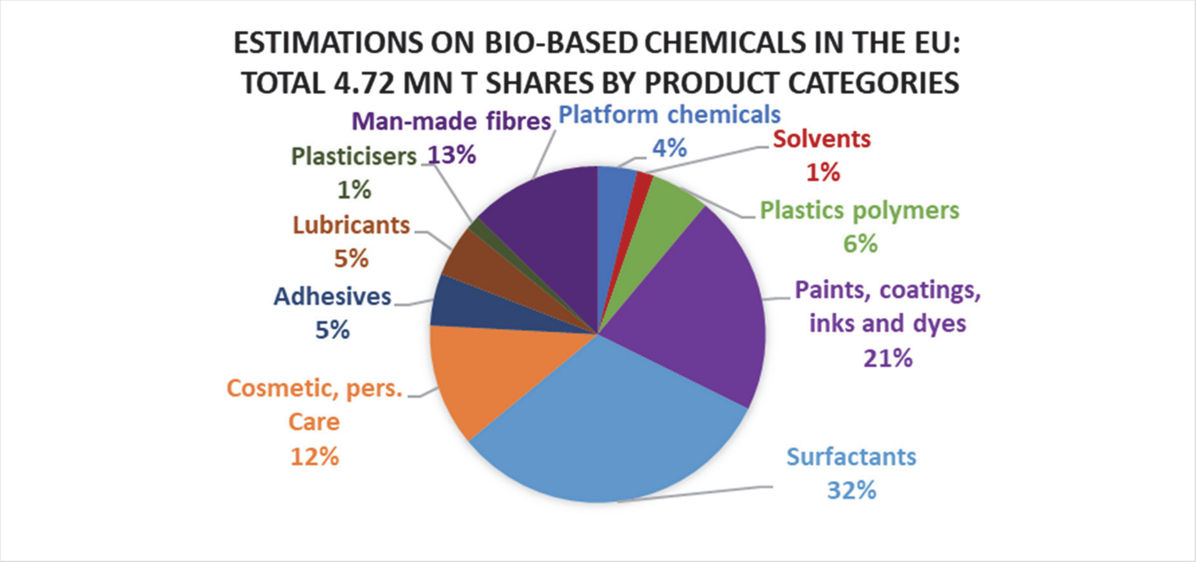


Figure 3: Estimations on bio-based chemicals markets and applications
Source: Own depiction based on Spekreijse et al. (Spekreijse, et al., 2019)

As it is calculated, the ten product categories achieve an annual turnover of around €9.17 billion. According to the SWOT analysis of the study, high research activities, as well as properties like biodegradability, more safety, lower toxicity and less greenhouse gas (GHG) emissions are considered to be beneficial for future development and commercialisation of the bio-based chemical industry. Apart from sometimes higher cost of, for example, biomass materials in comparison with fossil-based counterparts or new or less mature technologies, more efforts on purification processes of bio-based products, and quality deviations of some bio-based products are considered as weaknesses. Less consumer awareness on bio-based products except of bio-based plastics and bio-lubricants and land use are identified as drawbacks as well while legal measures to introduce biodegradable products could support their launch on the market. (Spekreijse, et al., 2019).

Since biomass is estimated to build globally 10-13% of chemical raw materials, sustainability has to be respected in particular according to resources traditionally used for food and feed markets (Kircher M., 2019) (Pietrowsky, et al., 2015).

4.5 Plastics – Moderate increase of the bioplastic capacities

Worldwide, the production of plastics reached 359 million tonnes while the EU contributed with more than 17% or 61.8 million tonnes in 2018 (Plastics Europe, 2019). Global production capacities of bioplastics are expected to increase by around 12% from a total of 2.152 million tonnes in 2020 to 2.426 million tonnes in 2024 (European Bioplastics, 2020a). More details are depicted in Figure 4. The main capacity is located at Asia (45%), for Europe it is about 25% and for America 30% (European Bioplastics, 2020a)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

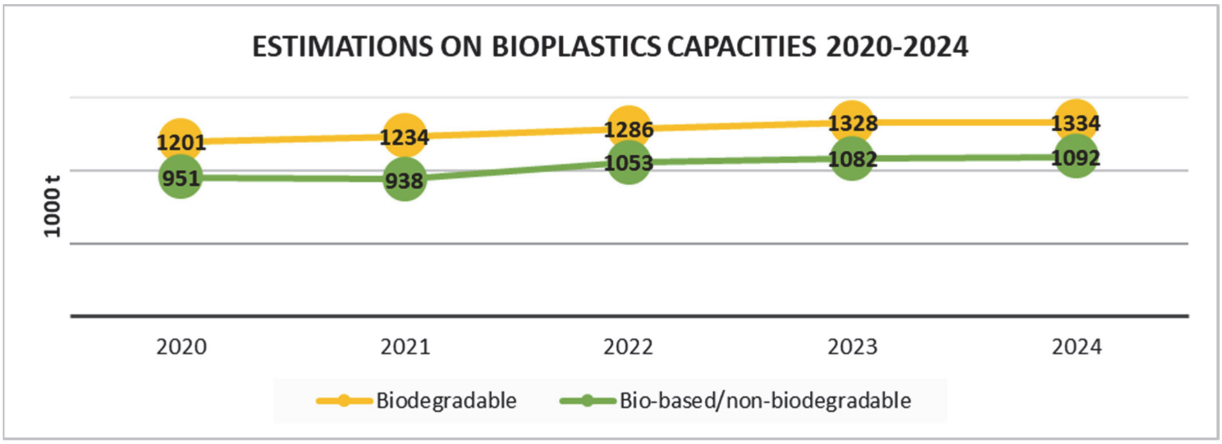


Figure 4: Estimations on development of bioplastics production capacities 2020-2024
Source: European Bioplastics (modified) (European Bioplastics, 2020a)

However, various estimations and forecasts exist. The IfBB – Institute for Bioplastics and Biocomposites projects a capacity of 4.35 million tonnes for 2023 for "new economy plastics" including bio-based and biodegradable plastics (IfBB, 2019), and a global amount of 3.8 million tonnes of bio-based structural polymers is reported by the Nova Institute for 2019 (Nova, 2019).

Following European Bioplastics, a high consumer acceptance is documented for these materials. Furthermore, less dependence from fossil resources and the effects of climate change support the acceptance of bio-based plastics (European Bioplastics, 2020a). The first bacterial derived plastic: polyhydroxybutyrate was already discovered by Maurice Lemoigne in 1925 (Seva Nayak, D. & Singh, B., 2018). Nowadays, a technological maturity is attested to the materials. Depending on the material, life cycle analysis has proven clear benefits of bioplastic against traditional products (European Bioplastics, 2020a).

4.6 Packaging as the most relevant bioplastics application

For 2019, the global packaging market was valued at $917 billion (Smithers, 2020). Flexible (16%) and rigid (30%) packaging are the most important market segments for bio-based plastics. If considering the biodegradable plastic sector, the flexible ones reach already 43% while the rigid types attain 16%. A total of 1.14 million tonnes of packaging constituting 53% of the entire bioplastics market is indicated for 2019 (European Bioplastics, 2020b). Due to environmental benefits and depending on the material, specific biodegradability properties, packaging is an excellent market opportunity for the bioeconomy.

4.7 Fertilisers market – the challenge of imported nutrients

A global value from around $155 billion to $200 billion is reported for the mineral fertilisers market (World Fertilizer Magazine, 2019), (Global Market Insights, 2019). In 2018, the production covered 250.9 million tonnes of nutrients at the international level while 18.1 million tonnes were generated in the EU-28. A consumption of 20 million tonnes and net imports are documented for the EU (Fertilisers Europe, 2020). To address these nutrient imports and to fill the gap display a chance for the bioeconomy since the legal terms for the use of anaerobically or aerobically treated organic waste are newly defined in the EU in 2019.

Moreover, the global market value of organic fertilisers is estimated to be in the range of $4.512 billion (P&S Intelligence, 2020) and $6.7 billion (2018) (Cohererent, 2018), and a growing demand is projected for Europe from $2.451 billion in 2016 to $3.260 billion in 2023 (Allied Market Research, 2018). Among the opportunities for organic fertilisers are precision farming better product quality, specific agriculture (e.g. flowers, viticulture), nutrient loss in soil and organic farming conditions (ECOFI, 2020).

4.8 Energy – Renewables conquered Europe

The evolvement of renewable energy markets is a success story in the EU, e.g. raising from 8.5% in 2005 to 17% in 2015 at the gross energy consumption. Bioenergy consumption is expected to reach 12% at renewable energy sources (RES) use of 2020. Electricity from RES has reached 397 GW in 2015 while 30 GW were gained from biological resources. Biogas takes a share of 20% of the global "biopower" and 4% of the heat production. The EU was the frontrunner in biogas electricity worldwide in 2015 producing 58 TWh from 80 TWh (Figure 5) (Scarlat, et al., 2018). More than 18,000 biogas plants and 1,600 biomethane plants were reported for Europe by the European Biogas Association (EBA) in 2018. Taking into account 6.000 biogas sites in 2009 (EBA, 2019), their number tripled within ten years.

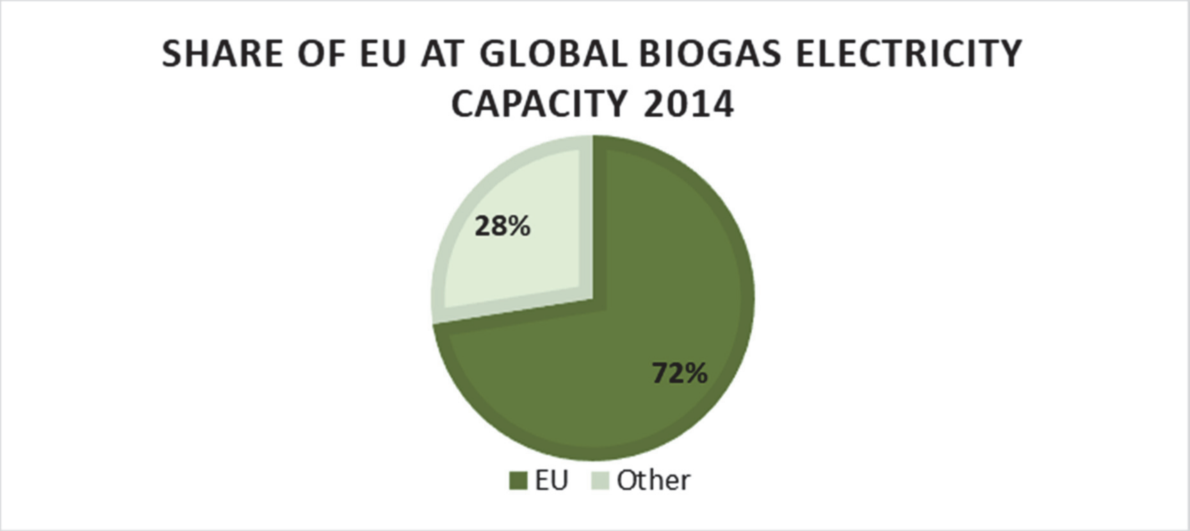


Figure 5: Share of EU at global biogas electricity production 2014
Source Own depiction based on Scarlat et al. (Scarlat, et al., 2018)

More than 119 million cbm of bioethanol were globally produced in 2016 (UFOP, 2018) and around 5.5 billion litres for fuels in the EU-28 in 2019 (USDA, 2019). As regards biodiesel production for transport, the EU is the world leader with more than 12 million tonnes out of a total of 34 million in 2016 and according to estimations of USDA, around 14 billion litres were produced in the EU in 2019 (USDA, 2019). Even though rape seed oil was the dominant feedstock for biodiesel, waste materials could already contribute by 15% in the EU in 2015 (UFOP, 2018). Biodiesel dominates the RES for transport sector in the EU (USDA, 2019) (USDA, 2020).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

The market developments and achievements of RES and bioenergy are very encouraging for further efforts on establishing a bioeconomy in Europe and worldwide. The need of clean energy and properties of certain materials (e.g. less toxicity) vote for the Bioeconomy while on the other hand, COVID-19 leads to less transport (USDA, 2020) and may trigger supply chain interruptions.

5. STARTING FROM WASTE - legal aspects of the EU Bioeconomy

Due to the complexity of the bioeconomy, it is affected by an array of legal areas and sources, respectively. This paper concentrates on legal aspects of value chains starting from biowaste (EU, 2018a) and biodegradable waste (EU, 1999) as legally defined at the EU level.

5.1 Facets of waste legislation

By introducing a mandatory separate collection of biowaste for the entire EU as of July 2023, a strong push signal was sent towards valorisation of this resource and advancing the bioeconomy. Furthermore, EU Member States could allow "similars to biowaste" to be collected with biowaste if, e.g. standards of compostable packaging are met (EU, 2018a), which is a positive impulse for bio-based products with biodegradable characteristics. The waste hierarchy has been established as a priority order and reprocessing of waste into substances, materials and products fits into the category recycling. From the EU legal point, there is no difference between recycling for the original purpose or other purposes (EU, 2008), as such no preference is expressed by this term for a possible added value of bio-based products on the basis of waste. In order to enable a smooth functioning of supply chains, end-of-waste criteria must be defined and met. To date, these criteria are legally elaborated for certain metal scraps and glass cullet at the EU scale, and case-by-case decisions will be needed as long as they are not nationally available for a waste type or decided for the whole EU territory (Johansson, N. & Forsgren, C., 2020). This complicates the transformation routes from biowaste to bio-based secondary resources. A strong reduction of biodegradable waste in landfill to 35% compared to data of 1995 was already legally decided more than 20 years ago (EU, 1999), and a target of 10% for municipal waste was set by 2035 (EU, 2018a). The difference on landfill and municipal waste management between 1995 and 2017 in the EU is shown in Figure 6 (Eurostat, 2019). On the other hand, more specific targets for biodegradable waste types, e.g. for sludges from wastewater treatment and coupling them to specific treatments, such as anaerobic digestion, could further support to build up the bioeconomy.

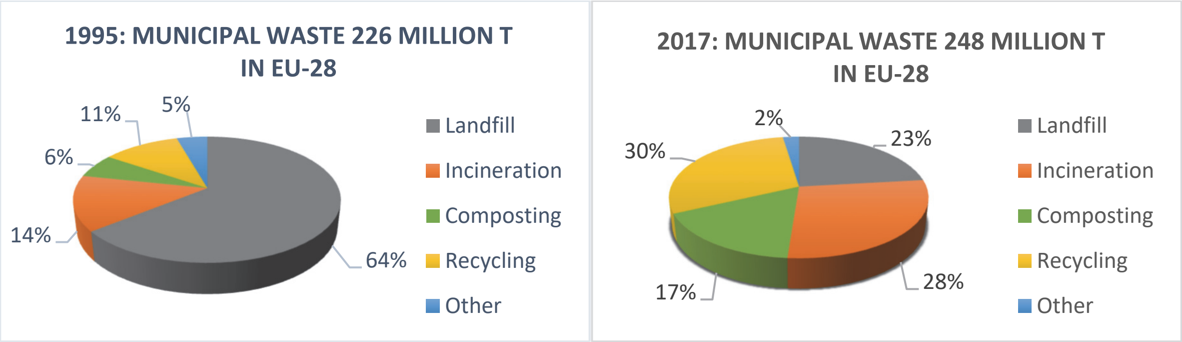


Figure 6: EU municipal waste management in 1995 and 2017
Source: Own depiction based on Eurostat (Eurostat, 2019)

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

5.2 Finding the right slots for food

General rules on the entire food and feed chain ensuring safety and high quality are laid down in Regulation (EC) No 178/2002 (EU, 2002). But a specific reference to food resulting from recovery of biowaste or follow up processes therefrom is not given in this legal source. If the food was not significantly placed on the market before 15th May 1997 and integrates microorganisms, fungi or algae for its development (de Boer, A. & Bast, A., 2018) as under use for biotechnological valorisation of organic waste (VOLATILE D2.6, (2020)), the stipulations for Novel Food come into play. Whereas an explicit authorisation by the EU is necessary, Member States can restrict the marketing of them (EC, 2020a), and as such affect the bioeconomy market.

5.3 Feed materials – between opportunities and limitations

The catalogue of feed materials has been altered at several times since 2010, and more recently conditions for the use of polyhydroxy butyrate based on *Ralstonia eutropha* have been added (EU, 2017). This measure provides a new opportunity for bio-based products. However, Regulation (EU) No 568/2010 formulates a prohibition on the use of wastes from WWT for feed purposes “irrespective of any further processing” and “solid urban waste, such as household waste” (EU, 2010). These rules would need to be examined with regard to the fostering of waste-based bio-refinery processes.

5.4 Chemicals - how to pass the entrance to secondary resources?

According to ECHA, all forms of recovery processes are considered as manufacturing processes if they result in substances, mixtures or articles which have ceased to be waste (ECHA, 2010). The REACH Regulation itself, which applies also to isolated intermediates, gives no indication on end-of-waste-criteria. Even though in some cases (e.g. for food, feed, medicinal products), certain exemptions from some Titles of REACH could be possible (EU, 2006), this legal act constitutes one of the most critical gateways if waste will be transformed to enter the market in terms of the bioeconomy.

5.5 Reducing plastic pollution – Is there a legal role for biomaterials?

Considering that 5 to 13 million tonnes of plastic end up in the oceans worldwide every year (EC, 2018b), significant prohibitions and restrictions for single-use plastics were legally passed in the EU. Currently, bio-based and biodegradable plastics are encompassed by the new law (EU, 2019a), which, indeed, does not favour the bioeconomy. An excerpt on products to be prohibited by July 2021 is presented in Table 2. For biodegradable plastics, an assessment on scientific and technical progress is planned by the European Commission by 2027 (EU, 2019a).

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 720777

Table 2: Examples of single-use plastics prohibited as of July 2021

Requirement	Examples of single-use plastics products covered
Art. 5 Products to be prohibited in the Member States as of 03.07.2021 (Excerpt)	Cotton bud sticks, unless they are for used for medical devices
	Cutlery including spoons, forks, knives, and chopsticks
	Plates
	Straws, unless they are used for medical devices
	Beverage stirrers
	Sticks for balloons including mechanisms relating thereto

Source: Own depiction based on Directive (EU) 2019/904 (EU, 2019a)

5.6 Appreciation for biodegradable packaging

Following the amended Packaging Directive, at least 65% of the packaging waste and 50% of the plastic waste have to be recycled by 2025. A clear statement is provided that biodegradable packaging waste could be counted as recycled if aerobically or anaerobically treated and leading to a substance, material or product (EU, 2018b). This will foster acceptance of bio-based packaging complying with biodegradability standards.

5.7 Fertilising products - New routes for biodegradable waste but without mulch films

With view on biodegradable waste valorisation, a new concept has been introduced by the Fertiliser Products Regulation. If undergone aerobically or anaerobically treatment and in accordance with the quality requirements, biowaste and sewage sludge can be converted into fertilising products. Criteria for the application of biodegradable mulch films will be defined by the European Commission by 2024 (EU, 2019b) which postpones the market entrance of bio-based products with properties suitable for this purpose.

5.8 Sustainable bioenergy and how to integrate material priorities

Apart from manufacturing of goods, the bioeconomy includes also the energy sector. Legal provisions on production of renewable energy are strongly oriented at sustainability criteria and the 40% GHG emissions reduction target of the Paris Agreement (EU, 2018c). Renewable energy sources (RES) have been lifted up and should achieve 32% in the gross energy consumption and 14% in the transport sector by 2030 while e.g. biowaste counts double to achieve the targets if deployed for transport (EU, 2018d). A specific sub-target is set for certain organic waste (e.g. biowaste) which need to achieve 3.5% of the "transport target". Depending on the calculation method and technology, GHG emission savings for biogas up to 84% are legally defined for this RES. Targets on RES vary significantly within the EU countries, and the commitments of Member States on these targets in 2020 compared with 2005 are laid down in Figure 7 (EU, 2018d).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

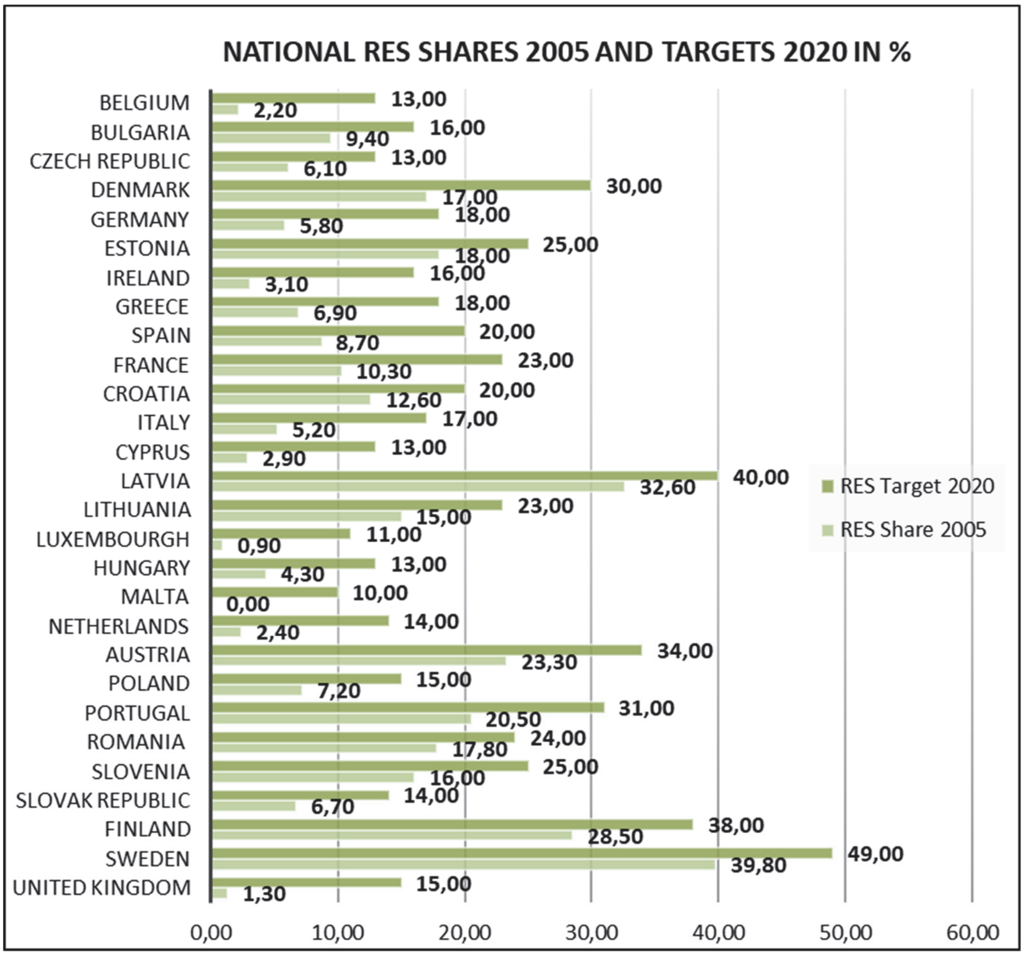


Figure 7: National RES shares 2005 and targets 2020 in % in the EU
Source: VOLATILE D2.6 (VOLATILE D2.6, (2020)) based on (EU) 2018/2001 (EU, 2018d)

Obviously, the bioeconomy could benefit from RES targets as it is expressed by the aforementioned and estimated bioenergy rate of 12% by 2020 (Scarlat, et al., 2018). On the other hand, targets do not exist for prioritising of materials from bio-based feedstock, e.g. by a committed cascading principle or minimum contents of bio-based carbon in materials. To further boost materials from renewable resources, legal preferences on “material against energy use” of bio-based feedstock could be supportive.

6. CONCLUSIONS

A multitude of deployments for the bioeconomy is offered by growing markets thus far for the sectors food, feed, bio-based products and energy scrutinized in the scope of this work. If value chains are derived on the basis of waste and cover one or both, gaining materials and energy, legal adaptions would be required. In particular, if revolutionary concepts are presented, e.g. those of the VOLATILE project www.volatile-h2020.eu, tailor-made solutions would be necessary to trigger investments in technologies valorising organic waste and representing a blue print not only for the bioeconomy but also for the circular economy.

7. REFERENCES

Allied Market Research. 2018. Europe Organic Fertiliser Market Overview. [Online] 2018. [Cited: 08. 11. 2020.] https://www.alliedmarketresearch.com/europe-organic-fertilizer-market.

Bioökonomierat. 2020. International Bioeconomy Strategies. [Online] 2020. [Cited: 23. 10. 2020.] https://biooekonomierat.de/en/international/index.html.

Camia, A. and Robert N., Jonsson R., Pilli R., García-Condado S., López-Lozano R., van der Velde M., Ronzon T., Gurría P., M'Barek R., Tamosiunas S., Fiore G., Araujo R., Hoepffner N., Marelli L., Giuntoli J. 2018. Biomass production, supply, uses and flows in the European Union. First results from an integrated assessment. Luxemburg: EUR 28993 EN, Publications Office of the European Union, 2018. ISBN 978-92-79-77237-5, doi:10.2760/539520, JRC109869.

CEFIC. 2020. 2020 Facts and Figures of the European Chemicals Industry. [Online] 2020. [Cited: 28. 10. 2020.] https://cefic.org/app/uploads/2019/01/The-European-Chemical-Industry-Facts-And-Figures-2020.pdf.

CISION. 2020. Dietary Supplements Market to Reach USD 216.3 Billion by 2026 | Reports And Data. [Online] 2020. [Cited: 08. 11. 2020.] https://www.prnewswire.com/news-releases/dietary-supplements-market-to-reach-usd-216-3-billion-by-2026--reports-and-data-300969115.html.

Cohererent. 2018. Coherent Market Insights; Global Organic Fertilizer Market to reach around US$ 12.5 Billion by end of 2027 - Coherent Market Insights. [Online] 2018. [Cited: 08. 11. 2020.] https://www.globenewswire.com/news-release/2020/03/30/2008502/0/en/Global-Organic-Fertilizer-Market-to-reach-around-US-12-5-Billion-by-end-of-2027-Coherent-Market-Insights.html.

de Boer, A. & Bast, A. 2018. Demanding safe foods – Safety testing under the novel food regulation (2015/2283). Trends in Food Science & Technology. Volume 72, February 2018, pp. 125-133, 2018.

EBA. 2019. European Biogas Association - EBA Annual Report 2019. [Online] 2019. [Cited: 29. 10. 2020.] https://www.europeanbiogas.eu/eba-annual-report-2019/.

EC. 2010. Communication from the Commission to the Council and the European Parliament on future steps in bio-waste management in the European Union {SEC(2010) 577}. /* COM/2010/0235 final */. [Online] 2010. [Cited: 27. 10. 2020.] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52010DC0235.

EC. 2002. Communication from the Commission to the Council, the European Parliament, the Economic and Social Committee and the Committee of the Regions - Life sciences and biotechnology - A Strategy for Europe. /* COM/2002/0027 final */. Official Journal C 055, 02/03/2002 P. 0003 - 0032, 2002.

EC. 2012. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS. Innovating for Sustainable Growth: A Bioeconomy for Europe; /* COM/2012/060 final */ Brussels, 13.2.2012. [Online] 2012.

EC. 2018a. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS A sustainable Bioeconomy for Europe: Strengthening the connection between. economy, society and the environment; COM/2018/673 final. [Online] 2018a.

EC. 2018b. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS. A European Strategy for Plastics in a Circular Economy; [Online] 2018b.

EC. 2019a. COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE EUROPEAN COUNCIL, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS The European Green Deal. COM/2019/640 final. [Online] 2019a.

EC. 2016. ECSIP Consortium; The competitive position of the European food and drink industry; Final Report. [Online] 2016. [Cited: 28. 10. 2020.] file:///C:/Users/Computer/Downloads/EA0416075ENN.pdf.

EC. 2019b. European Commission - Knowledge for Policy, 24.09.2019. Food, feed, fibres, fuels. Enough biomass for a sustainable bioeconomy? [Online] 2019b. [Cited: 07. 11. 2020.] https://ec.europa.eu/knowledge4policy/publication/food-feed-fibres-fuels-enough-biomass-sustainable-bioeconomy_en?page=1.

EC. 2020d. European Commission; Food; Animal Feed. [Online] 2020d. [Cited: 28. 10. 2020.] https://ec.europa.eu/food/safety/animal-feed_en.

EC. 2020a. European Commission; Food; Novel food catalogue. [Online] 2020a. [Cited: 26. 10. 2020.] https://ec.europa.eu/food/safety/novel_food/catalogue_en. accessed 26.10.2020.

EC. 2020c. European Commission; Internal Market, Industry, Entrepreneurship and SMEs; Food and drink industry. [Online] 2020c. [Cited:] https://ec.europa.eu/growth/sectors/food_en.

EC. 2020f. European Commission; Jobs and Wealth in the European Union Bioeconomy. [Online] 2020f. [Cited: 08. 11. 2020.] https://datam.jrc.ec.europa.eu/datam/mashup/BIOECONOMICS/index.html.

EC. 2020e. European Commission; Knowledge Centre for Bioeconomy; Browse Bioeconomy by country. [Online] 2020e. [Cited: 06. 11. 2020.] https://ec.europa.eu/knowledge4policy/bioeconomy_en#country.

EC. 2020b. European Commission; Knowledge for policy; Biomass; Biomass production in the EU by biomass type. [Online] 2020b. https://ec.europa.eu/knowledge4policy/bioeconomy/topic/biomass_en. accessed 27.10.2020.

ECHA. 2010. ECHA - European ChemiEuropean Chemicals Agency; Guidance on waste and recovered substances; Version 2, May 2010. [Online]. [Online] https://echa.europa.eu/documents/10162/23036412/waste_recovered_en.pdf. [Online] 2010. [Cited: 27. 10. 2020.] https://echa.europa.eu/documents/10162/23036412/waste_recovered_en.pdf.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

ECN. 2019. European Compost Network. Biowaste in Europe. [Online] 2019. [Cited: 27. 10. 2020.] https://www.compostnetwork.info/policy/biowaste-in-europe/.

ECOFI. 2020. European Consortium of the Organic Based Fertiliser Industry. Market Outlook for Organic-Based Fertilisers. [Online] 2020. [Cited: 08. 11. 2020.] http://www.ecofi.info/2020/03/market-outlook-for-organic-based-fertilisers/.

EIA. 2020. U.S. Energy Information Administration; International; Petroleum and other liquids. [Online] 2020. [Cited: 27. 10. 2020.] https://www.eia.gov/international/data/world.

EU. 2017. Commission Regulation (EU) 2017/1017 of 15 June 2017 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. [Online] 2017.

EU. 2010. COMMISSION REGULATION (EU) No 568/2010 of 29 June 2010 amending Annex III to Regulation (EC) No 767/2009 of the European Parliament and of the Council as regards the. prohibition to place on the market or use for animal nutritional purposes protein products obtained from yeasts of the Candida variety cultivated on n-alkanes. [Online] 2010.

EU. 1999. Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste. 1999.

EU. 2018d. Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the promotion of the use of energy from renewable sources. [Online] 2018d.

EU. 2018a. Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May 2018 amending Directive 2008/98/EC on waste. 2018a.

EU. 2018b. Directive (EU) 2018/852 of the European Parliament and of the Council of 30 May 2018 amending Directive 94/62/EC on packaging and packaging waste. [Online] 2018b.

EU. 2019a. Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment. [Online] 2019a.

EU. 2008. Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. 2008.

EU. 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food saf. establishing the European Food Safety Authority and laying down procedures in matters of food safety. [Online] 2002.

EU. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). establishing a European Chemicals Agency, amending Directive. 999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as. [Online] Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC, 2006.

EU. 2018c. Regulation (EU) 2018/1999 of the European Parliament and of the Council of 11 December 2018 on the Governance of the Energy Union and Climate Action, amending Regulations (EC) No 663/2009 and (EC) No 715/2009 of the European Parliament and of the Council, Directives 94/22/EC, 98/70/EC, 2009/31/EC, 2009/73/EC, 2010/31/EU, 2012/27/EU and 2013/30/EU of the European Parliament and of the Council, Council

Directives 2009/119/EC and (EU) 2015/652 and repealing Regulation (EU) No 525/2013 of the European. [Online] Parliament and of the Council, 2018c.

EU. 2019b. Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. [Online] 2019b.

EUBIA. 2020. European Biomass Industry Association. Bio-waste. [Online] 2020. [Cited: 06. 11. 2020.] https://www.eubia.org/cms/wiki-biomass/biowaste/.

European Bioplastics. 2020b. Facts and Figures. [Online] 2020b. [Cited: 28. 10. 2020.] https://docs.european-bioplastics.org/publications/EUBP_Facts_and_figures.pdf.

European Bioplastics. 2020a. Market drivers and development. [Online] 2020a. [Cited: 28. 10. 2020.] https://docs.european-bioplastics.org/publications/market_data/Report_Bioplastics_Market_Data_2019.pdf.

Eurostat. 2020. Eurostat; Recycling of biowaste; kg per capita. [Online] 2020. [Cited: 06. 11. 2020.] https://ec.europa.eu/eurostat/databrowser/view/cei_wm030/default/table?lang=en.

Eurostat. 2019. File: Municipal waste landfilled, incinerated, recycled and composted, EU-28, 1995-2017.png. [Online] 2019. [Cited: 06. 11. 2020.] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:Municipal_waste_landfilled,_incinerated,_recycled_and_composted,_EU-28,_1995-2017.png&oldid=439075.

FEFAC. 2020a. Compound Feed Production (1989-2019) (figures). [Online] 2020a. [Cited: 08. 11. 2020.] https://fefac.eu/statistics/.

FEFAC. 2020b. FEFAC Feed Sustainability Charter 2020-2030. [Online] 2020b. [Cited: 08. 11. 2020.] https://fefac.eu/newsroom/news/fefac-publishes-feed-sustainability-charter-2030-at-xxix-congress/.

Fertilisers Europe. 2020. Industry facts. [Online] 2020. [Cited: 28. 10. 2020.] https://www.fertilizerseurope.com/wp-content/uploads/2020/07/Industry-Facts-and-Figures-2020-spreads.pdf.

FoodDrink Europe. 2020. Data & Trends of the European Food and Drink Industry 2019. [Online] 2020. [Cited: 08. 11. 2020.] https://www.fooddrinkeurope.eu/publication/data-trends-of-the-european-food-and-drink-industry-2019/.

Fortune. 2020. Europe Dietary Supplements Market Size to Hit USD 33.80 Bn by 2027; Increasing Awareness about Health and Wellness to Accelerate Market Expansion, Says Fortune Business Insights™. [Online] 2020. [Cited: 08. 11. 2020.] https://www.globenewswire.com/news-release/2020/08/14/2078421/0/en/Europe-Dietary-Supplements-Market-Size-to-Hit-USD-33-80-Bn-by-2027-Increasing-Awareness-about-Health-and-Wellness-to-Accelerate-Market-Expansion-Says-Fortune-Business-Insights.html.

Fritsche, U. and Brunori, G., Chiaramonti, D., Galanakis, C.M., Hellweg, S., Matthews, R., Panoutsou, C. 2020. Future transitions for the Bioeconomy towards Sustainable Development and a Climate-Neutral Economy. Knowledge Synthesis Final Report. [Online] 2020. [Cited: 24. 10. 2020.] https://ec.europa.eu/jrc/en/publication/future-transitions-bioeconomy-towards-sustainable-development-and-climate-neutral-economy-knowledge.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Global Market Insights. 2020. Compound Feed Market Size, Industry Analysis Report, Regional Outlook. [Online] 2020. [Cited: 18. 11. 2020.] https://www.gminsights.com/industry-analysis/compound-feed-market.

Global Market Insights. 2019. Industry Overview. [Online] 2019. [Cited: 08. 11. 2020.] https://www.gminsights.com/industry-analysis/fertilizer-market.

IfBB. 2019. University of Applied Sciences and Arts, Hannover. Biopolymers- facts and statistics 2019. [Online] 2019. [Cited: 08. 11. 2020.] https://www.ifbb-hannover.de/files/IfBB/downloads/faltblaetter_broschueren/f+s/Biopolymers-Facts-Statistics-2019.pdf.

Johansson, N. & Forsgren, C. 2020. Is this the end of end-of-waste? Uncovering the space between waste and products. Resources, Conservation and Recycling. pp. Volume 155, April 2020, 104656., 2020.

Kamm B. & Kamm M. (2004). Principles of biorefineries. Applied Microbiology and Biotechnology. 64, pp.137–145, (2004).

Kircher M. 2019. Bioeconomy: Markets, Implications, and Investment Opportunities. Economies 2019(7):73. DOI: 10.3390/economies7030073, 2019.

Market Data Forecast. 2020. Compound Feed Market By Ingredients (Cereals, Cereals By-Product, Oilseed Meal, Oil, Molasses, Supplements And Others), By Supplements (Vitamins, Antibiotics, Antioxidants, Amino Acids, Feed Enzymes, Feed Acidifiers And Others), , . By Animal Type (Ruminant, Swine, Poultry, Aquaculture And Others), And By Region - Global Industry Analysis, Size, Share, Growth, Trends and Forecast |2020 to 2025. [Online] 2020. [Cited: 08. 11. 2020.] https://www.marketdataforecast.com/market-reports/global-compound-feed-market.

Nova. 2019. Nova Institut - Bio-based Building Blocks and Polymers – Global Capacities, Production and Trends 2019–2024. Data for 2019. [Online] 2019. [Cited: 08. 11. 2020.] file:///C:/Users/Computer/Downloads/20-02-04-Bio-based-Building-Blocks-and-Polymers-short-version.pdf.

P&S Intelligence. 2020. Organic Fertilizers Market Overview. [Online] 2020. [Cited: 08. 11. 2020.] https://www.psmarketresearch.com/market-analysis/organic-fertilizers-market-report.

Patermann, C. & Aguilar, A. (2018). The origins of the bioeconomy in the European Union. New Biotechnology. Volume 40, Part A; pp. 20-24, (2018), https://doi.org/10.1016/j.nbt.2017.04.002.

Pietrowsky, S., Carus, M. (Nova Institut) and Carrez, D. (BIC). 2015. European Bioeconomy in Figures; 2008 - 2015. [Online] 2015. [Cited: 28. 10. 2020.] https://biconsortium.eu/sites/biconsortium.eu/files/documents/Bioeconomy_data_2015_20150218.pdf.

Plastics Europe. 2019. The Facts - 2019. [Online] 2019. [Cited: 28. 10. 2020.] https://www.plasticseurope.org/application/files/1115/7236/4388/FINAL_web_version_Plastics_the_facts2019_14102019.pdf.

Ronzon, T. and Pietrowsky, S., Tamosiunas, S., Dammer, L., Carus, M., M'barek, R. 2020. Developments of Economic Growth and Employment in Bioeconomy Sectors across the EU. Sustainability 2020; 2(11), 4507; https://doi.org/10.3390/su12114507. 2020.

Ronzon, T. and Piotrowski, S., M'barek, R.,Carus, M., Tamošiūnas, S. 2018. Jobs and wealth in the EU bioeconomy / JRC - Bioeconomics. European Commission, Joint Research Centre (JRC). [Online] 2018. https://datam.jrc.ec.europa.eu/datam/mashup/BIOECONOMICS/index.html#. accessed 23.10.2020.

Ronzon, T., Piotrowsky, S. and Carus, M. 2015. DataM – Biomass estimates (v3): a new database to quantify biomass availability in the European Union. s.l. : Luxembourg: Publications Office of the European Union, 2015, 2015. ISSN 1831-9424 (online); Doi:10.2791/650215.

Scarlat, N. and Dallemand, J.-F., Monforti-Ferrario, F., Nita, V. 2015. The role of biomass and bioenergy in a future bioeconomy: Policies and facts. Environmental Development. 15 (2015) 3–34, 2015.

Scarlat, N., Dallemand, J.F. and Fahl, F. 2018. Biogas: Developments and perspectives in Europe. Renewable Energy. Volume 129, Part A, December 2018, pp. 457-472, 2018, Vol. https://doi.org/10.1016/j.renene.2018.03.006.

Seva Nayak, D. & Singh, B. 2018. Identification and Isolation of Acetoacetyl-CoA reducatase (phbB) gene - Involved in Poly (β-Hydroxybutyrate) Biosynthesis in Ralstonia eutropha - (IMTCC 1954 strain). International Journal of Current Microbiology and Applied Sciences. ISSN: 2319-7692 Special Issue-7 pp. 349-355, 2018.

Smithers. 2020. Smithers; The Future of Global Packaging to 2024. [Online] 2020. [Cited: 28. 10. 2020.] https://www.smithers.com/services/market-reports/packaging/future-of-global-packaging-to-2024.

Spekreijse, J. and Lammens, T., Parisi, C., Ronzon, T., Vis, M. 2019. Insights into the European market of bio-based chemicals. Analysis based on ten key product categories. EUR 29581 EN, Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-79-98420-4, doi:10.2760/549564, JRC112989. [Online] 2019. [Cited: 31. 10. 2020.] file:///C:/Users/Computer/Downloads/jrc_europeanmarket_biochemicals_online.pdf. accessed 31.10.2020.

UFOP. 2018. UFOP Report on Global Market Supply 2017/2018. Union zur Föderung der Öl- und Proteinpflanzen. [Online] 2018. [Cited: 29. 10. 2020.]

USDA. 2020. GAIN Report Number: E42020-0032; Biofuels Annual European Union, June 29,2020. [Online] 2020. [Cited: 09. 11. 2020.] https://apps.fas.usda.gov/newgainapi/api/Report/DownloadReportByFileName?fileName=Biofuels%20Annual_The%20Hague_European%20Union_06-29-2020.

USDA. 2019. GAIN Report Number: NL9022; EU Biofuels Annual 2019; Date:7/15/2019. [Online] 2019. [Cited: 09. 11. 2020.] https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Biofuels%20Annual_The%20Hague_EU-28_7-15-2019.pdf.

VOLATILE D2.6. (2020). Deliverable D2.6 - Outlook on future legislation affecting VFA based value chains, October 2020. [Online] (2020). https://www.volatile-h2020.eu/platform.php.

World Fertilizer Magazine. 2019. The worldwide fertilizer market to 2024, 21.03.2019. [Online] 2019. [Cited: 08. 11. 2020.]

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

https://www.worldfertilizer.com/environment/21032019/the-worldwide-fertilizer-market-to-2024/.

Worldbank. 2020. World Bank Commodities Price Data (The Pink Sheet). [Online] 2020. [Cited: 09. 11. 2020.] http://pubdocs.worldbank.org/en/843201604424898761/CMO-Pink-Sheet-November-2020.pdf.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

DEVELOPMENT OF A VFA PLATFORM

F. Velghe [a], C. Peral [b], I. Sellis [c], F. De Wilde [a], G. Spit [c], T. Dietrich [b]
[a] *OWS NV, Dok Noord 5, 9000 Gent, Belgium*
[b] *TECNALIA, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Álava, Leonardo Da Vinci 1, 01510 Miñano, Álava, Spain*
[c] *Twence BV, Boldershoekweg 51, Hengelo OV 7554 RT, Netherlands*
Corresponding author: Filip.Velghe@ows.be

Abstract

To match the growing problem of dealing with urban biowaste generation (household organics, sewage sludge, food waste…) and the increasing demand for bio-based raw materials, the VOLATILE project partners joined their efforts to develop a Volatile Fatty Acid Platform (VFAP) based on the conversion of urban biowaste with the aim of producing novel materials and chemicals (Polyhydroxyalkanoates (PHA), a bio-based, biodegradable bioplastic; Single Cell Oil (SCO), a sustainable alternative for plant and animal oils; omega-3 Fatty Acids (omega-3 FA), a nutraceutical with many health benefits). In this part of the research, the focus was on changing the well-known process of anaerobic digestion to exclude the final conversion step to methane, and to maximize the production of volatile fatty acids (containing 2-6 carbon atoms). This objective was reached by first characterizing > 40 different urban biowaste streams from seven countries after which several process parameters were optimized at lab scale to reach maximal VFA production. In a next step, a membrane filtration cascade (microfiltration, ultrafiltration, nanofiltration and reverse osmosis) was designed to recover, purify and concentrate the VFA from the reactor effluent. The lab scale findings were then translated to a TRL 5 pilot plant located at the Twence Waste Treatment Facility in the Netherlands.

Keywords: Volatile fatty acid platform, membrane filtration, pilot

1. INTRODUCTION

As long as man has lived, he has produced organic waste. But since the industrial revolution, population numbers have started to increase substantially and more and more people started living in cities, leading to a strong increase and concentration of organic waste production, up to a point where it became an environmental and sanitary burden. At the moment, over 100 million tonne or urban biowaste (i.e. household organics and sewage sludge) is produced annually in the European Union (EU-28). The cheapest way of dealing with this organic waste, at least on the short term, is landfilling. But on the long term, landfilling only brings negative aspects: ineffective use of valuable land area, leaching of often toxic compounds to the soil, surface waters and deep ground waters and gaseous emissions (CO_2, CH_4, N_2O, NH_4…) to the air. To remediate these harmful effects, costly measures are needed, yet no economic benefit is to be gained. Therefore, landfilling of municipal organic waste is to be reduced to 35% of the 1995 levels by 2016 (or 2020 for some countries) according to the Landfill Directive (1999/31/EC). Since many decades, composting has been recognized as a valuable alternative to treat urban biowaste. During the composting process, the organic matter is aerobically stabilized in a relative short timeframe (8-12 weeks), yielding a stable compost that contains most of the nutrients and stable organic carbon and can be used as a soil improver or slow release fertiliser. Despite its positive effects, the economic value of compost is low, and the

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

composting process requires a net energy input. Urban biowaste treatment was further improved by the introduction of anaerobic digestion, a technology developed in the ‘70s of the previous century but gaining more and more popularity since the 2000s. With this technology, the biowaste is converted anaerobically to produce biogas (CH_4 and CO_2), making this process energy positive, i.e. there is a net production of renewable energy. The remaining digestate still contains nearly all of the nutrients and can also be used as fertiliser, either directly, but mostly after a final composting step (which only takes 2-4 weeks to produce a mature compost due to the prior anaerobic treatment). At the moment, anaerobic digestion is regarded as state-of-the-art technology to treat urban biowaste but is nevertheless reaching its limits.

In view of a circular economy approach, only recovering energy and nutrients is no longer enough. This is where the VOLATILE project partners joined forces to develop a novel platform technology to turn heterogenous biowaste into building blocks for the bioeconomy. The basis for this novel platform technology is anaerobic digestion. In anaerobic digestion, urban biowaste is converted through 4 different steps, which typically occur simultaneously in one reactor (Figure 1). In the first step, organic particles are hydrolysed into their water-soluble building blocks (carbohydrates to sugar, fats and oils to fatty acids and proteins to amino acids) by extracellular enzymes. These building blocks are further converted in the acidogenesis phase, producing C3-C6 organic acids (76% of the COD), acetic acid (20% of the COD) and gas (mainly CO_2 and H_2; 4% of the COD). The C3-C6 organic acids are further degraded in the acetogenesis phase to acetic acid (± 2/3 of the C3-C6 COD) and CO_2/H_2 (± 1/3 of the C3/C6 COD). In the final step, methanogenesis, methane is produced through two pathways: acetoclastic methane production converts acetic acid into CH_4 and CO_2, whereas the hydrogenotrophic methane production combines CO_2 and H_2 to form CH_4 and H_2O. By blocking this final methanogenesis step, a volatile fatty acid (C2-C6) build-up is expected, together with the net production of H_2 gas.

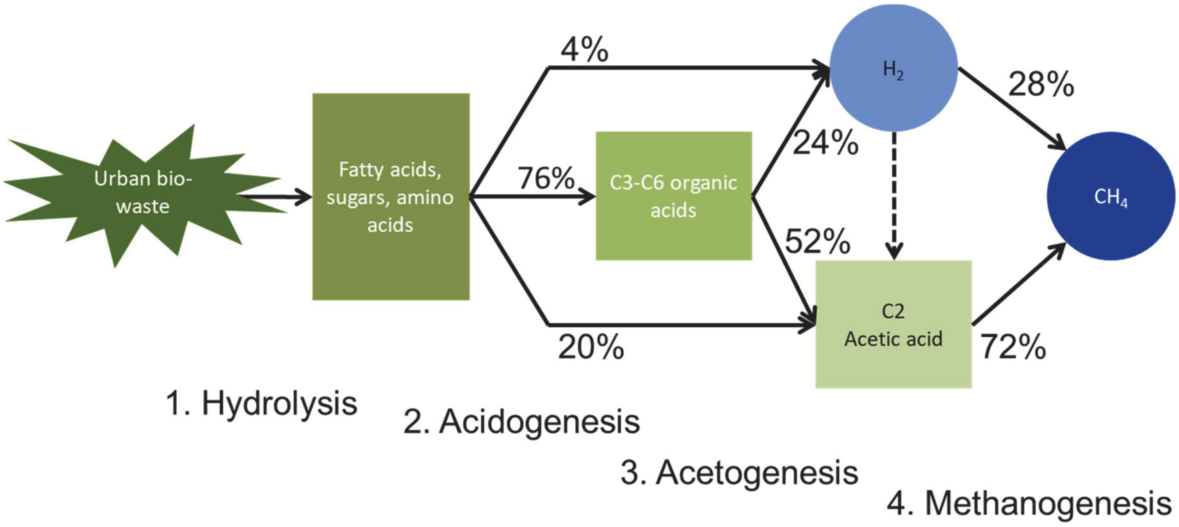


Figure 1: The four phases of anaerobic digestion and the distribution of COD content throughout the conversion steps (after Appels et al.)

2. VFA PRODUCTION

Before starting the production of volatile fatty acids (VFAs) from urban biowaste streams, a good characterization of these products was obtained to assess their potential as raw material. In total, 43 different waste samples were collected from 9 VOLATILE partners either producing or treating these waste streams in their facilities. As VFAs are the precursor to biogas production, the biogas potential of these waste streams was determined as a qualitative estimation of the VFA potential. Additionally, the heavy metal content was determined to assess possible barriers in using these products in the VFA Platform, and the macro- and micronutrient concentration was evaluated as these are determining the growth conditions of the VFA producing bacteria.

The 43 waste samples were divided over 7 categories: VGF waste (source separated household organics), OF-MSW (organic fraction obtained after mechanical treatment of mixed household waste), food waste (separately collected organic waste from the food and catering industry), green waste (source separated garden waste), roadside grass (grass from roadside and landscape maintenance), WWTP (waste water treatment plant) sludge before AD (secondary sludge or a mix of primary and secondary sludge from municipal WWTP) and WWTP sludge that has been anaerobically digested.

Based on the biogas potential (Figure 2) and the heavy metal content (Figure 3), the food waste samples show the highest potential for usage in a VFA Platform due to their high biogas potential and relatively low heavy metal content. On the downside, some of the micronutrients in the food waste samples are present at concentrations below the optimal range to allow a stable anaerobic digestion process.

Also, the VGF waste and OF-MSW show a high biogas potential and could therefore be regarded as having a high potential for VFA production. Contrary to the food waste samples, both types are characterized by a good macro- and micronutrient content which will improve the growth conditions of the VFA producing microbiome.

Finally, also the WWTP sludge before AD still shows a moderate biogas potential and has optimal macro- and micronutrient levels which could justify the use of this product in a VFA platform. Unfortunately, this biowaste category contained the highest levels of several heavy metals, which could hamper the final application. The green waste, roadside grass and WWTP sludge after AD had a too low biogas potential and therefore seem less suitable for VFA production.

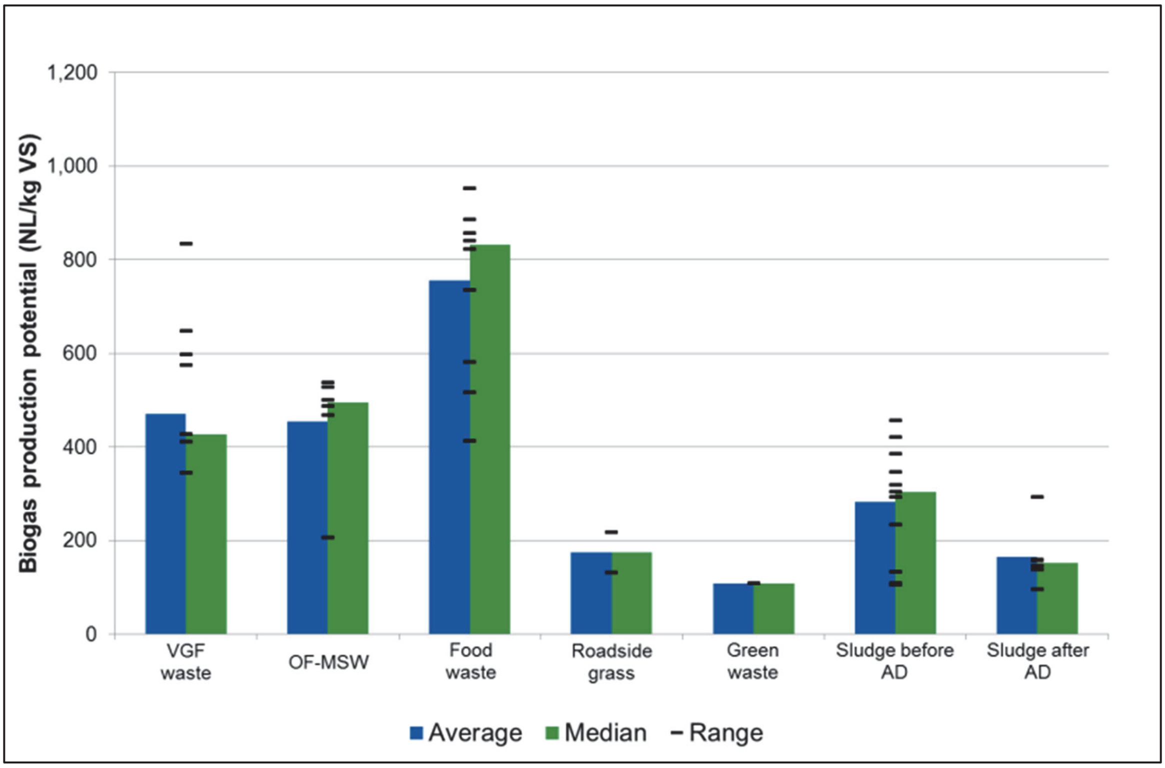


Figure 2: Range, average and median for the biogas production potential of all tested samples

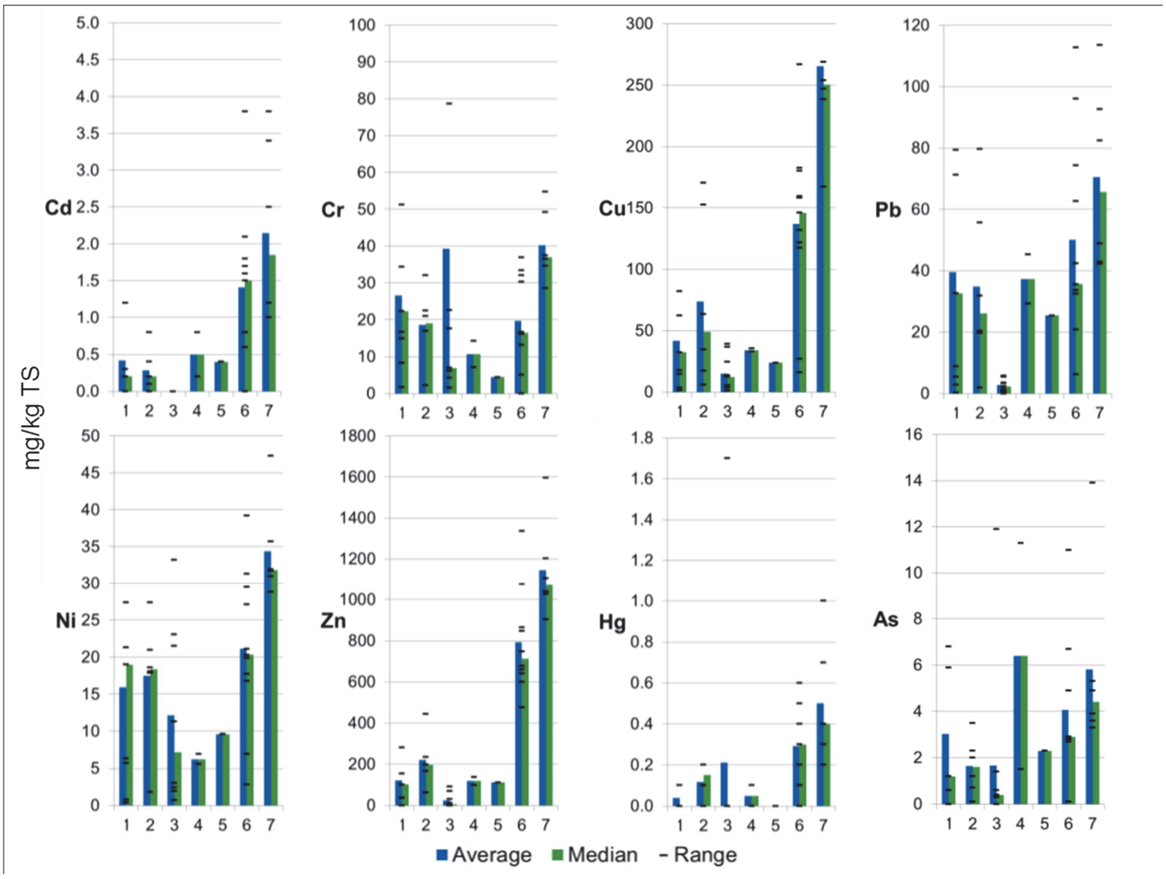


Figure 3: Range, average and median for the heavy metal content in all tested samples (1: VGF waste; 2: OF-MSW; 3: Food waste; 4: Roadside grass; 5: Green waste; 6: Sludge before AD; 7: Sludge after AD)

In a next phase, a laboratory test method (Volatile Fatty Acid Production Potential Test) was developed that yields, in a relatively short time interval (typically 11 days) information about the maximum VFA potential, organic matter to VFA conversion rate, VFA spectrum and the gas production (CO_2/H_2). The method is based on existing protocols to determine the maximum biogas potential of a substrate but is altered to suppress methanogenic activity and instead favour acidification and acetogenesis. The test method was developed and optimized with > 60 samples of urban biowaste and WWTP sludge, collected during two sampling campaigns from VOLATILE project partners. The same sample categories as defined before were used to aggregate all tested samples

The tests revealed that food waste showed the highest VFA potential, followed by VGF waste and OF-MSW, and secondary WWTP sludge with the lower potential. Digested WWTP sludge and roadside grass showed a low VFA potential and are deemed unfeasible at the moment to be integrated in a VFA platform (Figure 4). These results also indicate that the biogas potential is a good indication for a first evaluation of the suitability of a given substrate for VFA production (as the products with the highest biogas potential also resulted in the highest VFA potential). Another important conclusion from these extensive tests is that, under identical process conditions, the chemical composition of the biowaste determines the VFA spectrum that is obtained (Figure 4). On average, OF-MSW results in a slightly higher share of acetic acid compared to VFG waste, which is compensated by mainly a lower valeric and caproic acid content. Food waste has on average the lowest yield in acetic acid, but a highest yield in valeric and caproic acid. Propionic acid and butyric acid showed the least variation between these three waste categories. Also remarkable is that WWTP sludge, both before and after anaerobic digestion, are the only waste category that also led to the production of substantial amounts of the iso-forms of butyric and valeric acid.

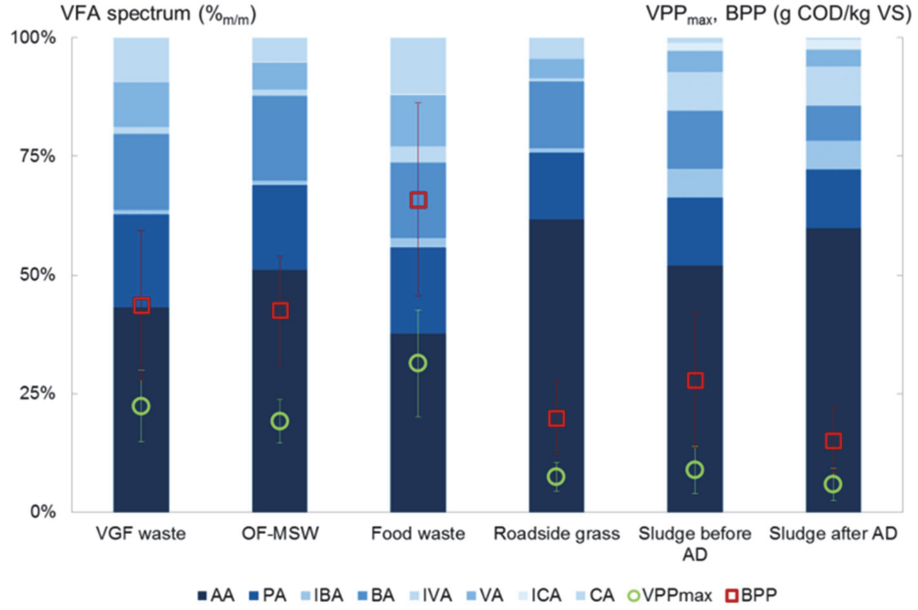


Figure 4: Average VPP max (green circle), average BPP (red square) and VFA spectrum composition for the six waste stream categories

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Next, a pre-treatment step was developed and tested to separate the heterogeneous bio-waste streams in a fraction for VFA recovery and a residual fraction to be used for biogas production, integrating material and energy recovery. The optimized pre-treatment process was able to retain 93% of the maximum VFA potential in an easy treatable (liquid) matrix and a secondary stream which still contained 48% of the biogas potential of the untreated waste (Figure 5).

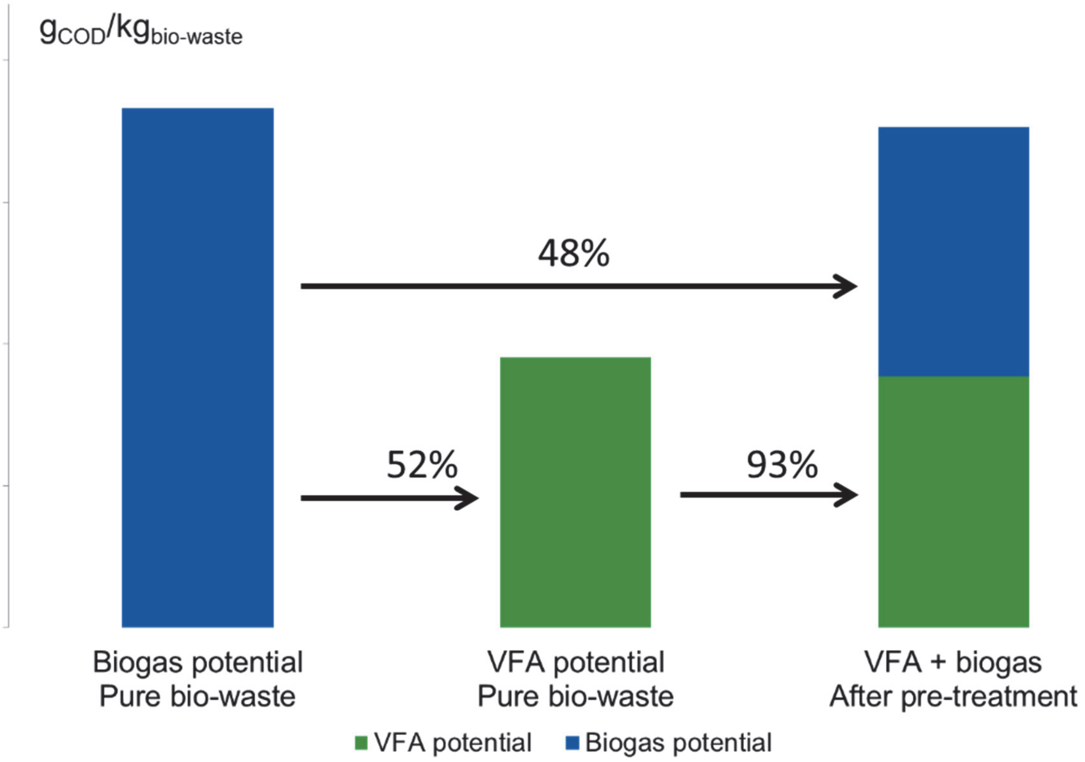


Figure 5: VFA and biogas potential after pre-treatment of bio-waste

3. VFA PURIFICATION AND CONCENTRATION

VFAs are promising feedstocks for multiple industrial applications. However, beside VFA generation via dark fermentation, sustainable downstream processing and VFA recovery and purification needs special attention. Especially for the use as carbon source for fermentation, it is necessary to purify and concentrate the VFA rich effluent from dark fermentation.

Considering the complex physicochemical nature of biowaste and resulting dark fermentation effluents, the separation of VFAs is not a straightforward process (Atasoy et al., 2018). VFA rich effluent is a mixture of soluble organic compounds such as carbohydrates, proteins, fats as well as salts and heavy metals in addition to VFAs and high amounts of particulate and suspended matter (Yin et al., 2016).

In order to recover the VFAs from the fermented broth different membrane technologies have been deeply studied such as electrodialysis (Baroi et al., 2015, 2017; Dai et al., 2019; Jones et al., 2015, 2017), pervaporation (Cheng et al., 2017; Choudhari et al., 2015; Fasahati & Liu, 2015; Işiklan & Şanli, 2005) as well as other technologies as distillation or vacuum distillation (Li et al., 2015).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Within the VOLATILE project pressure driven membrane technology was selected due to its numerous advantages. It is easy to scale-up and integrate into an already developed industrial process and it is expected to successfully filtrate and remove non-desirable substances from the VFA rich stream. VFA separation and concentration can be achieved through a sequential process of filtration: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The cascade scheme is shown in Figure 6.

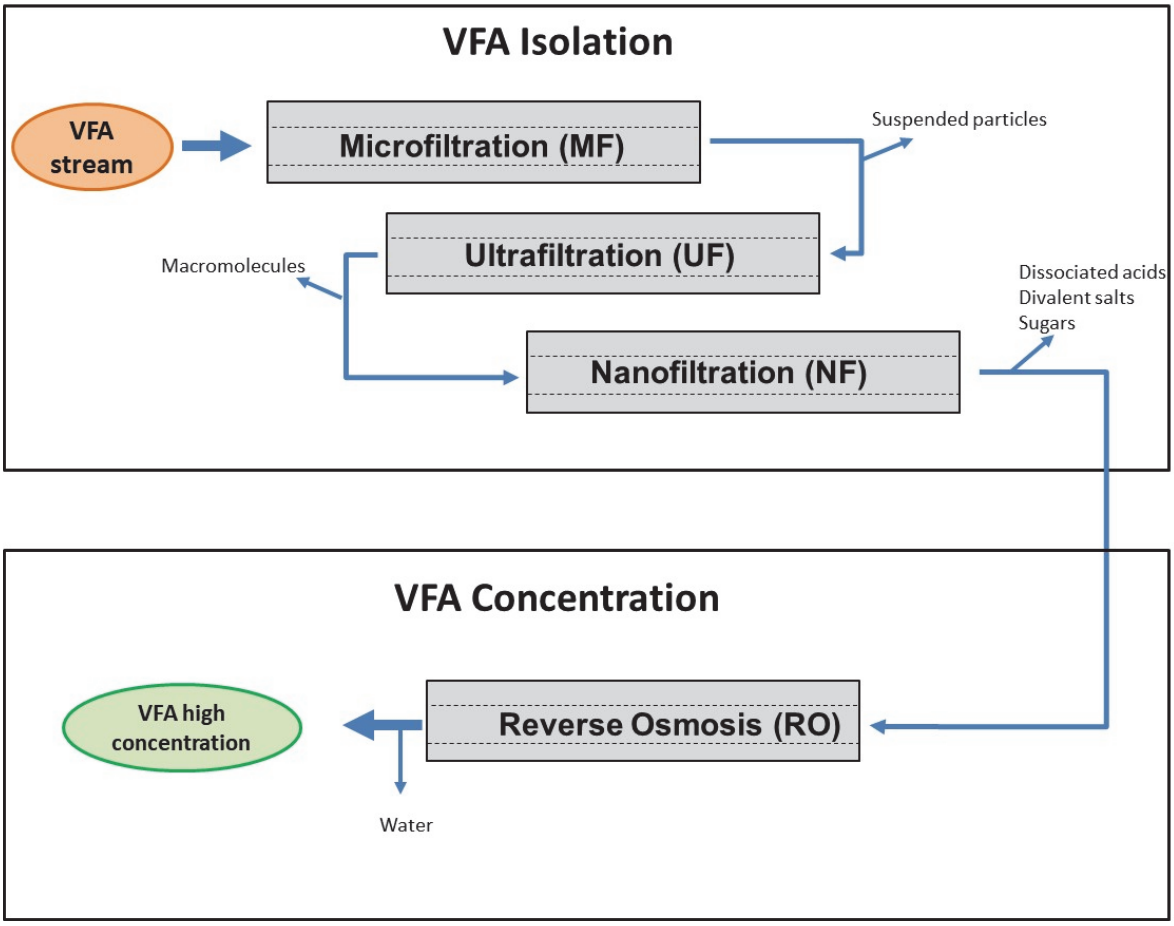


Figure 6: Sequential process of VFA membrane filtration

In a first step, a clarification of the dark fermentation effluent is performed using microfiltration to remove particulate matter and suspended solids. This procedure was followed by UF and NF steps. UF process retains macromolecules (proteins, polysaccharides, etc.) while NF leads to a permeate free of sugars and bigger molecules. Ceramic membranes were selected for processing the VFA fraction along the filtration cascade processes with pore sizes in a range from 70 nm to <1 nm.

Ceramic membranes belong to the group of inorganic membranes, more specifically the ceramic membranes for liquid filtration belong to the oxide ceramic membranes and are mainly made of aluminium oxide, zirconium oxide or titanium oxide. Compared with other typical membranes for liquid filtration, ceramic membranes provide a lot of advantages such as high chemical resistance (pH-value 0–14), thermal resistance as well as resistance against biological

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

attacks and microorganisms. They are less susceptible to biological degradation and have easy handling and storage. These characteristics facilitate effective cleaning with acidic or alkali solutions, indicating ceramic membranes as ideal candidates for processing complex effluent streams of sludge nature such as biowaste treatment sludge.

After sequential filtration steps it was possible to reduce the solids content up to 75% while maintaining more than 95% initial VFAs in the permeate stream.

After purification of the effluent a concentration step was implemented using reverse osmosis. Higher VFA concentrations are needed to be used as efficient carbon feed for fed-batch fermentation. RO removes water and allows to obtain a clean solution with the desired VFA concentration. A polyamide membrane was selected to develop the RO process, with a high rejection value and resistant to low pH values. Along the project a six times concentration factor was reached through the RO process.

4. UPSCALING

As a final step in the development of a biowaste based VFA Platform, the lab scale experiences were scaled up to a 5 m³ pilot VFA production reactor which was integrated with a 150 m³ AD reactor at the facilities of Twence in the Netherlands (Figure 7). From June to August 2020, 8 production runs were performed using the available food waste as input. Due to the COVID-19 restrictions at that time, the pilot could only be operated from Monday to Friday, and hence the residence time was lower than in the corresponding lab trials. Nevertheless, the VFA yield obtained at 5 m³ scale was comparable to the yields obtained in the lab, albeit slightly lower on average (Figure 8). The output of the last 2 VFA production runs was used to also validate the membrane purification and concentration at a larger scale (Figure 9). During these trial runs, ± 1000 L of fermentation effluent from the VFA pilot reactor was purified and concentrated to ± 200 L of RO concentrate, which was further used as input for pilot scale production of PHA, SCO and omega-3 fatty acids.

Figure 7: The VOLATILE pilot VFA production reactor

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

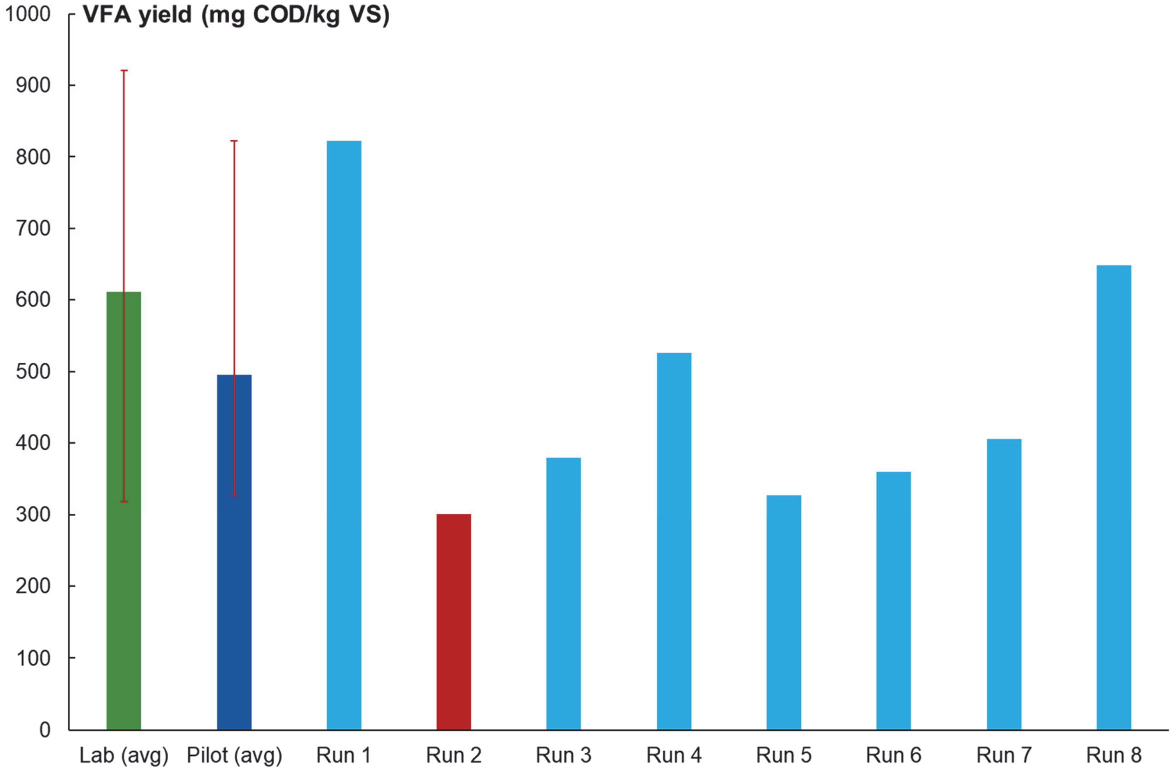


Figure 8: VFA yield of Twence food waste at lab scale (green, HRT of 11 days) and pilot scale (blue, HRT of 4 days); pilot run 2 (red) was stopped at an HRT of 2 days due to technical issues

Figure 9: Validation of centrifugation, ultra- and nanofiltration and reverse osmosis at pilot scale

5. CONCLUSIONS AND FUTURE CHALLENGES

During the 4 years of VOLATILE research, strong progress has been made in the development of a VFA platform using heterogenous biowaste as sole input stream. First of all, a reliable test protocol has been developed at lab scale to determine the VFA potential and expected composition of a given input stream. Next, the VFA production was successfully integrated with anaerobic digestion at lab scale, maximizing the combined production of chemical building blocks and biogas. In parallel, a membrane filtration cascade was optimized to purify and concentrate the VFAs from the reactor output. Finally, the lab results obtained during the first 3 years of the project were validated at pilot scale during the final project year, resulting in a TRL5 technology.

Despite the successes that were obtained during the VOLATILE project, new challenges lay ahead. A further upscaling to TRL7 is needed, but more importantly is to gain deeper insights in the biological process control to improve the stability of the VFA spectrum, and even to steer this VFA spectrum towards the desired composition for specific applications.

REFERENCES

Appels, L., Baeyens, J., Degrève, J., Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34(6): 755-781

Atasoy, M., Owusu-Agyeman, I., Plaza, E., & Cetecioglu, Z. (2018). Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. In *Bioresource Technology* (Vol. 268, pp. 773–786). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2018.07.042

Baroi, G. N., Gavala, H. N., Westermann, P., & Skiadas, I. v. (2017). Fermentative production of butyric acid from wheat straw: Economic evaluation. *Industrial Crops and Products*, *104*, 68–80. https://doi.org/10.1016/j.indcrop.2017.04.008

Baroi, G. N., Skiadas, I. v., Westermann, P., & Gavala, H. N. (2015). Continuous Fermentation of Wheat Straw Hydrolysate by Clostridium tyrobutyricum with In-Situ Acids Removal. *Waste and Biomass Valorization*, *6*(3), 317–326. https://doi.org/10.1007/s12649-015-9348-5

Cheng, X., Pan, F., Wang, M., Li, W., Song, Y., Liu, G., Yang, H., Gao, B., Wu, H., & Jiang, Z. (2017). Hybrid membranes for pervaporation separations. In *Journal of Membrane Science* (Vol. 541, pp. 329–346). Elsevier B.V. https://doi.org/10.1016/j.memsci.2017.07.009

Choudhari, S. K., Cerrone, F., Woods, T., Joyce, K., O'Flaherty, V., O'Connor, K., & Babu, R. (2015). Pervaporation separation of butyric acid from aqueous and anaerobic digestion (AD) solutions using PEBA based composite membranes. *Journal of Industrial and Engineering Chemistry*, *23*, 163–170. https://doi.org/10.1016/j.jiec.2014.08.010

Dai, K., Wen, J. L., Wang, Y. L., Wu, Z. G., Zhao, P. J., Zhang, H. H., Wang, J. J., Zeng, R. J., & Zhang, F. (2019). Impacts of medium composition and applied current on recovery of volatile fatty acids during coupling of electrodialysis with an anaerobic digester. *Journal of Cleaner Production*, *207*, 483–489. https://doi.org/10.1016/j.jclepro.2018.10.019

Fasahati, P., & Liu, J. J. (2015). Impact of volatile fatty acid recovery on economics of ethanol production from brown algae via mixed alcohol synthesis. *Chemical Engineering Research and Design*, *98*, 107–122. https://doi.org/10.1016/j.cherd.2015.04.013

Işiklan, N., & Şanli, O. (2005). Separation characteristics of acetic acid-water mixtures by pervaporation using poly(vinyl alcohol) membranes modified with malic acid. *Chemical Engineering and Processing: Process Intensification*, *44*(9), 1019–1027. https://doi.org/10.1016/j.cep.2005.01.005

Jones, R. J., Massanet-Nicolau, J., Guwy, A., Premier, G. C., Dinsdale, R. M., & Reilly, M. (2015). Removal and recovery of inhibitory volatile fatty acids from mixed acid fermentations by conventional electrodialysis. *Bioresource Technology*, *189*, 279–284. https://doi.org/10.1016/j.biortech.2015.04.001

Jones, R. J., Massanet-Nicolau, J., Mulder, M. J. J., Premier, G., Dinsdale, R., & Guwy, A. (2017). Increased biohydrogen yields, volatile fatty acid production and substrate utilisation rates via the electrodialysis of a continually fed sucrose fermenter. *Bioresource Technology*, *229*, 46–52. https://doi.org/10.1016/j.biortech.2017.01.015

Li, Q. Z., Jiang, X. L., Feng, X. J., Wang, J. M., Sun, C., Zhang, H. B., Xian, M., & Liu, H. Z. (2015). Recovery processes of organic acids from fermentation broths in the biomass-based industry. In *Journal of Microbiology and Biotechnology* (Vol. 26, Issue 1, pp. 1–8). Korean Society for Microbiolog and Biotechnology. https://doi.org/10.4014/jmb.1505.05049

Yin, J., Yu, X., Wang, K., & Shen, D. (2016). Acidogenic fermentation of the main substrates of food waste to produce volatile fatty acids. *International Journal of Hydrogen Energy*, *41*(46), 21713–21720. https://doi.org/10.1016/j.ijhydene.2016.07.094

PRODUCTION OF BIOPOLYESTERS FROM WASTE

A. Carolas [a], *B.S. Ferreira* [a],

[a] *BIOTREND - INOVACAO E ENGENHARIA EM BIOTECNOLOGIA SA, Biocant Park - Nucleo 4, Lote 2, 3060-197 Cantanhede, Portugal*

Corresponding author: alcarolas@biotrend.pt

Abstract

Most of the plastics currently used worldwide are produced from non-renewable and non-biodegradable sources. In an effort to reduce the impact of the plastics in the environment, alternative production methods and end-of life management have been studied for decades now. Several microorganisms have the ability to naturally produce plastics, using different substrates, which are biodegradable and biocompatible in certain conditions. In this work, the production of polyhydroxyalkanoates (PHA) using either commercial volatile fatty acids (VFA) or VFA produced from waste, has been demonstrated for a *Paraburkholderia* strain, in fed-batch fermentations. The effect of different feeding strategies, namely a pH-STAT and pulses/pre-programmed feed rates, as well as different feed profiles and composition has been assessed. Overall, a feeding strategy by pulses, using a balanced feed profile to avoid both carbon limitation and excessive accumulation of VFA, in combination with a feed supplemented with citric acid, resulted in relative improved results specially regarding PHA concentration, content in cells and productivity.

Keywords: VFA, PHA, *Paraburkholderia*, fed-batch, co-polymer

1. INTRODUCTION

In the last decades plastics have been playing an increasingly important role in almost all sectors of our life, with innovative applications being developed as stand-alone products or as parts of other devices. However, the vast majority of the currently used plastics depend on non-renewable fossil resources and are not biodegradable. Although a significant effort has been made in the recent years to find solutions for this waste, the toll of fossil fuel-based plastics to the environment is still dramatic, with a current estimate that 1200 million metric tonnes of plastic will be in landfill or the environment by 2050 (Geyer at al., 2017).

One promising alternative to the traditional plastics are biopolyesters such as polyhydroxyalkanoates, produced by naturally occurring microorganisms which have physicochemical properties similar to conventional plastics. However, contrary to the latter, PHA are biodegradable and/or bio compostable given the appropriate conditions (Szacherska et al., 2021).

Despite the advantages of the bio-based plastics, their current high production cost when compared to the fossil fuel alternative has been hampering the efforts for a large-scale PHA production and widespread use of the more environmentally friendly plastics. Further, most current production processes implemented commercially use sugars or oils as main carbon source, competing with food and feed uses. One way to reduce the production cost and improve the sustainability of production is to replace refined sugars with carbon sources derived from existing wastes or side-streams, namely VFA obtained from the treatment of household and/or agro-industrial wastes (Hasan et al., 2015), contributing for the transition to a circular economy.

The biosynthesis of PHA is achieved under controlled conditions in bioreactors, by inducing a nutrient limitation such as nitrogen, or phosphorous, with excess carbon (Steinbüchel at al., 2001). The production of PHA is regulated by several mechanics, namely at the enzymatic level, in which citrate synthase could be a control point for PHA production (Kessler at al., 2001). In the tri-carboxylic acid (TCA) cycle, citric acid is an intermediary resulting from the reaction of acetyl-CoA with oxaloacetate catalysed by citrate synthase. Furthermore, Passanha et al. (2013), described the importance of magnesium in the ability of several gram-positive and gram-negative bacteria to sustain growth and phosphate is a well-known key component in essential anabolic processes, such as the production of nucleic acids and phospholipids (Oliveira-Filho et al., 2020).

This work aimed at demonstrating that optimization of biomass and PHA production conditions is key to improve process productivity in fed-batch fermentations using VFA to feed the culture. The effect of different feeding strategies was tested (pH-STAT, pulses/pre-programmed feed rates), as well as different feed profiles, when feeding by pulses/pre-programmed feed rates was implemented. In addition, the effect of changing the feed composition was also assessed, considering the relevance of citric acid, phosphate and magnesium in metabolic processes either associated with PHA and biomass production.

2. MATERIALS AND METHODS

Paraburkholderia was maintained cryo-preserved at -80 ºC in 30 % glycerol. The fed-batch fermentations were carried out in 3 L BioFlo 115 fermenters (Eppendorf, Germany) equipped with dissolved oxygen probes and pH electrodes. Fermentations were controlled at 30 ºC and a fixed air flow of 1 vvm. Initially the dissolved oxygen was controlled at 20 % of the maximum oxygen saturation by varying the stirrer speed between 200 and 1200 rpm. pH was controlled at 6.45 by automatic addition of a base (NH_4OH or NaOH) and acid, that could be H_2SO_4 or a solution of VFA in the case of a pH-STAT fermentation strategy was being implemented. When a feed strategy by pulses/pre-programmed feed rates was implemented, an external peristaltic pump was used to feed the VFA solution into the fermenter. The VFA were either a mixture of commercial VFAs (39 % acetic acid, 30 % propionic acid, 20 % butyric acid and 10 % valeric acid) or VFA produced by anaerobic digestion of waste (OWS, Belgium) and concentrated by Tecnalia (Spain) with an average composition of 41 % acetic acid, 24 % propionic acid, 22 % butyric acid, 9 % valeric acid and 3 % caproic acid. The latter was used in fed-batch fermentations in which the feed strategy was tested (pH-STAT or by pulses/pre-programmed feed rates) while the former was used to assess the role of changing the feed profile and composition in biomass and PHA productions.

The initial medium was inoculated with 5 % inoculum. The inoculum for each fermentation was produced in shake flasks, incubated at 30 ºC and 150 rpm, in an orbital shaker (50 mm orbit diameter).

Throughout the fermentations, samples were collected and analysed. Growth was assessed by the measurement of the optical density at a wavelength of 600 nm in a spectrophotometer (Shimadzu UV-1700, Japan) after appropriate dilution using reverse-osmosis (RO) water. In

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

addition, the cell dry weight (CDW) was determined by transferring a known volume of sample into a dry microtube. After two washing steps with RO water, the pellet was allowed to dry at 70 ºC until no variation of weight was measured.

PHA concentration and monomeric composition were obtained by gas-chromatography coupled with mass spectrometry (Shimadzu GCMS-QP2020, Japan), using a ZB-5MS plus column and helium as carrier gas.

3. RESULTS AND DISCUSSION

3.1 *Effect of different feeding strategies – pH-STAT and Pulses/Pre-programmed feed rates*

The optimization of the process productivity and product quality is dependent on multiple factors such as the cultivation regime and process design, which have to be adapted to the physiological and kinetic particularities of the biological system, as well as the substrate used (Koller, 2018).

When the substrate is fed using a pH-STAT strategy, the VFA are automatically added as agent to control the pH at the desired set-point. This means that low amounts of VFA are added to the culture at once and only when they are needed, avoiding accumulation of VFA, which are detrimental to the culture due to their toxicity (Koller, 2018). On the other hand, the supply of carbon is always limiting, leading to lower productivities and limiting the PHA accumulation potential since carbon is not in excess.

Figure 1 shows the maximum results obtained for the fermentations using different feeding strategies. The pH-STAT strategy resulted in a significant improvement in terms of biomass and PHA production when compared to the strategy in which VFA were fed by pulses/pre-programmed feed rates. In the pH-STAT, an automatic addition of VFA is triggered whenever the pH rises above the set-point due to the uptake of VFA, thus supplying more carbon to the culture when needed. Contrarily, a feeding strategy by pulses/pre-programmed feed rates require an optimization of the feed rates to take into account the VFA consumption rates, also to accommodate any changes in the culture metabolism. In this fermentation there were periods in which carbon was limiting, certainly affecting the culture performance.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

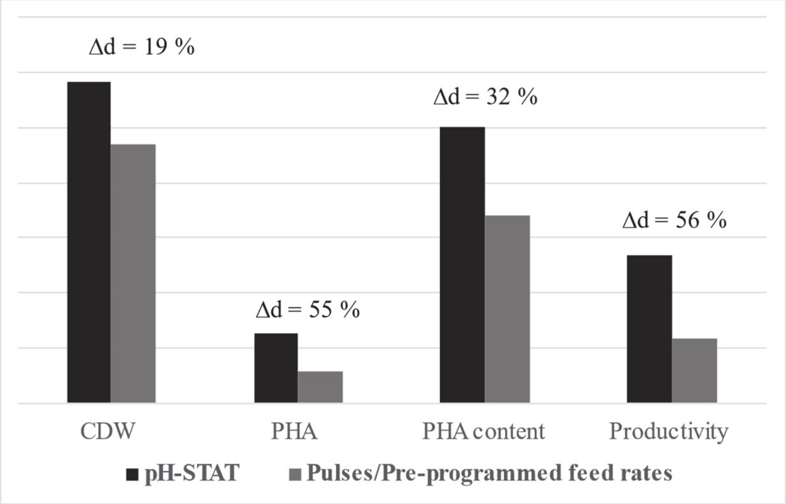


Figure 1: Comparative maximum results obtained in fed-batch fermentations using a pH-STAT or pulses/pre-programmed feeding rates as feeding strategies, using as feed VFA produced from waste. The decrease (Δd) or increase (Δi) in the production of biomass (CDW) and production of PHA (concentration, cell content and productivity) are shown.

Another important parameter to consider is the monomeric composition of the polymer, as it affects the final mechanical properties of the polymer and consequently its intended application. A polymer mostly composed by hydroxybutyrate will be brittle. As the hydroxyvalerate (HV) content increases the resultant polymer will be more elastic in nature (Singh at al., 2015). In both fermentations, a copolymer of P(3HB:3HV) was obtained, although the feeding strategy by pulses/pre-programmed feed rates resulted in a polymer with higher content of hydroxyvalerate, far more versatile than the homopolymer PHB. Figure 2 shows an improvement of 41 % in the HV content in the fermentation in which VFA were added by pulses/pre-programmed feed rates.

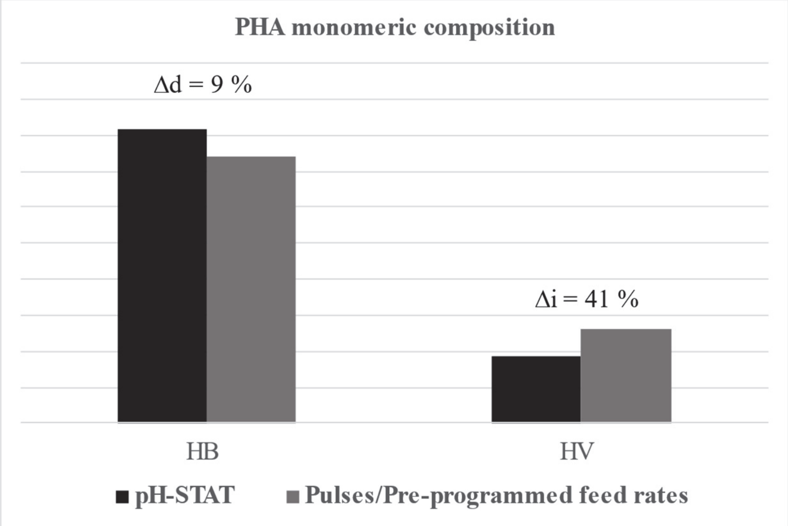


Figure 2: Comparative monomeric composition of the PHA obtained in fed-batch fermentations using a pH-STAT or pulses/pre-programmed feed rates as feeding strategies, using as feed VFA produced from waste. The decrease (Δd) or increase (Δi) in the hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers content are shown.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

3.2 *Effect of different feed profiles*

Despite the overall best results observed in the pH-STAT feeding strategy, the polymer resulting from a fermentation fed by pulses/pre-programmed feed rates was richer in HV. Thus, optimization of the feed profiles was carried out in order to improve biomass and PHA yields and productivity. Two feed profiles were tested, one of which was less conservative (Feed profile 1) than the other (Feed profile 2). By better responding to the culture demand for carbon, through avoiding carbon limitation but also excessive accumulation of VFA, it was possible to improve both biomass and PHA production, as shown in Figure 3.

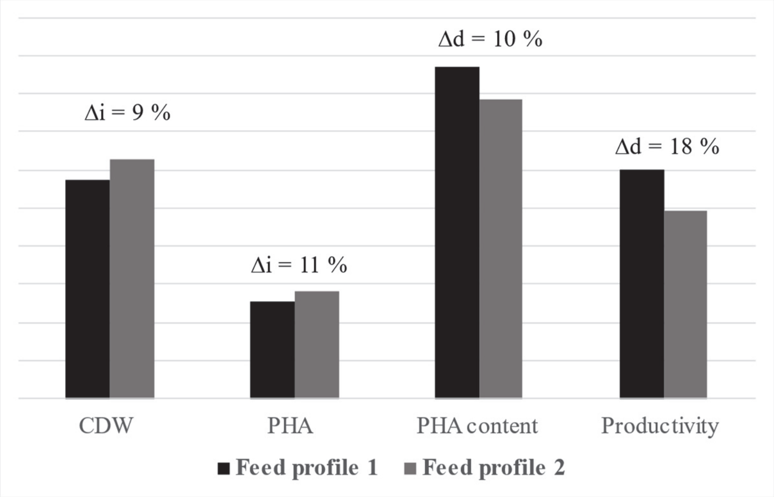


Figure 3: Comparative maximum results obtained in fed-batch fermentations fed with a mixture of commercial VFA by pulses, with different feed profiles (Profile 1 and Profile 2). The decrease (Δd) or increase (Δi) in the production of biomass (CDW) and production of PHA (concentration, cell content and productivity) are shown.

However, the PHA content and productivity decreased, as a result of the more conservative feed rates imposed. On the contrary, the HV content was favoured by the more conservative feed, as Figure 4 shows, although the differences were not as substantial as the ones observed in fermentations using different feeding strategies.

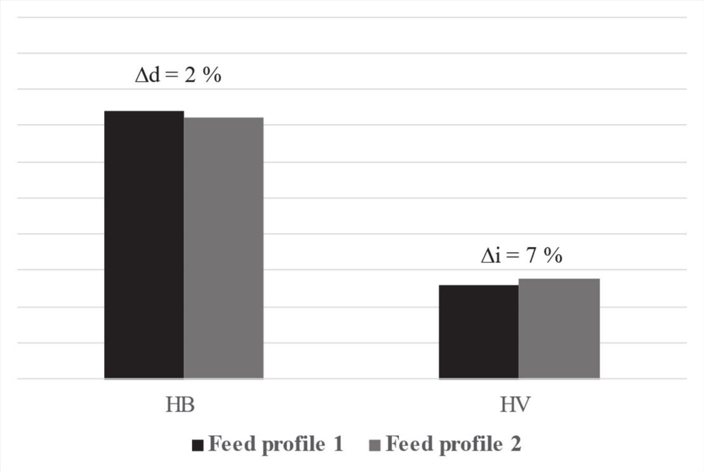


Figure 4: Comparative monomeric composition of the PHA obtained in fed-batch fermentations fed with a mixture of commercial VFA by pulses, with different feed profiles (Profile 1 and Profile 2). The decrease (Δd) or increase (Δi) in the hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers content are shown.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

3.3 Effect of different feed composition

Using the same feeding strategy, solutions of commercial VFA were supplemented either with citric acid or with KH_2PO_4 and $MgSO_4$ and their effect in biomass and PHA accumulation was assessed. Figure 5 shows the effect of changing the feed composition.

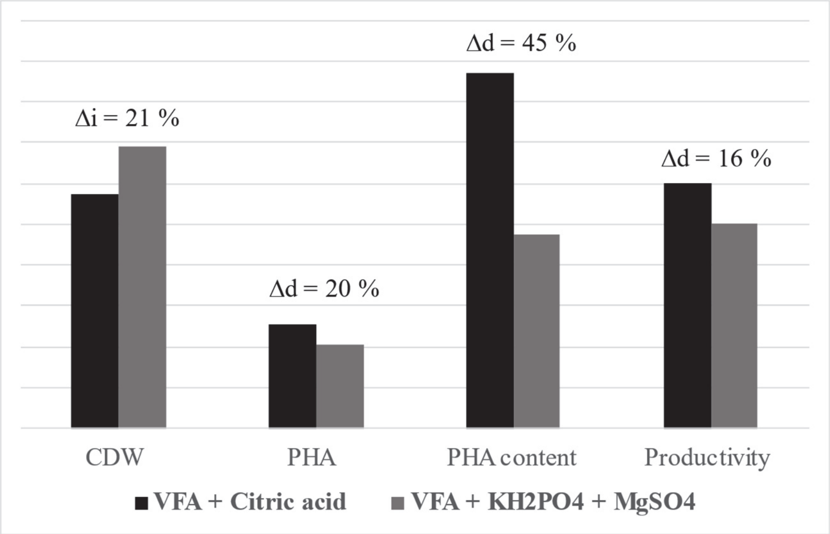


Figure 5: Comparative maximum results obtained in fed-batch fermentations fed with a mixture with different feed compositions (VFA+citric acid or VFA+KH_2PO_4+$MgSO_4$). (Profile 1 and Profile 2). The decrease (Δd) or increase (Δi) in the production of biomass (CDW) and production of PHA (concentration, cell content and productivity) are shown.

Supplementing the feed with phosphate and magnesium showed only improved results regarding biomass accumulation, confirming their role in metabolic processes associated with cell growth. Overall, the citric acid had a major impact in the performance of the culture, particularly in the PHA content in cells, with an improvement of 45 %, thus supporting the results obtained by Lee (1995) for a *Cupriavidus necator* strain cultivated in nitrogen-limited media. In these conditions, the NADPH/NADP and NADH/NAD ratios and the intracellular concentrations of NADH and NADP were higher than those found under nitrogen sufficient conditions, resulting in the inhibition of citrate synthase, thus funnelling the carbon to PHA instead of being directed to the tricarboxylic acid cycle. By supplementing the culture with citrate, a bypass to the reaction of oxaloacetate and acetyl-CoA allows closing the loop in the TCA cycle maintaining the culture ability to sustain growth while producing PHA, hence explaining the results obtained. Figure 6 shows that no significant changes were observed regarding the polymer monomeric composition.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

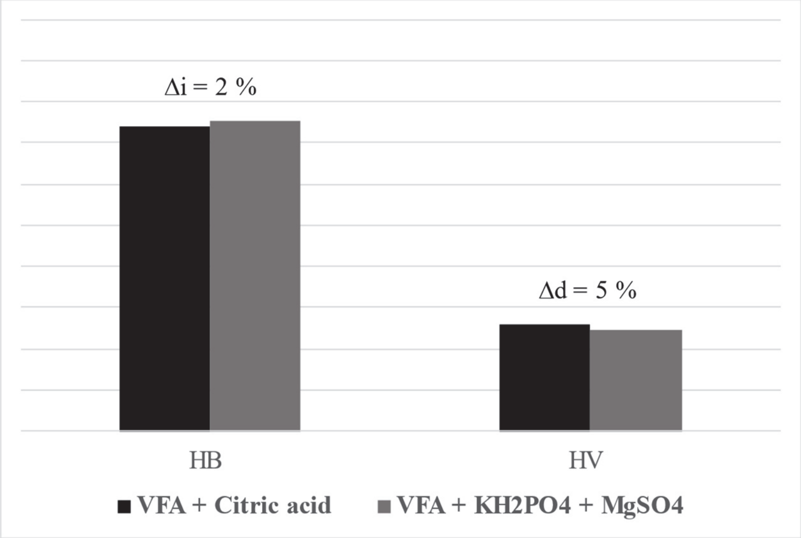


Figure 6: Comparative monomeric composition of the PHA obtained in fed-batch fermentations fed with a mixture of commercial VFA by pulses, with different feed compositions (VFA+citric acid or VFA+KH_2PO_4+$MgSO_4$). The decrease (Δd) or increase (Δi) in the hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers content are shown.

4. CONCLUSION

The effect in biomass and PHA production of different feed strategies using VFA as feed, including the implementation of different feed profiles and feed compositions, have been successfully demonstrated in this work for fed-batch fermentations. Overall, improved biomass and PHA production were reached by implementing a feed regimen using pre-programmed feed rates, in which the feed profile implemented avoids significant accumulation of VFA. Moreover, supplementing the VFA stream with citric acid resulted in improved PHA production, namely PHA cell content, concentration and productivity. This work shows that the optimization of PHA production is a complex process which involves multiple variables, some of which were not addressed. Nonetheless, it validated the use of VFA from waste as a possible inexpensive carbon source for the sustainable production of PHA, eventually allowing closing the loop in the circular economy concept for biopolyester production.

5. REFERENCES

Geyer, R., Jambeck, J.R., Law, K.L. (2017) Production, use, and fate of all plastics ever made. Science Advances 3,7, 1-5. doi: 10.1126/sciadv.1700782

Hasan, S. D. M., Giongo, C., Fiorese, M. L., Gomes, S. D., Ferrari, T. C., Savoldi, T. E., (2015). Volatile fatty acids production from anaerobic treatment of cassava waste water: effect of temperature and alkalinity, Environmental Technology. doi: 10.1080/09593330.2015.1041426

Kessler, B., Witholt, B. (2001). Factors involved in the regulatory network of polyhydroxyalkanoate metabolism. Journal of Biotechnology 86, 97-104. doi:10.1016/s0168-1656(00)00404-1

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Lee, I. (1995). Regulation of poly-β-hydroxybutyrate biosynthesis by nicotinamide nucleotide in *Alcaligenes eutrophus*. *FEMS Microbiology Letters.* 131, 35–39. doi: 10.1016/0378-1097(95)00231-S

Oliveira-Filho, E.R., Silva, J.G.P., de Macedo, M.A., Taciro, M.K., Gomez, J.G.C. and Silva LF (2020) Investigating Nutrient Limitation Role on Improvement of Growth and Poly(3-Hydroxybutyrate) Accumulation by *Burkholderia sacchari* LMG 19450 From Xylose as the Sole Carbon Source. Frontiers in Bioengineering and Biotechnology. 7, 416. doi: 10.3389/fbioe.2019.00416

Passanha, P., Esteves, S. R., Kedia, G., Dinsdale, R. M., & Guwy, A. J. (2013). Increasing polyhydroxyalkanoate (PHA) yields from *Cupriavidus necator* by using filtered digestate liquors. Bioresource Technology, 147, 345–352. doi:10.1016/j.biortech.2013.08.050

Singh, M., Kumar, P., Ray, S., & Kalia, V. C. (2015). Challenges and Opportunities for Customizing Polyhydroxyalkanoates. Indian journal of microbiology, 55(3), 235–249. https://doi.org/10.1007/s12088-015-0528-6

Steinbüchel, A., and Hein, S. (2001). Biochemical and molecular basis of microbial synthesis of polyhydroxyalkanoates in microorganisms. Byopolyesters. Advances in Biochemical Engineering/Biotechnology, 71,81–123. doi: 10.1007/3-540-40021-4_3

Szacherska, K.; Oleskowicz-Popiel, P.; Ciesielski, S.; Mozejko-Ciesielska, J. (2021). Volatile Fatty Acids as Carbon Sources for Polyhydroxyalkanoates Production. Polymers 13, 321. https:// doi.org/10.3390/polym13030321

CULTIVATION OF OLEAGINOUS YEASTS FOR SINGLE CELL OIL PRODUCTION

L. Burgstaller [a], *C. Pais* [b], *P. Sampaio* [b], *L. Oliver* [c], *M. Neureiter* [a]
[a] *Universität für Bodenkultur Wien, Gregor Mendel Strasse 33, 1180 Wien, Austria*
[b] *Universidade do Minho, Largo do Paco, 4704 553 Braga, Portugal*
[c] *TECNALIA, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Álava, Leonardo Da Vinci 1, 01510 Miñano, Álava, Spain*
Corresponding author: *markus.neureiter@boku.ac.at*

Abstract

The use of waste as a resource is a cornerstone of the transition from a linear to a circular economy. A technology that allows the utilization of various organic wastes is the production of volatile fatty acids (VFAs) via anaerobic digestion (AD). The produced VFAs can subsequently be used as substrate for the biotechnological production of various materials. Amongst others, lipids can be produced from VFAs by some yeasts. Although most of the described processes for the production of these so-called single cell oils (SCOs) are based on the utilization of various sugar rich substrates, AD derived VFAs are also an attractive carbon source. While literature on VFA-derived SCOs mostly focuses on a few selected oleaginous yeast strains, this work demonstrates the SCO production potential of two yeast strains, *Apiotrichum brassicae* V134 and *Pichia kudriavzevii* V194 that have only recently been described as oleaginous. For this purpose, a pH-stat fed batch process using a mixture of acetic, propionic and butyric acid as carbon source was implemented. This way, high volumetric SCO productivities of 0.245 g/Lh and 0.386 g/Lh could be demonstrated for strains V134 and V194 respectively. Maximal SCO concentrations reached were 16.2 g/L and 25.5 g/L, respectively. It could thus be shown, that both strains are well suited for the VFA based SCO production, increasing the range of strains available for this application.

Keywords: volatile fatty acids, lipids, fed-batch process

1. INTRODUCTION

One of the main challenges of this century will be the transition from a linear economy – 'take, make and dispose' – to a circular economy due to the finite nature of natural resources available. A major factor in implementing a circular economy is the sustainable use of waste as a resource. The concept of such 'waste biorefineries' can be employed to produce biofuels, bioplastics, platform chemicals or other materials (Mohan et al., 2019). A technology that can be used for the transformation of a wide range of organic wastes to the platform chemicals acetic acid, propionic acid and butyric acid is anaerobic digestion (Bettencourt et al., 2020; Llamas et al., 2020a). These VFAs can for instance be used as substrate for the biotechnological production of polyhydroxyalkanoates by bacteria (Cerrone et al., 2014), omega 3-fatty acids by microalgae (Chalima et al., 2019) or lipids by so called oleaginous yeasts (Kolouchová et al., 2015). The lipids produced by these yeasts are similar in composition to various plant oils and can thus be used in a similar fashion. For these yeasts, the lipids act as storage compound for carbon and energy. The SCO is usually accumulated when carbon is available in excess but other nutrients like nitrogen or phosphorous are limited. Given the right conditions, SCO contents above 70% of the yeasts dry cell weight (DCW) have been reported (Beopoulos et al., 2011). While ample

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

literature is available on the production of SCO using various sugars, sugar containing hydrolysates or glycerol (Papanikolaou and Aggelis, 2011), literature on the utilization of VFAs for SCO production is somewhat scarce. In available literature the focus is mainly put on batch cultivation of a few selected strains like *Cryptococcus curvatus*, *Cryptococcus albidus* or *Yarrowia lipolytica* (Fei et al., 2011; Llamas et al., 2020b; Park et al., 2017). This work, however, aims to demonstrate the suitability of the two recently described oleaginous yeast strains *Apiotrichum brassicae* V134 and *Pichia kudriavzevii* V194 for VFA based SCO production. To allow for high volumetric lipid productivities a pH-stat fed-batch approach is proposed in this work.

2. MATERIALS AND METHODS

2.1 Yeast strains

Using a high-throughput Nile red fluorescence assay Miranda et al. (2020) screened 366 yeast isolates for their ability to produce SCO with acetic acid as carbon source. *Apiotrichum brassicae* V134 and *Pichia kudriavzevii* V194 were identified as the most promising strains in this screening. DNA sequences of both strains V134 and V194 are deposited in the NCBI GenBank database. The respective accession numbers are MN913458 and MN913463 (Miranda et al., 2020). Cultures of both strains were maintained on a yeast extract, peptone, dextrose (YPD) medium supplemented with glycerol (20% v/v) as cryo-culture (–80 °C). YPD medium contained 5 g/L yeast extract (Ohly KAT; Ohly, Hamburg, Germany), 10 g/L peptone from casein (Casein Peptone E1 19546; Organotechnie, La Courneuve France) and 20 g/L glucose.

2.2 Temperature optimum of selected strains

Both yeast strains were cultivated in 300 mL baffled shake flasks containing 100 mL of YPD medium. The medium was autoclaved at 121 °C for 30 min. Glucose was autoclaved separately as a 200 g/L stock solution. Each shake flask was inoculated with 50 µL of cryo-culture (V134 OD_{600}: 35; V194 OD_{600}: 27). Shake flasks were incubated in a rotary shaker at 130 rpm for 40 h. Temperature was controlled either at 20 °C, 25 °C, 30 °C, 35 °C or 40 °C. Cultivation at each temperature was performed in triplicate.

2.3 Cultivation of selected strains in bioreactor

For cultivation of both strains a lab-scale parallel bioreactor system (DASGIP; Eppendorf, Hamburg, Germany) was used. The reactors were equipped with a temperature probe, a pH electrode (405-DPAS-SC-K8S, Mettler Toledo, Columbus, USA) and a dissolved oxygen (DO) sensor (VisiFerm DO ECS 225 H0, Hamilton, Bonaduz, Switzerland). DO was maintained above 20% of the maximum oxygen saturation at the given conditions by varying the stirrer speed between 400 and 1600 rpm. Aeration was kept constant at 35 L/h. pH was maintained at 6 by automated addition of either 2M NaOH or a VFA solution (95 g/L HAc, 45 g/L HPr, 50 g/L HBu). Using this pH-stat fed batch system VFA concentration in the reactor was kept low, while ensuring constant supply of substrate. Temperature was maintained at 30 °C. Each reactor was initially filled with 500 mL of medium containing 10 g/L of peptone from casein and 5g/L of yeast extract. 100 µL of antifoam (Glanapon DG160; Bussetti, Vienna, Austria) were added to the reactor at the beginning of all fermentations. Reactors were inoculated with 50 mL of

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

pre-culture (V134 OD_{600}: 30; V194 OD_{600}: 25). This pre-culture was prepared in 300 mL baffled shake flask containing 100 mL YPD medium. These flasks were inoculated with 500 µL cryo-culture and incubated in a rotary shaker for 24h at 30 °C and 130 rpm.

2.4 Analytics – Biomass

When cultivated in shake flasks, growth was only assessed by measuring OD_{600}. For cultivation of the yeasts in bioreactors, a defined amount of sample was centrifuged at 2750 rcf for 7 min, the pellet was washed twice in RO-H_2O, frozen at –80 °C and subsequently freeze dried (Alpha 2-4 LSCplus; Christ, Osterode am Harz, Germany) for 24 h at 0.5 mbar. Based on the pellets weight, the DCW concentration in the fermentation broth was calculated. The supernatant was used for subsequent determination of VFA concentration, Total-Kjehldal Nitrogen (TKN) and phosphate concentration.

2.5 Analytics - VFAs

VFA concentrations in feed and fermentation broth were measured by HPLC analysis (Agilent 1100, Santa Clara, USA). Samples were diluted to appropriate concentrations and Carrez-precipitation was performed. A CARBOSep COREGEL 87H3 (Transgenomic, Omaha, USA) was used for separation, column temperature was set to 65 °C. 5 mM H_2SO_4 was used as mobile phase. Flow rate was set to 0.9 mL/min. Refractive index detection combined with an external calibration were used for quantification of all analytes.

2.6 Analytics – FAME

Determination of the SCO content of the yeast biomass was based on a one step method for simultaneous hydrolysis of the cell biomass and acid catalysed methylation of the fatty acids described by Meesters et al. (1996). The fatty acid methyl esters (FAME) were analysed using a GC-FID system (7890B; Agilent, Santa Clara, USA). For separation, a HP-88 column (length: 100 m, inner diameter: 250 µm, film thickness 0.2 µm; Agilent, Santa Clara, USA) was used. Carrier gas was H_2. Flowrate was kept at of 2 mL/min. Column temperature was 120 °C initially. Subsequently temperature was increased to 230 °C in several steps. Using an internal standard and an external calibration with a 37 component FAME mix (Supelco CRM 47885; Sigma-Aldrich, St. Louis, USA) FAME amounts were quantified. Furthermore, recovery was determined using palm oil (Palm oil analytical standard, Sigma-Aldrich St. Louis, USA)

2.7 Analytics – Free Ammonia and Phosphate

TKN content of the fermentation broth was determined using an AutoKjeldahl Unit K-370 and a Digest Automat K-438 (both Büchi, Flawil, Switzerland). For each sample 2 mL of cell free supernatant were used for the determination. Total phosphorous content was determined photometrically using a LCK350 cuvette test kit (Hach, Loveland, USA).

3. RESULTS AND DISCUSSION

3.1 Temperature optimum of selected strains

For both yeast strains – *Apiotrichum brassicae* V134 and *Pichia kudriavzevii* V194 – growth at several temperatures from 20 °C to 40 °C was assessed. Both yeast strains exhibited limited growth at 35 °C and no growth at all could be detected after 20 h of cultivation at 40 °C (see Figure 1). After 40 h of cultivation at 25 °C, an OD_{600} of 10.4 was reached for strain V134. For

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

cultivation at 30 °C OD_{600} was 11.0. While these values are quite similar, growth behaviour varied. When cultivated at 30 °C the OD_{600} reached a value of 7.6 after 16 h, whereas at 25 °C the OD_{600} was only 2.0. For strain V194 the pattern was similar with the OD_{600} reaching 9.1, 10.9 and 10.0 for cultivation at 20 °C, 25 °C and 30 °C for 40 h. Like for strain V134 growth however was faster at 30 °C reaching an OD_{600} of 7.3 in 16 h. In comparison, the OD_{600} reached at 20 °C and 25 °C after 16 h was considerably lower with values of 0.17 and 1.0, respectively. This data shows that both strains are able to grow in a temperature range of 20 °C to 35 °C. Due to the highest growth speed at 30 °C it is concluded that for both strains 30 °C is the optimal cultivation temperature. This temperature was thus used for subsequent experiments.

3.2 Cultivation of selected strains in bioreactor

Both strains were able to utilize the VFA mixture for growth and SCO production. In 66 h a DCW of 32.9 g/L could be reached for strain V134, the DCW reached for strain V194 was 40.5 g/L (see Figure 2). All VFAs were consumed and no significant accumulation of any VFA was observed for strain V134. For strain V194 the concentration of HPr initially increased from 0.48 g/L to 0.77 g/L in the first 18 h of fermentation. Subsequently the HPr concentration steadily decreased again, reaching a final HPr concentration of 0.24 g/L after 66 h. This suggests that the use of HAc and HBu as carbon source is preferred to the utilization of HPr. However, both strains seem to be able to simultaneously utilize all three VFAs used. If this would not be the case, an accumulation of HPr followed by a phase of HPr utilization would be observed, which is not the case. Significant amounts of SCO could be produced in both strains. After 18 h of fermentation, the phosphorus concentration was virtually depleted by both strains while TKN concentration stayed between 0.3 g/L and 0.6 g/L throughout the fermentation (data not shown). This indicates that the observed SCO production was induced by phosphorous limitation, rather than nitrogen limitation.

While limiting the amount of nitrogen available is a more common strategy for the induction of SCO production (Papanikolaou and Aggelis, 2011), it has also been shown that phosphate limitation can be an equally effective strategy (Wu et al., 2010). The SCO content reached a maximum of 0.49 g_{SCO}/g_{DCW} in strain V134 and 0.63 g_{SCO}/g_{DCW} in strain V194. Combined with the respective DCW concentrations this results in SCO concentrations of 16.2 g/L and 25.5 g/L and a volumetric lipid productivity of 0.386 g/Lh for strain V194 and 0.245 g/Lh for strain V134. Product yield was comparable for both strains reaching 0.184 g_{SCO}/g_{VFA} for strain V194 and 0.172 g_{SCO}/g_{VFA} for strain V134. When compared to values reported in literature, it stands out that the productivities reported in this work are higher than most values reported for VFA based SCO production (Fei et al., 2011; Kolouchová et al., 2015; Llamas et al., 2020b; Park et al., 2017). This most likely is a result of the cultivation mode used. While it is the most commonly reported approach for VFA based SCO production, batch cultivation only allows the use of low VFA concentrations. This is a result of the toxic nature of VFAs at high concentrations and low pH values (Gao et al., 2020). Due to the low substrate concentrations however, only low DCW concentrations can be achieved. The possible volumetric lipid productivity is thus limited as well.

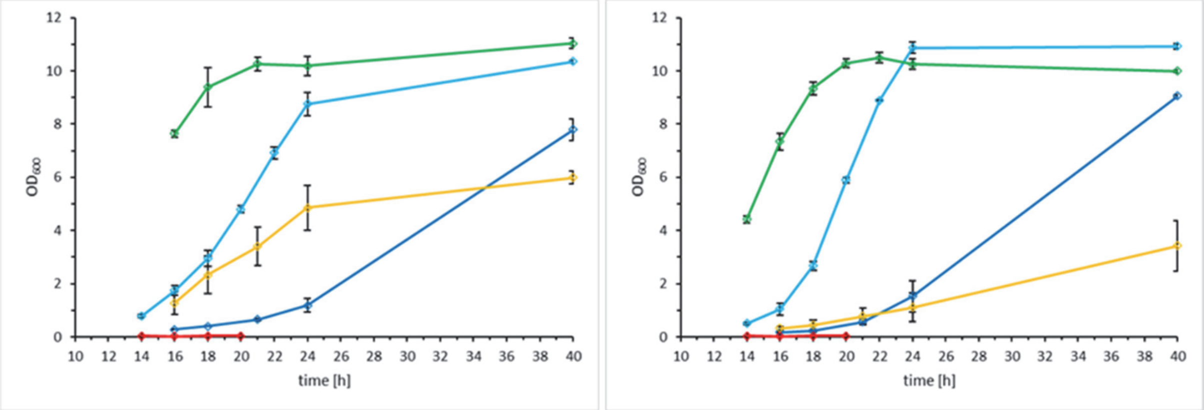


Figure 1: Time course of OD_{600} over 40 h of cultivation for strain V134 (**left**) and V194 (**right**) at 20 °C (dark blue), 25 °C (light blue), 30 °C (green), 35 °C (yellow) and 40 °C (red). For 40 °C cultivation was stopped after 20 h due to a lack of observable growth. Cultivation at all temperatures was performed in triplicate.

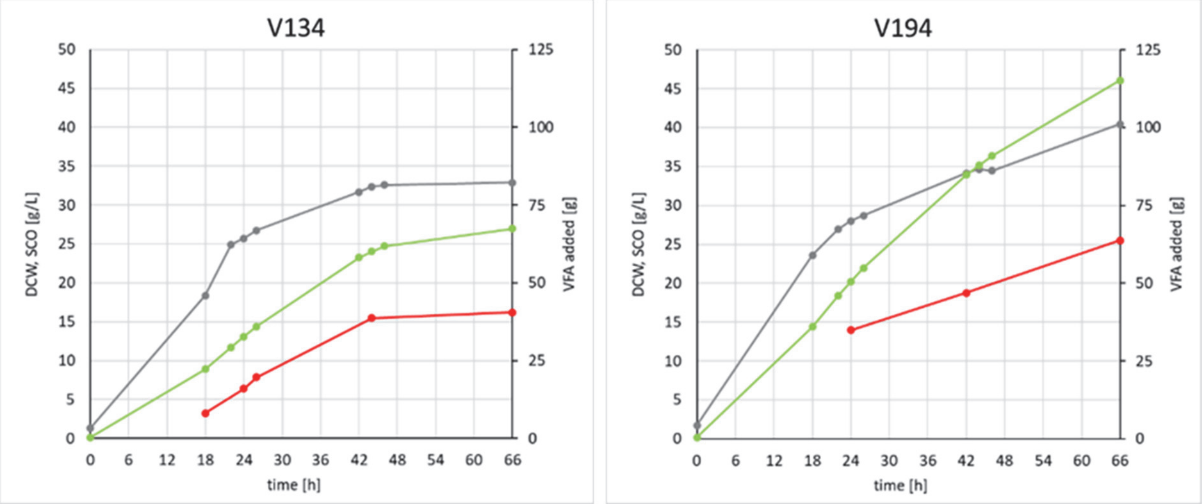


Figure 2: Development of DCW (grey) and SCO (red) concentration over time for strains *A. brassicae* V134 (**left**) and *P. kudriavzevii* V194 (**right**). Furthermore, the amount of VFA added to the reactor (green) is given as cumulative value.

In contrast, a pH-stat fed batch process allows for the continuous supply of VFA while keeping the VFA concentrations in the fermentation broth low. Béligon et al. (2015) reported lipid concentrations of 28.4 g/L for such a pH-stat fed batch process. While the lipid productivity achieved is comparable with the productivities reported in this work, only acetic acid, not a mixture of several VFAs was used as carbon source. Chi et al. (2011) achieved an exceptionally high volumetric lipid productivity of 0.66 g/Lh by also using a pure acetic acid solution. While this is, to our knowledge, the highest productivity reported for VFAs-based SCO production, several other factors have to be taken into account when assessing this process. The use of acetic acid as sole carbon source is contrary to the concept of using waste derived VFAs for SCO production. VFA solutions derived from anaerobic digestion are usually a mixture of several VFAs, with acetic acid, propionic acid and butyric acid being the most prominent ones. This VFA mix can have an influence on the process performance, since acetic acid is often preferred to other VFAs as carbon source (Llamas et al., 2020a). In addition, the acetic acid concentration of the feed used by Chi et al. (2011) is exceptionally high. While this allows for exceptionally high volumetric productivities, such concentrations are not reached by anaerobic digestion of organic wastes. Concentration of the VFAs however is – although necessary to some extent – an expensive process, decreasing the feasibility of the SCO production process (Strazzera et al., 2018).

4. CONCLUSIONS

In this work, the suitability of the two novel yeast strains *Apiotrichum brassicae* V134 and *Pichia kudriavzevii* V194 for VFA based SCO production could be demonstrated. It was shown that strain V134 and V194 are both able to fully utilize a moderately concentrated VFA mixture. The strains respectively produced 16.2 g/L and 25.5 g/L of SCO, resulting in comparably high volumetric lipid productivities of 0.245 g/Lh and 0.386 g/Lh.

5. REFERENCES

Béligon, V., Poughon, L., Christophe, G., Lebert, A., Larroche, C., Fontanille, P. (2015). Improvement and modeling of culture parameters to enhance biomass and lipid production by the oleaginous yeast *Cryptococcus curvatus* grown on acetate. Bioresource Technology 192: 582–591. https://doi.org/10.1016/j.biortech.2015.06.041

Beopoulos, A., Nicaud, J.-M., Gaillardin, C. (2011). An overview of lipid metabolism in yeasts and its impact on biotechnological processes. Applied Microbiology and Biotechnology 90 (4): 1193–1206. https://doi.org/10.1007/s00253-011-3212-8

Bettencourt, S., Miranda, C., Pozdniakova, T.A., Sampaio, P., Franco-Duarte, R., Pais, C. (2020) Single cell oil production by oleaginous yeasts grown in synthetic and waste-derived volatile fatty acids. Microorganisms 8 (11): 1809. https://doi.org/10.3390/microorganisms8111809.

Cerrone, F., Choudhari, S.K., Davis, R., Cysneiros, D., O'Flaherty, V., Duane, G., Casey, E., Guzik, M.W., Kenny, S.T., Babu, R.P., O'Connor, K. (2014). Medium chain length polyhydroxyalkanoate (mcl-PHA) production from volatile fatty acids derived from the anaerobic digestion of grass. Applied Microbiology and Biotechnology 98 (2): 611–620. https://doi.org/10.1007/s00253-013-5323-x

Chalima, A., Hatzidaki, A., Karnaouri, A., Topakas, E. (2019). Integration of a dark fermentation effluent in a microalgal-based biorefinery for the production of high-added value omega-3 fatty acids. Applied Energy 241: 130–138. https://doi.org/10.1016/j.apenergy.2019.03.058

Chi, Z., Zheng, Y., Ma, J., Chen, S. (2011). Oleaginous yeast *Cryptococcus curvatus* culture with dark fermentation hydrogen production effluent as feedstock for microbial lipid production. International Journal of Hydrogen Energy 36 (16): 9542–9550. https://doi.org/10.1016/j.ijhydene.2011.04.124

Fei, Q., Chang, H.N., Shang, L., Choi, J.-d.-r., Kim, N., Kang, J. (2011). The effect of volatile fatty acids as a sole carbon source on lipid accumulation by *Cryptococcus albidus* for biodiesel production. Bioresource Technology 102 (3): 2695–2701. https://doi.org/10.1016/j.biortech.2010.10.141

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Gao, R., Li, Z., Zhou, X., Bao, W., Cheng, S., Zheng, L. (2020). Enhanced lipid production by *Yarrowia lipolytica* cultured with synthetic and waste-derived high-content volatile fatty acids under alkaline conditions. Biotechnology for Biofuels 13: 3. https://doi.org/10.1186/s13068-019-1645-y

Kolouchová, I., Schreiberová, O., Sigler, K., Masák, J., Řezanka, T. (2015). Biotransformation of volatile fatty acids by oleaginous and non-oleaginous yeast species. FEMS Yeast Research 15 (7). https://doi.org/10.1093/femsyr/fov076

Llamas, M., Magdalena, J.A., González-Fernández, C., Tomás-Pejó, E. (2020a). Volatile fatty acids as novel building blocks for oil-based chemistry via oleaginous yeast fermentation. Biotechnology and Bioengineering 117 (1): 238–250. https://doi.org/10.1002/bit.27180

Llamas, M., Tomás-Pejó, E., González-Fernández, C. (2020b) Volatile fatty acids from organic wastes as novel low-cost carbon source for *Yarrowia lipolytica*. New Biotechnology 56: 123–129. https://doi.org/10.1016/j.nbt.2020.01.002

Meesters, P.A.E.P., Huijberts, G.N.M., Eggink, G. (1996). High-cell-density cultivation of the lipid accumulating yeast *Cryptococcus curvatus* using glycerol as a carbon source. Applied Microbiology and Biotechnology 45 (5): 575–579. https://doi.org/10.1007/s002530050731

Miranda, C., Bettencourt, S., Pozdniakova, T., Pereira, J., Sampaio, P., Franco-Duarte, R., Pais, C. (2020). Modified high-throughput Nile red fluorescence assay for the rapid screening of oleaginous yeasts using acetic acid as carbon source. BMC Microbiology 20 (1): 60. https://doi.org/10.1186/s12866-020-01742-6

Mohan, V.S., Dahiya, S., Amulya, K., Katakojwala, R., Vanitha, T.K. (2019). Can circular bioeconomy be fueled by waste biorefineries — A closer look. Bioresource Technology Reports 7: 100277. https://doi.org/10.1016/j.biteb.2019.100277

Papanikolaou, S. and Aggelis, G. (2011). Lipids of oleaginous yeasts. Part I: Biochemistry of single cell oil production. European Journal of Lipid Science and Technology 113 (8): 1031–1051. https://doi.org/10.1002/ejlt.201100014

Park, G.W., Chang, H.N., Jung, K., Seo, C., Kim, Y.-C., Choi, J.H., Woo, H.C., Hwang, I.-j. (2017). Production of microbial lipid by *Cryptococcus curvatus* on rice straw hydrolysates. Process Biochemistry 56: 147–153. https://doi.org/10.1016/j.procbio.2017.02.020

Strazzera, G., Battista, F., Garcia, N.H., Frison, N., Bolzonella, D. (2018). Volatile fatty acids production from food wastes for biorefinery platforms: A review. Journal of Environmental Management 226: 278–288. https://doi.org/10.1016/j.jenvman.2018.08.039

Wu, S., Hu, C., Jin, G., Zhao, X., Zhao, Z.K. (2010). Phosphate-limitation mediated lipid production by *Rhodosporidium toruloides*. Bioresource Technology 101 (15): 6124–6129. https://doi.org/10.1016/j.biortech.2010.02.111

MICROBIAL PRODUCTION OF ω-3 FATTY ACIDS: THE ROLE OF MICROALGAE IN THE VOLATILE PROJECT

E. Topakas [a], *A. Chalima* [a], *L. Fernandez de Castro* [b], *L. San Vicente* [b], *L. Oliver* [b]

[a] *National Technical University of Athens, Heroon Polytechniou 9, Zographou Campus, 15780 Athens, Greece*

[b] *TECNALIA, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Álava, Leonardo Da Vinci 1, 01510 Miñano, Álava, Spain*

Corresponding author: *vtopakas@chemeng.ntua.gr*

Abstract

The establishment of a sustainable circular bioeconomy requires the effective material recycling from biomass and biowaste beyond composting/fertiliser or anaerobic digestion/bioenergy. Recently, low-cost volatile fatty acids (VFA) derived from digested biowaste attracted much attention due to their potential application as carbon source for the microbial production of functional commodities, such as bioactive compounds of high nutritional value. A highly industrially required compound, docosahexaenoic acid (DHA), is one of the two most known omega-3 fatty acids and has been found to be necessary for a healthy brain and proper cardiovascular function. The main DHA source is currently fish, which obtain the fatty acid from the primary producers, microalgae, through the food chain. Specific heterotrophic microalgal strains such as *Crypthecodinium cohnii* and *Schizochytrium limanicum* are known for accumulating high amounts of DHA, while offering the advantage of assimilating various carbon sources, such as glucose, ethanol, glycerol and acetic acid. Based on their ability to assimilate different substrates, the two strains have been successfully cultivated on a dark fermentation effluent, rich in VFA. The biomass produced accumulated substantial amount of DHA, while efficiently consuming the carbon content of the waste stream. Experimental efforts have succeeded in establishing the conditions for an optimized fed-batch cultivation process that allows bioconversion of waste-derived VFA to the maximum amount of valuable omega-3 fatty acid.

Keywords: *Crypthecodinium cohnii*, *Schizochitrium limanicum*, docosahexaenoic acid, fed-batch

1. INTRODUCTION

Omega-3 fatty acids (FA) comprise a group of polyunsaturated fatty acids (PUFA) well known for their benefits to human health. The two most examined omega-3 fatty acids are the eicosapentaenoic acid (EPA; C20:5) and the docosahexaenoic acid (DHA; C22:6) [1]. DHA is an essential nutrient for the development of the nervous and visual system during infancy, and therefore it is a basic ingredient of infant formulas [2]. Moreover, DHA has been proven to have antithrombotic and anticancer properties [3]. Owning to its benefits, coupled with the human's organism's deficiency in synthesizing high amounts of the acid, various national and international health authorities, such as the American Heart Association and the European Society of Cardiology, have recommended special uptakes of DHA, as well as EPA, on a daily basis [4].

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Under the light of these events, production of DHA supplements is a growing industrial field. Even nowadays, the main PUFA source is fish oil, which encloses several unappealing characteristics. Apart from the fish odor, the quality of the oil depends on many factors, such as the season and location of fishing and the degree of sea contamination. Still, fish tissue rich in omega-3 FA include a mixture of DHA and EPA, which, in specific applications that require only one of the two FA, appears as a disadvantage. Furthermore, the oceans' fish stock cannot satisfy the increasing demand for omega-3 FA [5]. The presence of contaminants, like dioxins, polychlorinated biphenyls and heavy metals in fish oils, as a result of bioaccumulation, also poses a major issue [6]. An obvious solution is aquaculture, but farmed fish require a special diet in order to synthesize and accumulate appropriate EPA and DHA amounts. Some of the nutritional additives- rich in omega-3- used in fish food are krill and microalgae [7].

Microalgae are an extremely varied group of eukaryotic microorganisms found in marine and freshwater ecosystems. Although fish is the most common dietary source of omega-3 FA, microalgae are the primary natural producers of them, with the ability of accumulating total lipids of up to 70% of their dried biomass [8]. *Crypthecodinium cohnii* is a heterotrophic dinoflagellate marine microalgae species that is known for its ability to accumulate high amounts of DHA. Martek Biosciense in Maryland was the first to commercialize the production of DHA by *C. cohnii* [9, 10]. *Schizochytrium limanicum*, a member of the *Thraustochytrids* group, is another promising DHA source of increasing interest. *S. limacinum* is a unicellular heterotrophic protist/ microalga commonly found in marine environment and capable of accumulating considerable amounts of omega-3 FA, especially DHA [11].

Although the current process followed for the production of omega-3 from microalgae poses a solution to the increasing fish demand, it does not provide a sustainable application for the future. The high cost of glucose, which is the main carbon source used in the majority of the reports published in literature, needs to be replaced by a cheaper alternative nutrient. Research so far has shown *C. cohnii* and *S. limanicum* to grow well on other substrates, such as ethanol, glycerol and acetic acid [11, 12], which, however, in their pure, commercial form are equally expensive.

Acetic acid is the most abundant product of dark fermentation (DF), a process that enables the production of volatile fatty acids (VFA) from biowaste. The procedure follows the same principles as typical anaerobic digestion, but the last step of methanogenesis is inhibited. That way the three steps of hydrolysis, acidogenesis and acetogenesis lead finally to the production of high amounts of VFA such as acetic, propionic and butyric acid [13, 14].

Since they are derived from biowaste, the production cost of these VFA mixtures is very low and their utilization for the synthesis of more expensive, high added-value products is a desirable step towards a sustainable and circular economy. Therefore, the valorization of waste-derived VFA for the biomass production, as in case of microalgae, and the harvesting of their valuable metabolites inspires a lot of research [14, 15, 16]. The two microalgal strains seem to be a potential tool for the valorization of VFA towards omega-3 production, since they have already exhibited relatively high growth yields under pure acetic acid feed [18][19]. For the purposes of a research project funded by the European Commission under the Horizon 2020 programme entitled "VOLATILE - Biowaste derived volatile fatty acid platform for biopolymers, bioactive compounds and chemical building blocks" the two strains were successfully cultivated on mixtures of VFA, as well as on a DF effluent, derived from Vegetable Garden and Food (VGF) biowaste.

2. MATERIALS AND METHODS

C. cohnii ATCC 30772 was purchased and maintained according to the ATCC protocols. Seed cells were maintained in batch cultures and used for inoculating fed-batch pH-auxostat bioreactors of 2 L total volume. The feed was either 33% v/v glacial acetic, 25% v/v butyric or propionic acid or a DF effluent, acidified with HCl. The DF liquid fraction from VGF waste, provided by Organic Waste Systems NV (OWS, Belgium) and processed by Tecnalia Research & Innovation (Spain).

Apart from examining the ability of the strain to assimilate the various VFA, the effect of the culture conditions, and specifically the temperature, initial C/N ratio and nitrogen source, on the intracellular DHA production was also examined. The optimization experiments were carried out in fed-batch bioreactors of 150 mL total working volume.

S. limanicum ATCC MYA-1381 was maintained by sub-cultivation on a medium recommended by the culture collection. Cells were grown in batch culture and were used to inoculate the bioreactor of 5 and 30 L total volume. The DO-stat fed-batch strategy was used in the bioreactor and the feed was DF effluent (20.5g/L VFA) or synthetic medium with a similar composition of VFA.

3. RESULTS AND DISCUSSION

3.1 VFA assimilation by C. cohnii

C. cohnii was able to assimilate the main VFA present in a DF effluent as main carbon source in fed-batch cultures and produce DHA at a percentage of 33.3 ± 0.2% (w/w) of Total Fatty Acids (TFA) for a feed of acetic acid, 35.8 ± 0.6% (w/w) TFA for propionic and 31.1 ± 1.0% (w/w) TFA for butyric acid. The strain grew also on the acidified DF effluent, as well as on a synthetic medium mimicking the VFA composition of the effluent (Figure 1.) Although the total carbon content of the effluent was consumed, the low VFA concentration didn't allow the application of a fed-batch cultivation process very effectively due to low pH control. A more concentrated effluent was examined and resulted in a bioconversion yield of 0.21 g CDW/ g VFA consumed.

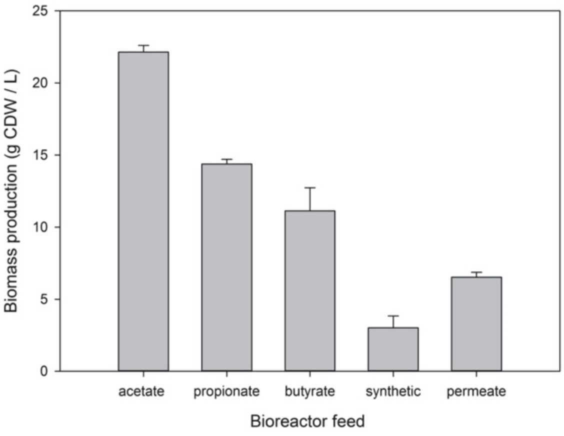


Figure 1: Final dry biomass production of the different bioreactors.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

3.2 DHA production optimization in *C. cohnii*

Optimization of temperature revealed that DHA percentage of the cells and DHA production are maximized at an incubation temperature of 23 ºC. The substitution of yeast extract with ammonium, as the nitrogen source, was deemed more economic, however it was proven to reduce the final DHA production by the cells in fed-batch cultures. Finally, a high initial availability of nitrogen with an excess of carbon increased the dry biomass production, but however decreased the TFA production, without effecting substantially the DHA content (per CDW) (Figure 2). Based on the above results we concluded that the maximum omega-3 production is achieved with a high C/N ratio equal to 8.9, with yeast extract used as a carbon source, at 23 ºC.

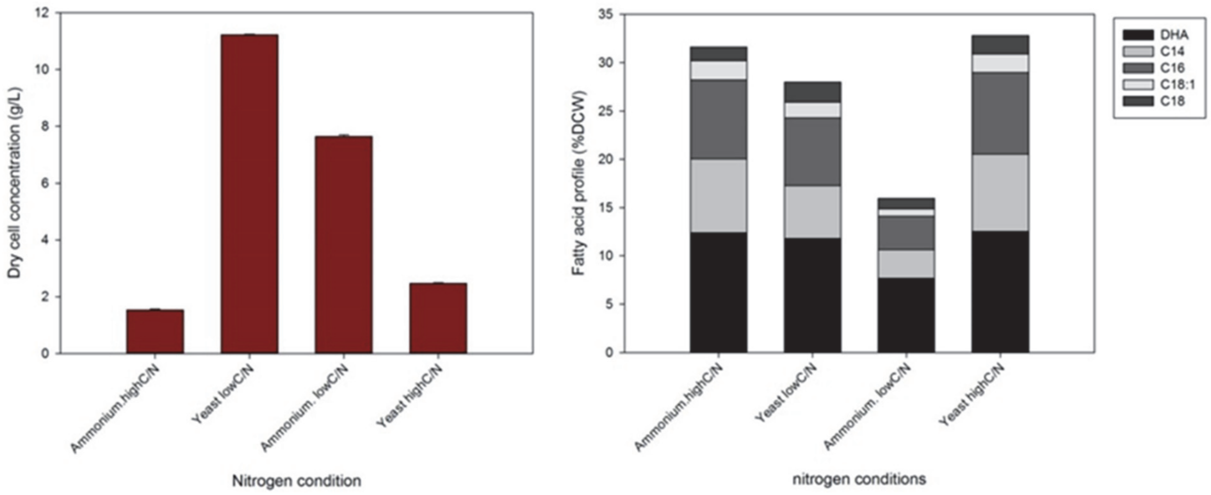


Figure 2: Dry cell concentration and FA profile of the fed-batch cultures of *C. cohnii* under different nitrogen sources and initial C/N ratios

3.3 VFA assimilation by *S. limanicum* in bioreactors

Biomass growth, lipids production and VFA consumption were followed during the time course of the cultivation. The first experiments were aimed to identify optimal concentration of VFA in initial media and the other conditions for fed-batch implementation. A fed-batch mode of operation was carried out using a C-source feeding regime based on the decrease of the stirring speed which occurs due to the increase of the dissolved oxygen concentration as a result of C-source exhaustion (DO stat).

S. limacinum assimilated as carbon source the VFA present in the effluent (VFA permeate) and in the synthetic medium mimicking the VFA composition of the effluent (VFA synthetic). Figure 3 shows the growth of *S. limacinum* on both medium in 5-L laboratory bioreactors (Biostat B) at 25 ºC: The cell growth was a little more rapid on VFA synthetic medium, resulting in 11,12g/l DCW at 60h, and the cultivations on VFA permeate reached 10.4g/l at 74h.

At 70h, lipid content and the TFA are show in Figure 3. Some differences between both media were found, possibly due to the effect of growth speed and nutrient depletion on lipid content.

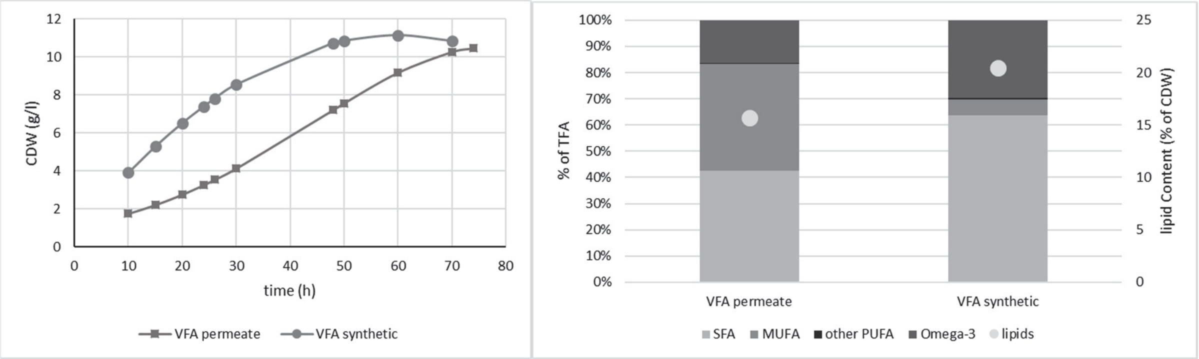


Figure 3: Growth and FA profile (70h) of the fed-batch cultures of *S limacinum* in 2L Bioreactor with VFA

In 30L scale bioreactor (Biostat C), process optimization with DO-stat fed batch cultivations was realized. Concerning biomass growth registered in these trials using synthetic VFA as feed, the biomass accumulation was higher than in previous cultivations in 2-L bioreactors. The cell dry weight reached 14.6g/L in 70h (Figure 4). In Figure 4, the values and evolution for dry cell weight show an optimization of the process. In addition, the lipid content was increased to 49.45% of CDW, containing a profile of TFA very similar to that obtained in 5L Bioreactor.

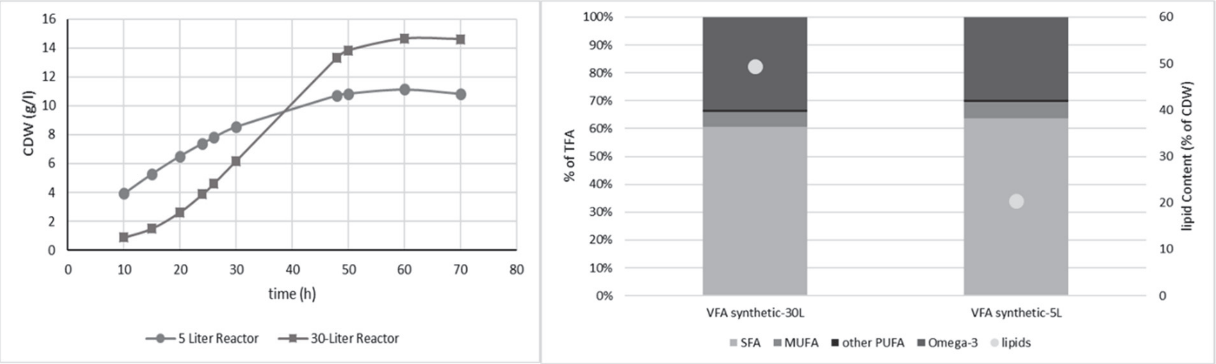


Figure 4: Growth and FA profile (70h) of the fed-batch cultures of *S limacinum in 5L and 30L Bioreactor with synthetic VFAs*

4. CONCLUSIONS

During the project VOLATILE it could be successfully shown that heterotrophic microalgae can use biowaste derived volatile fatty acids as carbon source transforming them into added-value compounds such as polyunsaturated long chain Omega-3 fatty acids. Furthermore, the availability of propionic acid in the VFA permeate leads most likely to the fact that the heterotrophic microalgae also accumulate odd-chain fatty acids such as C15 and C17. These kinds of fatty acids have wide application potential in therapeutic and nutritional industries as well as in chemical industry [20].

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

5. REFERENCES

[1] Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, Schenk PM. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact 2012;11:96. doi:10.1186/1475-2859-11-96.

[2] Li X, Pei G, Liu L, Chen L, Zhang W. Metabolomic analysis and lipid accumulation in a glucose tolerant Crypthecodinium cohnii strain obtained by adaptive laboratory evolution. vol. 235. Elsevier Ltd; 2017. doi:10.1016/j.biortech.2017.03.049.

[3] Swaaf ME De, Rijk TC De, Eggink G, Sijtsma L, De Swaaf ME, De Rijk TC. Crypthecodinium cohnii. J Biotechnol 1999;70:185–92. doi:10.1016/S0168-1656(99)00071-1.

[4] Ghasemifard S, Turchini GM, Sinclair AJ. Omega-3 long chain fatty acid "bioavailability": A review of evidence and methodological considerations. Prog Lipid Res 2014;56:92–108. doi:10.1016/j.plipres.2014.09.001.

[5] Sijtsma L, De Swaaf ME. Biotechnological production and applications of the ω-3 polyunsaturated fatty acid docosahexaenoic acid. Appl Microbiol Biotechnol 2004;64:146–53. doi:10.1007/s00253-003-1525-y.

[6] Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr 2014;2:443–63. doi:10.1002/fsn3.121.

[7] Adarme-Vega TC, Thomas-Hall SR, Schenk PM. Towards sustainable sources for omega-3 fatty acids production. Curr Opin Biotechnol 2014;26:14–8. doi:10.1016/j.copbio.2013.08.003.

[8] Cuellar-Bermudez SP, Aguilar-Hernandez I, Cardenas-Chavez DL, Ornelas-Soto N, Romero-Ogawa MA, Parra-Saldivar R. Extraction and purification of high-value metabolites from microalgae: Essential lipids, astaxanthin and phycobiliproteins. Microb Biotechnol 2015;8:190–209. doi:10.1111/1751-7915.12167.

[9] Ratledge C. Microbial oils: an introductory overview of current status and future prospects. Ocl 2013;20:D602. doi:10.1051/ocl/2013029.

[10] Vazhappilly R, Chen F. Eicosapentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. J Am Oil Chem Soc 1998;75:393–7. doi:10.1007/s11746-998-0057-0.

[11] Abad S, Turon X. Biotechnological production of docosahexaenoic acid using aurantiochytrium limacinum: Carbon sources comparison and growth characterization. Mar Drugs 2015;13. doi:10.3390/md13127064.

[12] de Swaaf ME, Pronk JT, Sijtsma L. Fed-batch cultivation of the docosahexaenoic-acid-producing marine alga Crypthecodinium cohnii on ethanol. Appl Microbiol Biotechnol 2003;61:40–3. doi:10.1007/s00253-002-1118-1.

[13] Safdar W, Zan X, Song Y. Synergistic Effects of pH , Temperature and Agitation on Growth Kinetics and Docosahexaenoic Acid Production of C . cohnii Cultured on Different Carbon Sources. Int J Res Agric Sci 2017;4:94–101.

[14] Chalima A, Oliver L, Fernández de Castro L, Karnaouri A, Dietrich T, Topakas E. Utilization of Volatile Fatty Acids from Microalgae for the Production of High Added Value Compounds. Fermentation 2017;3:54. doi:10.3390/fermentation3040054.

[15] Lee WS, Chua ASM, Yeoh HK, Ngoh GC. A review of the production and applications of waste-derived volatile fatty acids. Chem Eng J 2014;235:83–99. doi:10.1016/j.cej.2013.09.002.

[16] Turon V, Trably E, Fouilland E, Steyer JP. Potentialities of dark fermentation effluents as substrates for microalgae growth: A review. Process Biochem 2016;51:1843–54. doi:10.1016/j.procbio.2016.03.018.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

[17] Turon V, Trably E, Fayet A, Fouilland E, Steyer JP. Raw dark fermentation effluent to support heterotrophic microalgae growth: Microalgae successfully outcompete bacteria for acetate. Algal Res 2015;12:119–25. doi:10.1016/j.algal.2015.08.011.

[18] Ratledge C, Kanagachandran K, Anderson AJ, Grantham DJ, Stephenson JC. Production of docosahexaenoic acid by Crypthecodinium cohnii grown in a pH-auxostat culture with acetic acid as principal carbon source. Lipids 2001;36:1241–6. doi:10.1007/s11745-001-0838-x.

[19] Ganuza E, Anderson AJ, Ratledge C. High-cell-density cultivation of Schizochytrium sp. in an ammonium/pH-auxostat fed-batch system. Biotechnol Lett 2008;30:1559–64. doi:10.1007/s10529-008-9723-4.

[20] Young-Kyoung Park Y-K, Dulermo T, Ledesma-Amaro R, Nicaud J-M (2018). Optimization of odd chain fatty acid production by *Yarrowia lipolytica*. Biotechnol Biofuels, 11: 158. https://doi.org/10.1186/s13068-018-1154-4

EN ROUTE TO STANDARDISATION – THE CEN WORKSHOP AGREEMENT 'evaVolatile'

J. Michels [a], S. Hiessl [a]

[a] *DECHEMA Gesellschaft für Chemische Technik und Biotechnologie e.V., Theodor Heuss Allee 25, 60486 Frankfurt (Germany)*

Corresponding author: *jochen.michels@dechema.de*

Abstract

To facilitate later Volatile Fatty Acid Platform (VFAP) implementation and to support standardisation in the area of sustainable biomass / biowaste upcycling, the project consortium initiated a CEN workshop. The aim of the workshop was to agree on a procedure which can be applied to evaluate if this kind of coupled energetic and material use is economically and ecologically reasonable for a given type of biowaste at a given location. The finalised CEN workshop agreement (CWA) is providing support for interested stakeholders to assess their respective test case accordingly.

Keywords: standardisation, CWA

1. INTRODUCTION

The separation of volatile fatty acids (VFA: acetic acid among others) from the biogas process is currently object of research and innovation and demonstration in many European funded projects like VOLATILE. Since the derived technological applications are now on the threshold of commercialization a framework is needed for the feasibility assessment of integrating the VFAP technology into anaerobic digestion plants.

Therefore, a CEN Workshop called "*CEN/WS - Procedure for evaluating if the use of a Volatile Fatty Acid Platform technology for a given type of biowaste at a given location is economically and ecologically reasonable (EvaVOLATILE)*" has been conducted from Sep. 2018 to May 2020 and has been approved by an open, independent workshop structure within the CEN-CENELEC system. The intension of the Workshop was to develop a simple evaluation methodology that allows biogas plant operators to assess whether the changeover of a given biogas plant to a coupled energetic and material use is ecologically and economically reasonable under certain conditions. The consensus document has been published as CEN/CENELEC Workshop Agreement (CWA 17484:2020). Responsible for the content were the registered participants, who are mainly actors of the entire value chain of municipal biowaste treatment by anaerobic digestion and related scientific research disciplines.

The framework considered in the CWA has been described for managers of anaerobic digestion plants in general, and those treating solid and sludgy biowaste in particular. It is divided into a non-technical and a technical part for the introduction of criteria and dimensions necessary for the evaluation of feasibility and accompanied by a section of assessment methodology.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

2. DEVELOPMENT OF THE CWA 'evaVOLATILE'

As stated in the CEN/CENELEC Guideline *"Workshop Agreements – A rapid way to standardization"* a CEN Workshop agreement is not a full standard and shall not conflict with a European Standard. It is designed with a focus on R&I outcomes, meet an immediate need, can be quickly developed and can be used as fast track to future standardization activities. It is developed and agreed by the participants in a temporary working group (CEN/CENELEC Workshop). Stakeholder involvement is limited to those directly interested in the subject and in elaboration of the CWA. Once published and if the topic shows to be market relevant, the CWA can be used as source for a future standard (CEN/CENELEC, 2020).

The process steps and timelines of initiation and development of the CWA 'evaVOLATILE' are depicted in Figure 1.

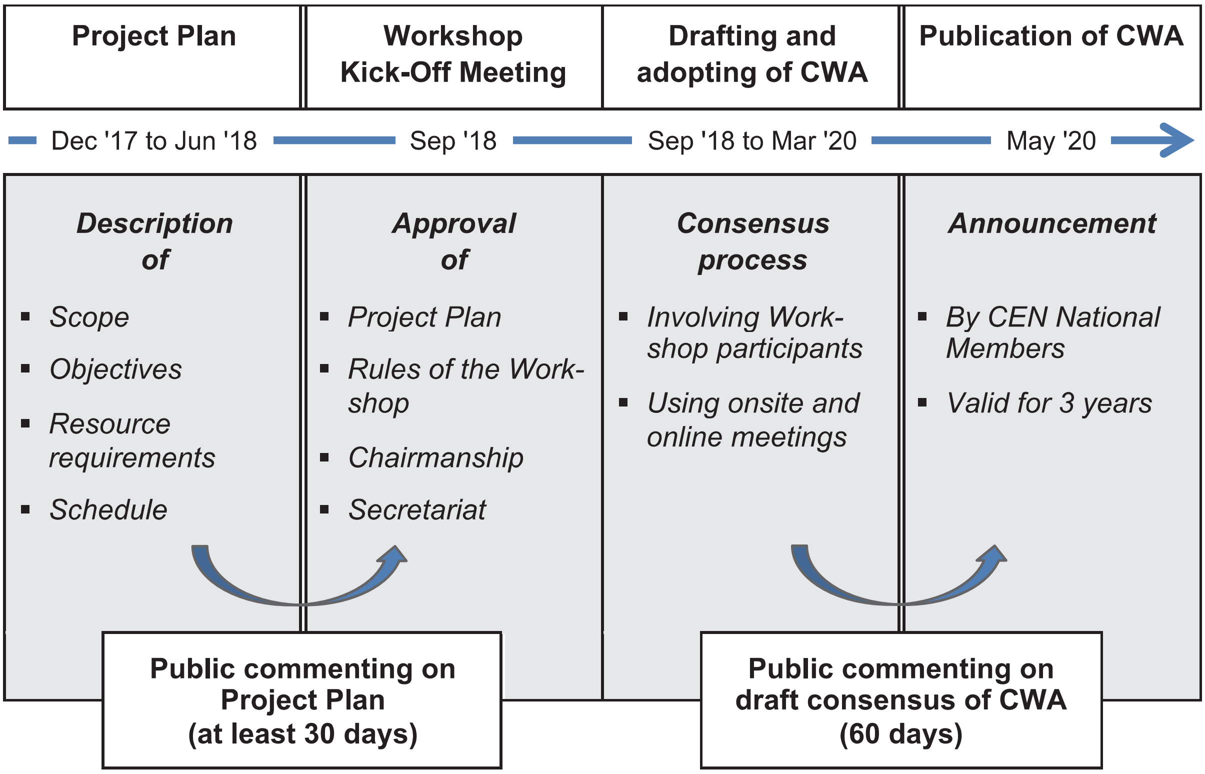


Figure 1: Process steps of Initiation and development of the CWA evaVOLATILE

DECHEMA as the contact point of the proposers of the CWA has prepared its proposal with the assistance of the CEN-CENELEC national member DIN (German Institute for Standardization). During the kick-off Meeting, DIN was elected as the secretariat to support the CWA development process. The kick-off meeting also represents the formal launch of the Workshop.

After the kick-off meeting, the participants, wishing to continue contributing to the development of the draft CWAs, were requested to officially register to the Workshop. Actors of the entire value chain of municipal AD-driven biowaste treatment and related scientific research disciplines have jointly developed the CWA according to the specifications laid down in the Project Plan. Five additional (virtual) Workshop meetings were necessary in the consensus process until the chairperson decided that an agreement has been reached amongst the registered WS participants on the final text of the CWA. This has finally been approved by the WS Secretariat in an official voting amongst the registered WS participants. As foreseen in the Project Plan an open commenting phase of 60 days has been launched thereafter. The comments were considered by the WS participants in an additional virtual Workshop meeting. After a final formal agreement, the CEN Workshop has completed its Project Plan and decided to disband itself.

The consensus document (CEN Workshop Agreement), named “Anaerobic digestion plants – Feasibility assessment methodology for integrating a Volatile Fatty Acid Platform Technology” (evaVOLATILE) will be valid for 3 years (from May 2020). Afterwards the former Workshop participants and the relevant CEN/CENELEC technical bodies will determine whether the CWA shall be confirmed for another 3 years, revised, transformed into another deliverable, or, however, withdrawn. The CWAs maximum lifetime is 6 years.

An established CWA can be proposed for transformation into a European Standard on the condition that it has all the characteristics of a European Standard and that the standards development process is correctly followed. Transformation into another deliverable may be proposed by anyone through a CEN/CENELEC (national) Member at any time during the 6-year lifetime (CEN/CENELEC, 2020).

3. THE CWA 'evaVOLATILE' AT A GLANCE

The CWA's framework has been designed for managers of AD plants in general and those treating solid and sludgy bio-waste in particular. The document is composed of a non-technical and a technical part introducing criteria and dimensions necessary for the assessment of feasibility as well as an evaluation part.

Economic criteria and arguments related to a company’s image as well as the social components are provided which make VFAP attractive for AD plant managers who look for new value chains in order to be able to continue their work in an economically viable way. Beside from gaining more revenue from additional high value products, the most important aspect is that installing a VFA platform will also boost the plant’s capacity, since the methanogenic step, which is now omitted or reduced, is the time limiting step of a conventional biogas plant. AD plant managers could make the shift from being processors of solid and sludgy biowaste and producers of energy, towards producers of new materials for the chemical industry, while still treating the waste streams and producing energy. Therefore, AD plant managers would become pioneers in the circular economy, giving them the image of being innovative, agile, and showing that their company has a vision.

Many fold contextual factors like subsidies, political vision and regulatory framework are important to be considered for the investment decision. Therefore, a web-based decision support tool has been developed by the VOLATILE consortium. The VOLATILE web-based decision support tool (DST) is specially developed for operators of wastewater, municipal solid waste and biowaste treatment facilities to identify the specific local potential of the VOLATILE technology for their plants. Information about the free tool can be found via the VOLATILE

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 720777

homepage (https://www.volatile-h2020.eu). Besides economic indicators and technical aspects, the decision support tool also takes into account non-technical criteria for VFAP technology implementation.

A multi-criteria decision-making guide is provided for assessing technical criteria: In a first part, the quality requirements, substrate categories and availability of various bio-based raw materials of municipal waste streams that are suitable for anaerobic degradation are discussed. Secondly, the impact of an integration of a VFAP technology in AD plants is assessed. This part mainly focuses on the underlying microbial processes as well as biogas recovery. Thirdly, available VFA conversion technologies including direct application, different fermentation routes using VFA as substrates as well as chemical conversion routes are presented.
Within the last section of the CWA several methods, important to assess the economic and ecological viability of the VFAP technology integration into existing or new biogas plants are introduced. This set of methods includes, inter alia, SWOT analysis, Life Cycle Assessment, Cost Analyses and Economic Feasibility Studies.

To conclude, the CWA provides a simple evaluation methodology for biogas plant operators, investors, and municipalities to assess “whether the changeover of a given biogas plant to a coupled energetic and material use is ecologically and economically reasonable”, and as such could provide an important tool to help bring a novel technology to the market.

4. REFERENCES

CEN/CENELEC (2020). CEN/CENELEC Workshop Agreements – A rapid way to standardization. Available at: ftp://ftp.cencenelec.eu/EN/EuropeanStandardization/Guides/29_CENCLCGuide29.pdf. Accessed 24 February 2021.

CWA 17484 (2020). Anaerobic digestion plants – Feasibility assessment methodology for integrating a Volatile Fatty Acid Platform Technology (VOLATILE). Available at: ftp://ftp.cencenelec.eu/EN/ResearchInnovation/CWA/CWA17484_2020.pdf. Accessed 24 February 2021.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 720777

ENVIRONMENTAL IMPACT OF BIOECONOMY (Life Cycle Assessment) - VOLATILE CASE STUDY

C. Boukouvalas [a], V. Oikonomopoulou [a], K. Laina [b], M. Krokida [a]

[a] *National Technical University of Athens, Heroon Polytechniou 9, Zographou Campus, 15780 Athens, Greece*

[b] *KPAD Ltd., Francis House 2 Park Road Barnet, England EN5 5RN, UK*

Corresponding author: *vasiaoik@central.ntua.gr*

Abstract

Bioeconomy is meant to reduce the dependence on natural resources, transform manufacturing, promote sustainable production of renewable resources from land, fisheries and aquaculture and their conversion into food, feed, fibre, bio-based products and bio-energy, leading to the creation of new jobs and industries. The utilization of production technologies for the optimal use of renewable resources, the reduction of pollution, and the enhancement of food security is needed, followed by less inputs, less environmental impact and reduced greenhouse gas emissions. Life Cycle Assessment (LCA) can be used to monitor, evaluate and forecast the environmental performance of all bioeconomy sectors. In VOLATILE project, LCA was applied to all the developed products, a) the Volatile Fatty Acids (VFA) produced from organic waste, b) the VFA produced from wastewater sludge, c) the polyhydroxyalkanoates (PHA) for biomaterials, d) the single cell oil for oleochemical industry (SCO), e) the omega-3 fatty acids produced by heterotrophic microalgae (OM3). The goal of the LCA study was the assessment of the environmental impacts of the whole process line used for the production of VOLATILE products and their comparison with current methodologies. The inputs and outputs (materials, energy, water, emissions to air, soil and water) for all the examined processes were collected from industrial scale processes or extrapolated from pilot scale. For the impact assessment, ReCiPe 2016 methodology was selected. VOLATILE VFA platform presented significantly lower footprint to all categories compared to an alternative methodology for VFA production from food waste, mainly due to the produced electricity credits. VOLATILE PHA, SCO and OM3 systems performed similarly with other examined alternative systems. VOLATILE technologies are new and can be further optimized leading to even improved environmental behavior. As a result, VFA platform can be a sustainable solution for the treatment of municipal solid and sludgy biowaste.

Keywords: carbon footprint, sustainability, VFA platform

1. INTRODUCTION

Bioeconomy is meant to reduce the dependence on natural resources, promote sustainable production of renewable resources and their conversion into bio-products and bio-energy, while providing economy goods and services in an environmentally-friendly way. In order to achieve resource efficiency and increase the efficiency of biomass use, the cascading use of biomass and raw bio-materials in recycling and reuse is required. Biomass can be provided as primary material to various sectors, such as food, chemicals, energy, etc., whereas agriculture sector provides most of the biomass (approximately 65%) (Camia A. et al., 2018, europa.eu/horizon2020). Apart from the economic factors, bioeconomy also includes environmental and ecological aspects that have to be taken into account in order to minimize and manage negative impacts. Life Cycle Assessment (LCA) is a powerful tool that can be used to evaluate the environmental performance of bioeconomy sectors and compare the environmental impacts of several products or processes.

The management of municipal solid waste (MSW) is one of the major environmental challenges worldwide. Waste management must follow sustainable and environmentally friendly processes for the transition of MSW into bio-based products. Until now, landfilling and incineration are the most widely used waste disposal methods, while anaerobic digestion (AD) is gaining ground, due to the production of bioenergy (Elginoz et al., 2020). Apart from AD, production of volatile fatty acids (VFA) from MSW is gaining attention for proper waste management. VOLATILE project is dealing with the establishment of a volatile fatty acid platform (VFAP) based on AD for the conversion of municipal solid biowaste fractions and sludgy biowaste from other industries such as food industry or waste water treatment facilities into volatile fatty acids (VFA) and their conversion into building blocks for oleo-chemical industry (SCO), biopolymer PHA for biomaterial application as well as bioactive compounds (Omega-3 fatty acids).

LCA is being used to evaluate the environmental impact of waste management systems (Sharma and Chandel, 2017). As a result, the objective of this study was the determination of the environmental performance of VFAs production from municipal solid biowaste, as well as their transformation into PHA biopolymers, using LCA.

2. MATERIALS AND METHODS

2.1 VOLATILE approach

In order to evaluate the valorisation schemes that were developed in the context of VOLATILE project, LCA was performed. In this work, production of VFA from VGF, as well as PHA production were evaluated for their environmental performance. LCA study was performed on GaBi ts (v8.7.0.18) commercial package as a leading tool of the examined system.

Production of VFA from VGF

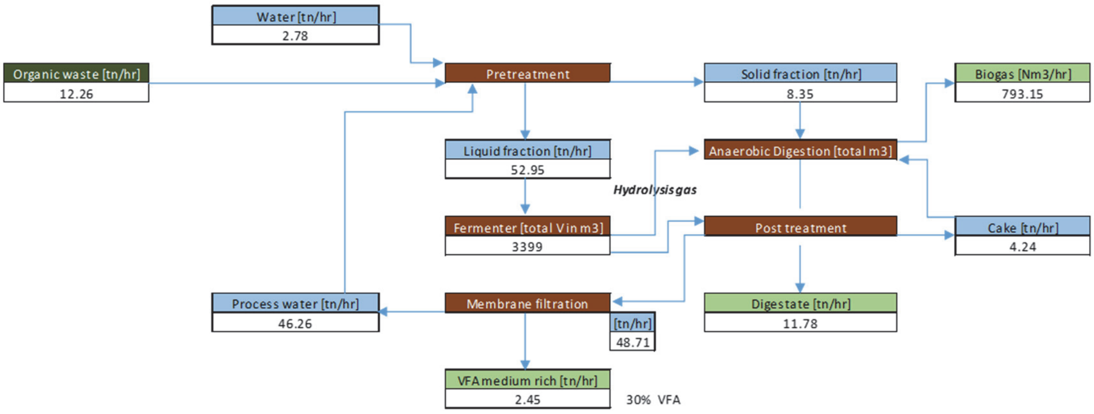


Figure 1: Process diagram for the production of VFA from VGF

The first VOLATILE approach was the production of VFA from VGF. The process diagram was set, VGF was pre-treated and the liquid fraction went to the fermenter. The produced hydrolysis gas went to the AD, the liquid part was centrifuged, and the solid residue went to

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

AD as well. The liquid flow of the centrifuge went to membrane filtration in order to get concentrated VFA medium, the water was recycled and the digestate from AD can be used for composting.

PHA production

The next VOLATILE approach was the production of PHA. There are two stages, the fermentation stage where raw PHA is produced and the extraction stage where the purified PHA is produced.

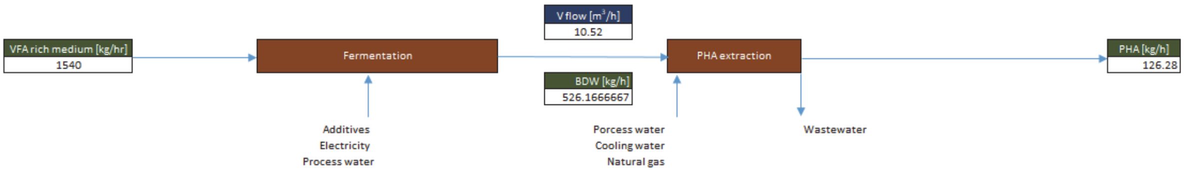


Figure 2: Process diagram for the production of PHA

2.2 *Life-Cycle Assessment*

LCA implementation includes four steps: a) Goal and Scope Definition, b) Life-Cycle Inventory, c) Life-Cycle Impact Assessment and d) Life-Cycle Interpretation (ISO 14040:2006).

Goal and Scope Definition: The goal of the LCA study was the assessment of the environmental impacts of the process line used for the production of VFA platform from VGF or the production of PHA. The functional unit selected was 1.0 kg of product. The examined systems were defined as all relevant life cycle stages and processes involved in the production of final products. Allocation of resources was based on the income that results from the selling of final products. Thus, products with no or low value (compost, pellets, etc.) have not been assigned any impact.

Life-Cycle Inventory: The inputs and outputs (materials, energy, water, emissions to air, soil and water) for all the examined processes were collected from: directly measured data by VOLATILE consortium partners, through completion of questionnaires, specific data from GaBi database, literature data, calculations based on specific formulas taken from literature or simulations. The data were taken from industrial scale processes or extrapolated from pilot scale.

Life-Cycle Impact Assessment: ReCiPe 2016 (H) methodology has been selected.

Life-Cycle Interpretation: Results were then interpreted in order to obtain the "hotspots".

3. RESULTS AND DISCUSSION

VFA from VGF

The processes that were included in the LCA analysis were: a) Pre-treatment, b) Fermentation, c) Post-Treatment, d) VFA recovery, e) Other processes (AD, Composting, Biogas production). The analysis was performed either taking into account the impact of VGF or not. Each process formed a plan or a unit process on GaBi ts (v8.7.0.18). Figure 3 presents the flow diagram for producing VFA from VGF waste, without taking into account biowaste impact.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

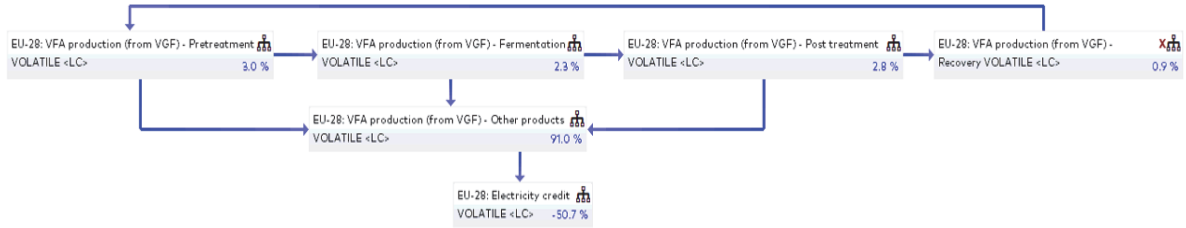


Figure 3: Plan for the production of VFA from VGF in GaBi software without the VGF impact

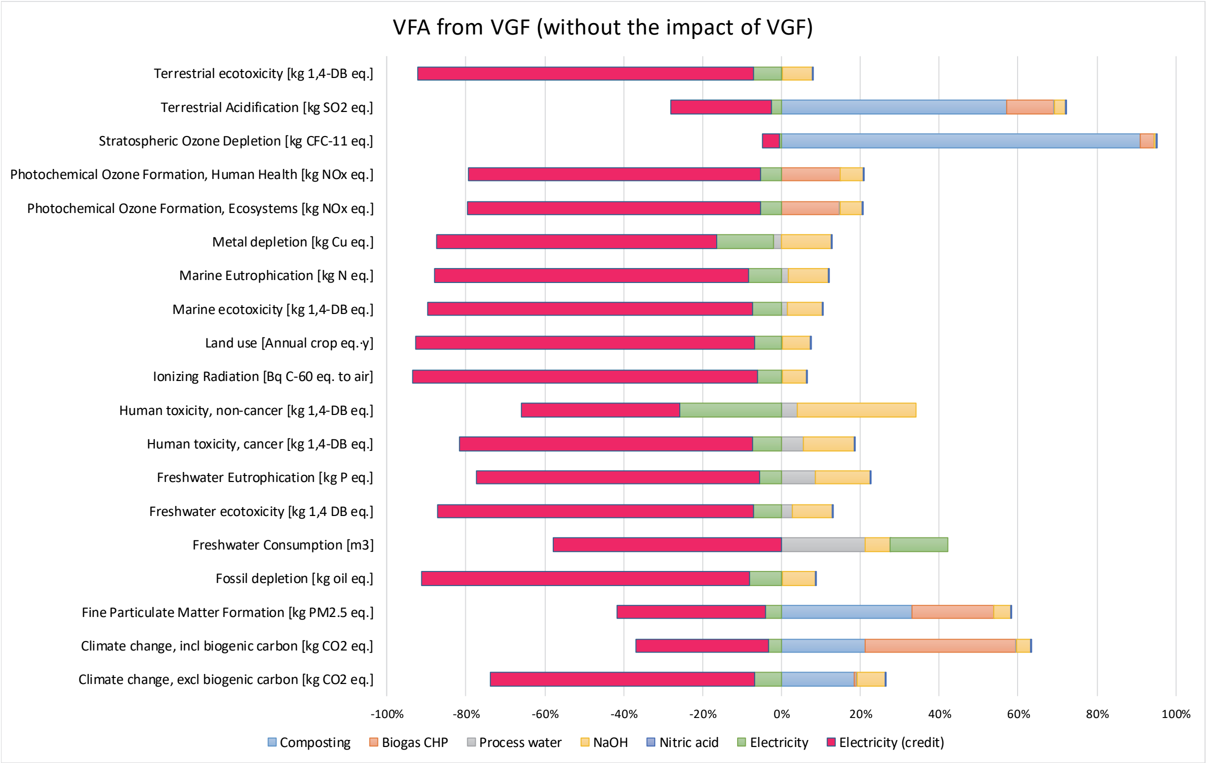


Figure 4: Environmental footprint for VFA production from VGF, without the impact of waste

In Figure 4, the percentage contribution of the different processes is presented. The higher percentage of footprint is attributed to the processes of compost and cogeneration (other products, more than 90% in climate change). Composting has significant contribution to several impact categories, due to the high impact of emissions produced. If emission preventing measures (e.g. biofilters) are used the footprint of the final product is presented in the Figure 5.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

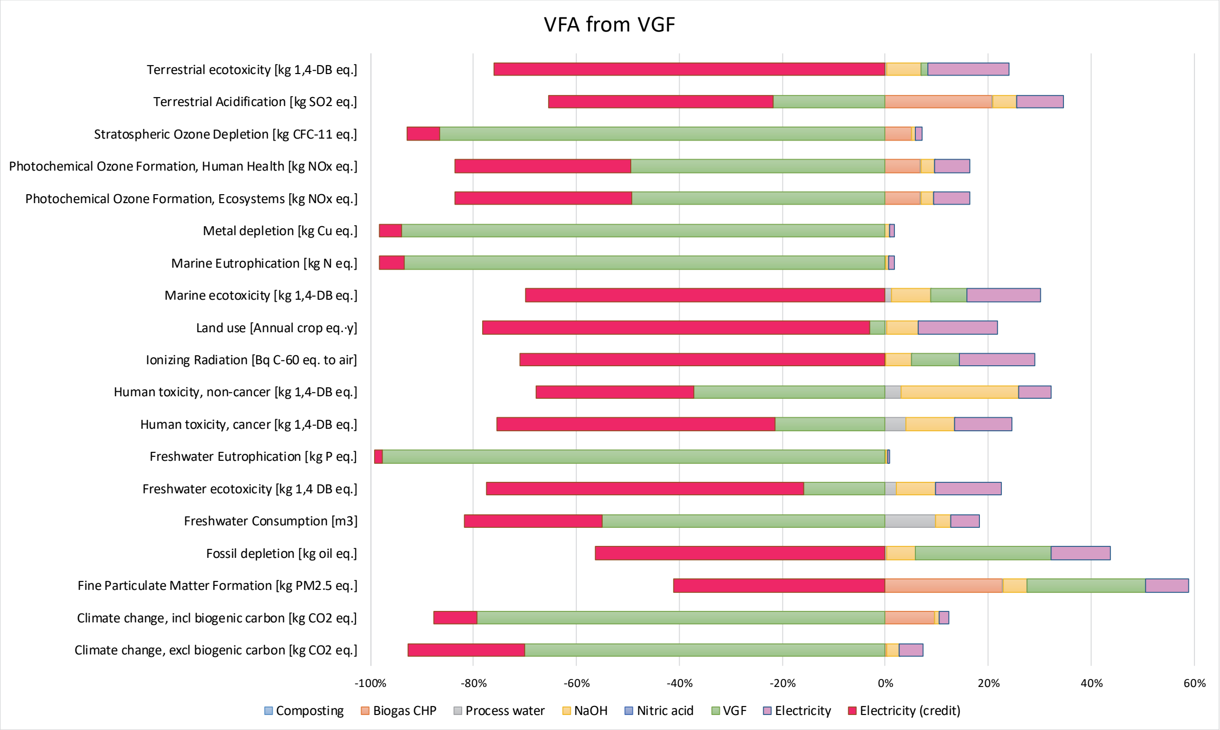


Figure 5: Environmental footprint for VFA production from VGF (no emissions from composting process – impact of VGF waste is taken into account)

The production of VFA using VOLATILE platform, from VGF (without the impact of the biowaste), was compared with an alternative methodology for VFA production from food waste presented by Elginoz et al., 2020. Indicative impact categories are presented in Figure 6. VOLATILE VFA platform presents significantly lower footprint to all categories, mainly due to the produced electricity credits.

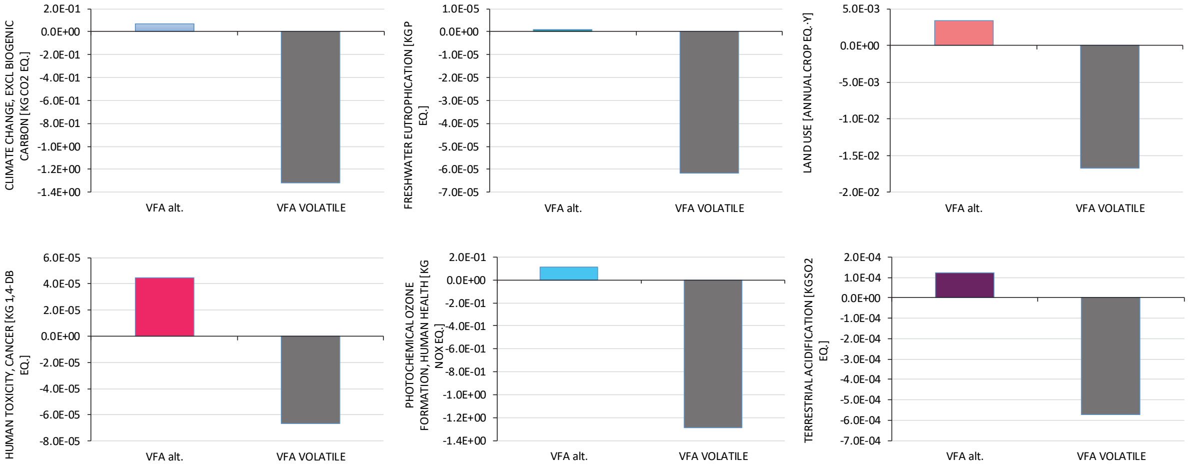


Figure 6: Comparison of several environmental impact categories for the production of VFA (without the impact of VGF) of VOLATILE VFA production system to an alternative system.

PHA production

The processes that were included in the LCA analysis were: a) Extraction, b) Fermentation, c) VFA production (with and without the impact of VGF). Figure 7 presents the process diagram for producing PHA, without taking into account the impact of biowaste.

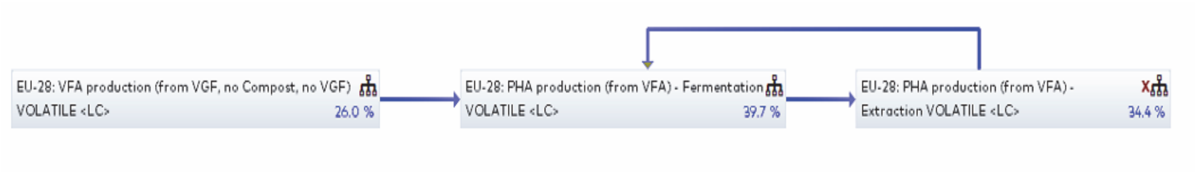


Figure 7: Plan for the production of PHA in GaBi software without the VGF impact

In Figure 8, the percentage contribution of the different processes is presented. Most of the impact comes from the bleaching agents and sugar.

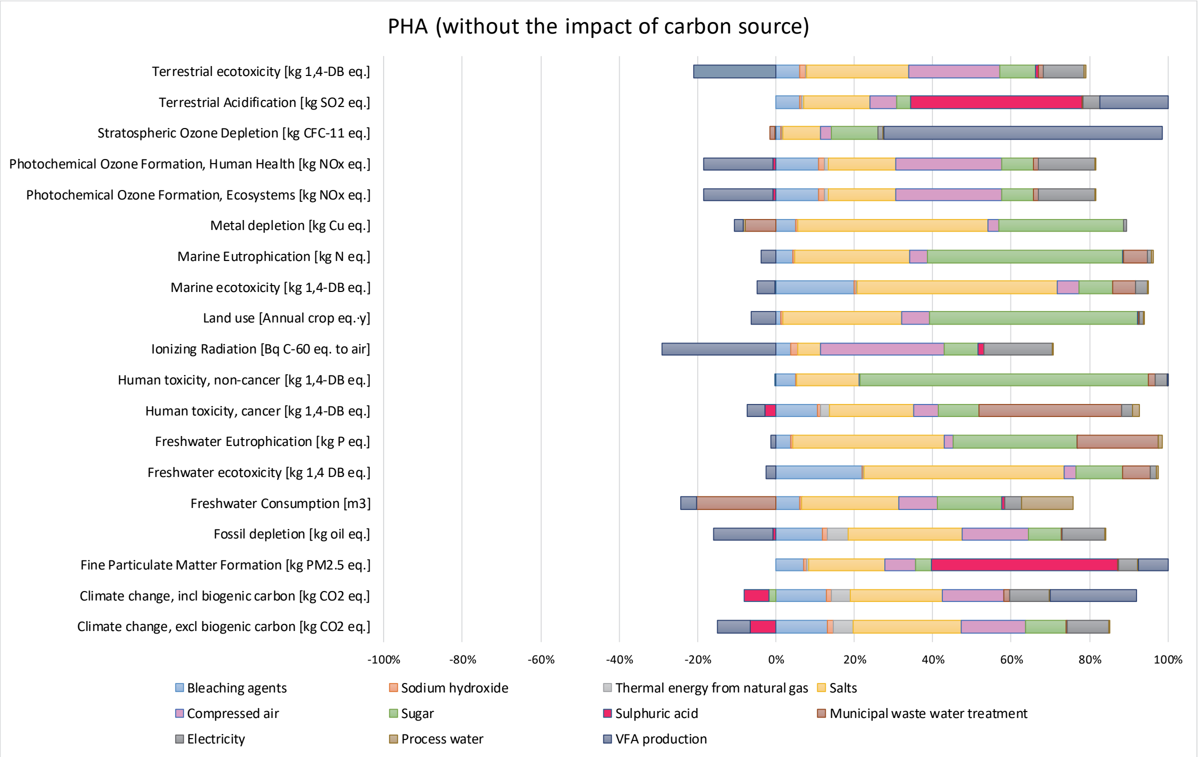


Figure 8: Environmental footprint for PHA production, without the impact of waste

Sensitivity analysis was performed for the variation of sugar consumption. The effect of different sugar consumption (±10%) on environmental impact was examined. Indicative impact categories are presented in Figure 9. The variation of sugar changes the impact categories less than 4%, apart from human toxicity, land use, metal depletion and marine eutrophication (less than 8%).

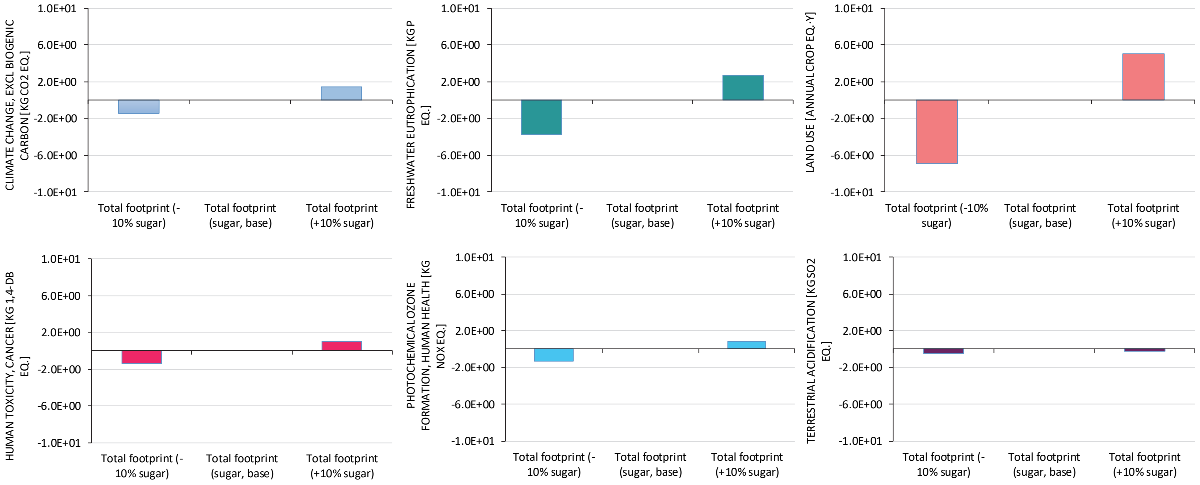


Figure 9: Several environmental impact categories for the production of PHA, without the impact of carbon source for various sugar consumption

The production of PHA using VFA platform was also compared with current methodologies used for PHA (or PHB) production from sugar cane (Harding et al., 2007), glycerin by-products (Leong et al., 2017) and wastewater sludge (Gurieff and Lant, 2007).

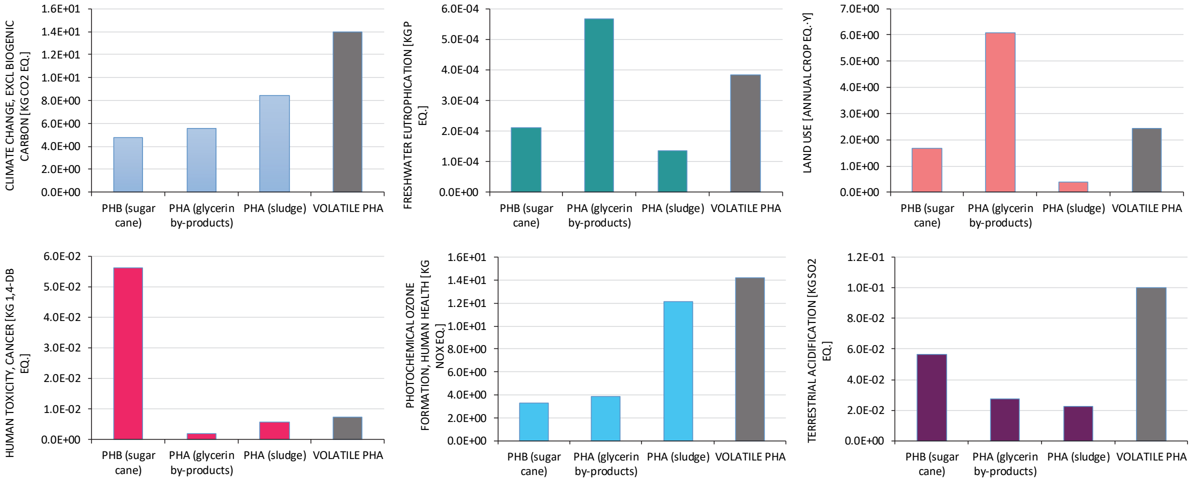


Figure 10: Comparison of several environmental impact categories for the production of PHA (without the impact of VGF) of VOLATILE PHA production system to an alternative system

4. CONCLUSIONS

VOLATILE VFA platform presents significantly lower footprint to all categories compared to an alternative methodology for VFA production from food waste, mainly due to the produced electricity credits. VOLATILE PHA systems performs similarly with other examined alternative systems. The VOLATILE technologies are new and can be further optimized leading to even improved environmental behavior. VOLATILE technologies are new and can be further optimized leading to even improved environmental behavior.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

5. REFERENCES

Camia, A. et al., Biomass production, supply, uses and flows in the European Union. First results from an integrated assessment, EUR 28993 EN, Publications Office of the EU, Luxembourg, 2018.

Elginoz, N., Khatami, K., Owusu-Agyeman, I. and Cetecioglu, Z., (2020). Life Cycle Assessment of an Innovative Food Waste Management System. Frontiers in Sustainable Food Systems 4: 23.

https://ec.europa.eu/programmes/horizon2020/en/h2020-section/bioeconomy

Gurieff, N. and Lant, P. (2007). Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoate production. Bioresource Technology 98 3393–3403.

Harding, K.G., Dennis, J.S., von Blottnitz, H. and Harrison, S.T.L. (2007). Environmental analysis of plastic production processes: Comparing petroleum-based polypropylene and polyethylene with biologically-based poly-B-hydroxybutyric acid using life cycle analysis. Journal of Biotechnology 13057–66.

International Standard, ISO 14040, 2006. Environmental Management -Life Cycle Assessment-General Principles and Framework. International Organization of Standardization.

Leong, Y.K., Show, P. L., Chi-Wei Lan, J., Loh, H.-S., Lam, H.L. and Ling, T.C. (2017) Economic and environmental analysis of PHAs production process. Clean Technologies and Environmental Policy 19:1941–1953.

Sharma, B.K. and Chandel, M.K. (2017). Life cycle assessment of potential municipal solid waste management strategies for Mumbai, India. Waste Management & Research 35: 79–91.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

A MULTILEVEL APPROACH IN ASSESSING CIRCULAR GREEN TECHNOLOGY ADOPTION: Deriving plausible business case scenarios through business modelling and agent-based modelling

S. Farahbakhsh [a], A. Santamaria [b], J. San José [b], J. Van Meensel [a], S. Snellinx [a], Edward Belderbos [a], A. Mertens [a]
[a] *EIGEN VERMOGEN VAN HET INSTITUUT VOOR LANDBOUW EN VISSERIJONDERZOEK, Burgemeester van Gansberghelaan 96, 9820 Merelbeke, Belgium*
[b] *ODEI SA, Plaza del Renacimiento 9, Planta 4, Oficina 20, 01004 Vitoria-Gasteiz, Spain*
Corresponding author: Siavash.Farahbakhsh@ilvo.vlaanderen.be

Abstract

While the attention to the circular economy is increasing, little research has been done to explore the socioeconomic mechanisms underlying the adoption of circular economy technologies. Against this backdrop, in this research, we aim to derive plausible adoption scenarios at both company and ecosystem levels for circular economy technologies. In doing so, we rely on the case of the waste-treatment plant industry using VOLATILE project data. The circular economy technology in this project is the Volatile Fatty Acid Platform (VFAP), which is about the introduction of a valorisation process technology for the production of high-end products at waste-treatment plants. We analyse our case deploying business modelling and agent-based modelling. Our results show the important role of gate-fee, cost of investment, subsidies, technology improvements, pilot installations and social pressure in the adoption of VFAP.

Keywords: circular economy, valorisation technologies, socioeconomic analysis, waste-treatment industry

1. TRANSITION TOWARDS A CIRCULAR ECONOMY

The circular economy has been introduced as a solution to environmental degradation resulting from the current linear take-make-dispose model (EC, 2014). Its focus is on closing material loops and increasing resource efficiency, which could lead to environmental sustainability as well as new economic opportunities (Moreau et al., 2017). In this respect, the waste treatment sector is of high importance and has a lot of potential for the circular economy. By re-using and recycling products and materials, the amount of waste and emissions can be reduced as well as the amount of primary resources needed (Korhonen et al., 2018). Consequently, adopting circular economy practices in waste treatment plants can reduce resource and energy costs as well as costs due to environmental legislation (e.g. landfilling taxes) (Korhonen et al., 2018).

Nowadays, anaerobic digestion is a prominent organic waste treatment practice. It can be seen as a part of a circular economy system since it deals with problems of waste management and emissions (renewable energy with low carbon footprint) as well as (local) energy demand (replacing fossil fuels) (Gutberlet et al., 2020; Pan et al., 2015). Most often, residues can be composted and used for soil improvement. Therefore, anaerobic digestion is already a suitable waste management practice. However, the waste hierarchy described in the European Commission's 2008 waste framework directive (2008/98/EC) requires waste management to go a step further. Rather than just recovering energy from waste, materials should be recycled

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

where possible. Therefore, there is a need for new technologies to recycle organic waste in a sustainable manner.

With this context, in this paper, we aim to investigate scenarios, which foster the adoption of circular economy technologies in the waste-treatment industry. In doing so, we rely on the VOLATILE project data (H2020 VOLATILE, 2020), which includes collected socioeconomic information about waste-treatment plants with regard to an innovative circular economy technology. During the VOLATILE project, an innovative technology was developed aiming to transform existing waste treatment plants into providers of volatile fatty acids (VFAs), which could be used for the production of four high-end products (VOLATILE products); purified acetic acid (AA), polyhydroxyalkanoates (PHA), single cell oils (SCO) and polyunsaturated fatty acids (PUFA). The technology aims to achieve higher levels of resource efficiency, by creating more value with the same input. That value is both environmental and economic. Nonetheless, the adoption of such technology contains risk-taking and positive evaluations of economic feasibility, which decision-makers at the waste-treatment plants face.

In the later sections, we show the used methodology, data analyses approach and results which provide plausible scenarios fostering the economic feasibility and reducing the adoption risk of VFAP.

2. DATA, METHODOLOGY, ANALYSIS

We rely on the VOLATILE project data: both qualitative and quantitative data were gathered. The qualitative data were obtained, mainly through semi-structured interviews with the business cases of the consortium and technology experts along with knowledge from project meetings. The quantitative data used in this analysis come from techno-economic analyses carried out in other work packages of the VOLATILE project as well as internet documents, specialised press and handbooks.

Analysing the collected data, we focused on the two levels of business and the ecosystem to come to a conclusion about the economic feasibility and potential for adoption of the VFAP.

Business level. We use business modelling to analyse the proposed VOLATILE products on their value proposition, with a special focus on customer and market, and on their cost structure and revenue streams. Firstly, adding value is the purpose of every organization. In the context of a business, the focus is put on providing economic value. The end result of the value proposition evaluation is a clear description of the VOLATILE product, its value in relation to the market in which it can be sold and its value in comparison to directly competing products. The current market price and/or the price of direct competing products will lead to a price estimation of the VOLATILE products. A second item in the business model analysis is an assessment of the cost structure and revenue stream analyses conducted in the VOLATILE project.

Ecosystem level. We use agent-based modelling (ABM) (Wilensky & Rand, 2015) to investigate the socioeconomic conditions necessary for the adoption of the VFAP. ABM is a strong tool to simulate and investigate complex social systems, where multiple heterogeneous hierarchical interacting units exist (Holland, 2014). Using this method and relying on the

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

collected data in the VOLATILE project, we have developed a model, where agents are waste-treatment plants of different kinds simulated in a community network. Agents face the problem of whether to adopt VFAP technology. They make the adoption decision based on the economic feasibility assessment and the social pressure (i.e. number of adopting neighbours of an agent). In this way, we analyse the socioeconomic conditions at an ecosystem level for industry wide VFAP adoption.

3. RESULTS

3.1 Business modelling

The business modelling results show the importance of attention to the structure of the targeted markets for VOLATILE products and the gate-fee required for a plausible VFAP adoption scenario. In this respect, Table 1 describes the market opportunity for each VOLATILE product.

Table 1: Market size, commodity price and supporting movements for each investment scenario

Scenarios	**Market size (# investments needed to reach 5% of the target market)**	**Estimated price (euro/kg)**	**Supporting movements**
VFA investment	-	0.2 – 1.25	-
Combined VFA-AA investment	275	0.75	-
Combined VFA-PHA investment	5	4	*Trend toward biodegradable packaging and discussions around plastic soup and micro-plastics*
Combined VFA-SCO investment	4,100	0.6-0.7	*Global trend against palm oil and calls for alternative sources*
Combined VFA-PUFA investment	70	1.7	*Global trend against overusing fish oil / fishing and calls for alternative sources*

Table 1 puts forward that each of the end products has a lot of potential for a waste treatment company to invest in, albeit each for its own reasons. For example, the size of the PHA market is rather small: 5 companies investing in this technology would make up for 5% of production in the market. In this market, a company that aims to invest and grow, can accumulate significant market power but that also comes with more risk. The size of the SCO market, on the other hand, is rather large. A company that enters this market, faces a lower risk that changes in the market will have a large effect on it. The large market size also signifies that there is enough demand for this product already to make investments to produce more. How markets will evolve in the future is difficult to quantify, which is why the supporting movements' column is included in Table 1. There is a trend towards biodegradable plastics, and there are trends against palm oil and overfishing favouring VOLATILE products.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777

Furthering the business modelling analysis, we identified the level of gate fees required for each scenario in order to reach the break-even point, where the revenue generated from the VOLATILE products reaches and equals the cost of production. Currently, the VFAP is not yet at the same technology readiness level (TRL) as biogas or composting. For these mature technologies, there are subsidies in place, that do not yet exist for the VFAP. Since it is important to follow EU legislation regarding the waste management hierarchy and the ban on landfilling, early innovators may need to find additional income to make the VFAP work. For waste treatment plants, increasing gate fees could be an effective way to obtain the needed funding to adopt the VFAP while continuing to make this new practice become more profitable. The average gate fee calculated based on all of the business cases involved in the project, is ca. 44.7 euro/ton. Moderate to significant increases in gate fees are needed to break even when producing each of the VOLATILE products. This increase needed ranges from ca. 16 to 39 euro/ton. However, when the technology evolves further, this could be reduced.

3.2 Agent-based modelling

The ecosystem level is analysed through an agent-based model. With this model, we attempt to gain insight into the drivers and barriers for technology adoption in the waste treatment sector. Therefore, we simulated waste treatment plants with different characteristics (types of waste, public/private, volumes of waste). The waste treatment plants exist in a network with neighbours that they can observe as a representation of changes in the sector and society. In this model, waste treatment plants make their decisions on two broad parameters. First, they make economic calculations: potential cost and revenues, gate fees and return on investment to learn about the economic feasibility of investment in the VFAP. Secondly, they base their decisions on social reasoning. They pay attention to how many of their neighbours are adopting the VFAP. This is a representative for – not only knowing what companies in their region are doing – but also for institutional change, changes in market demand, and all societal changes that could affect which technology a company would desire to invest in.

Four relevant scenarios for the sector wide adoption of the VFAP were considered in this analysis:

1) Increase in *cost of investments* and *role of subsides*.
2) Increase in *gate-fee*.
3) Increase in the *attention to social reasoning* while reducing the attention to economic reasoning.
4) Increasing *the number of installed pilots* while increasing the attention to social reasoning.

Figure 1 shows the results from Scenario 1. These results suggest that the roles of investment costs and subsidies are straightforward and linear. The higher the cost, the lower the eventual adoption rate. The higher the investment subsidies, the higher the eventual adoption rate.

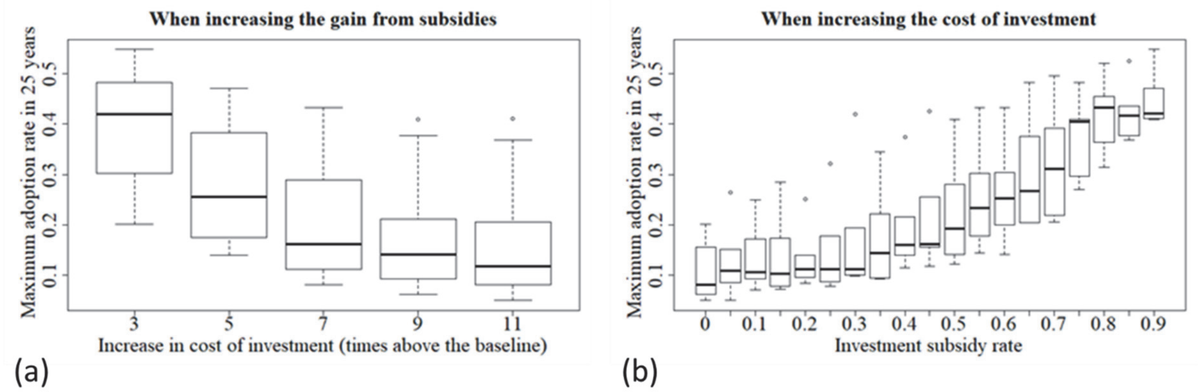


Figure 1: Effect of costs and subsidies

Figure 1.a depicts higher average adoption rates when investment costs are lower. The variation portrayed by the boxplots shows the impact of subsidies on that adoption rate. Figure 1.b. suggests that insufficient subsidies have the potential to reduce adoption rates, while higher subsidies can increase adoption rates.

Continuing with the ABM analysis, Figure 2 shows the results from Scenario 2. An increase in gate fee leads to higher adoption rates, up to a certain point. The higher the gate fee, the higher the adoption rates.

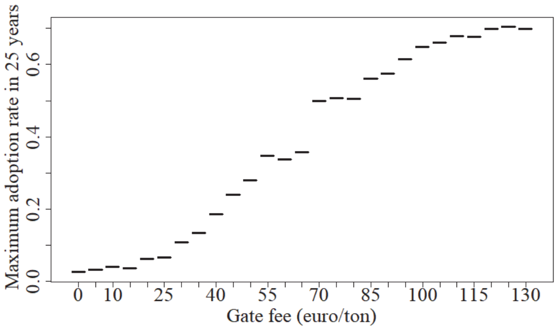


Figure 2: Gate fee effect

Notably, the effect of gate fee increase on adoption is mostly visible from ca. 40 ca. 80 euro per ton. Below this range, the effect is minor, and above this range, there is a plateau effect.

Furthermore, the results from Scenario 3 are shown in Figure 3.

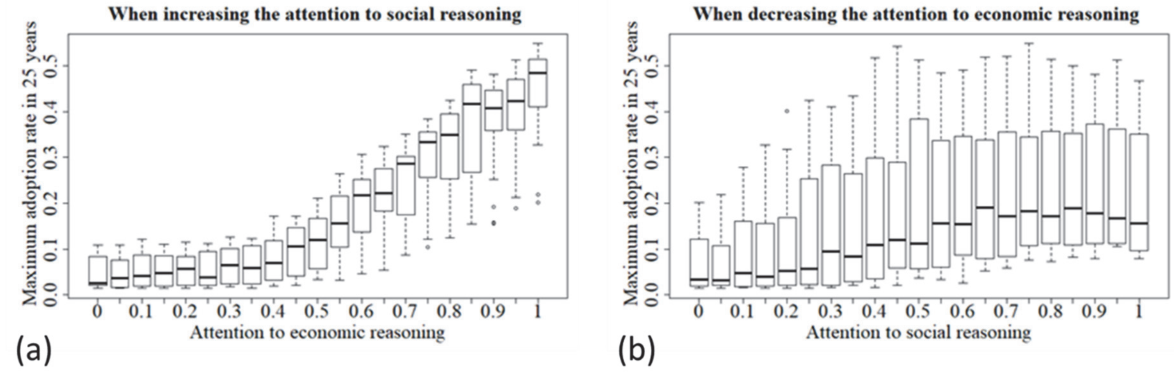


Figure 3: Covariation of attention to economic and social reasoning

Scenario 3 represents a covariation between social and economic reasoning because both of these parameters are considered in the decision-making model of treatment plants and their combined effect influences adoption rates. Figure 3.a shows the higher the attention to economic reasoning, the higher the adoption rate in 25 years. The variation in this graph demonstrates the change in the level of attention to social reasoning. Similarly, Figure 3.b shows that increasing attention to social reasoning increases adoption rates, however, only up to a certain point. After ca. 0.6, a plateau is reached. Therefore, after this point, other factors need to be changed in order to increase adoption rates (e.g. gate fee).

Moreover, Figure 4 shows the results of Scenario 4. With this scenario, we attempt to gain insight into the covariation effect of pilots and social pressure stimulating the environment into adopting the technology.

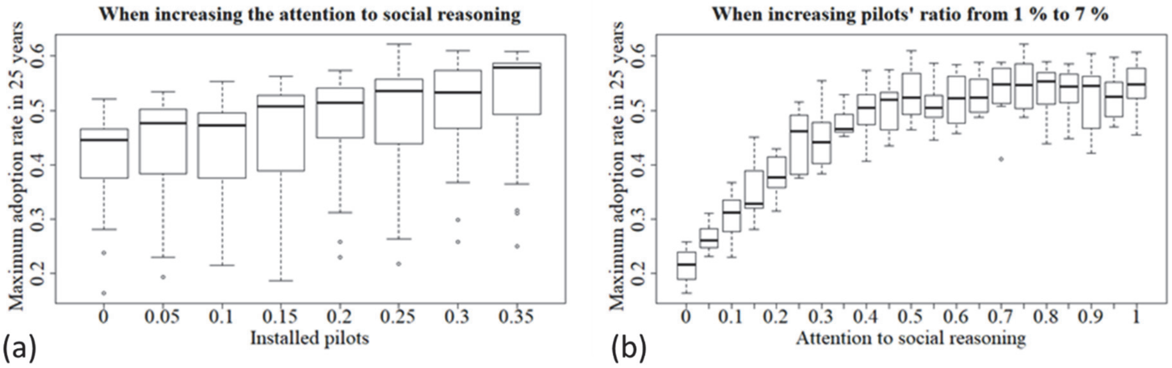


Figure 4: Covariation of increase in the number of pilots and attention to social reasoning

Figure 4.a puts forward that a higher percentage of installed pilots leads to higher eventual adoption rates. However, when the attention to social reasoning is very low, pilots do not have their desired effect. Moreover, the results from Figure 4.b show the effect of an increase in the attention to social reasoning while increasing the ratio of installed pilots. This result suggests that the effect of attention to social reasoning, in the presence of an increase in the pilot ratio, fosters the VFAP adoption rate. Thus, having more or few pilots in a network with significant social reasoning has the potential to increase or decrease adoption rates, as it pressures the agents into considering the technology.

4. CONCLUSIONS

Investment in a circular technology such as VFAP opens a venue to new markets with certain sizes, for waste treatment companies. However, such technology adoptions require careful investment assessment together with business modelling. Moreover, improvements in VFAP technology is an important factor as it will result in higher yields and more profitable scenarios. Such improvements could be reached through pilot installation, where plants could assess and improve the scalability of the technology. Currently, gate-fee plays a crucial role in VFAP adoption. Nevertheless, its effect is visible in a certain range (moderately low to moderately high). That is, in order to support the adoption of VFAP technology, a minimum and a maximum level of increase in gate fee should be considered.

At a more network level, social pressure in the form of peer pressure or movements in society has a strong effect on the VFAP adoption pattern. But similar to the gate-fee effect, the social pressure effect disappears after a certain degree. Therefore, in order to achieve higher levels of VFAP adoption, social-pressure needs to be accompanied by other economic factors such as gate-fee, pilot installations and subsidies.

5. REFERENCES

European Commission. (2014). Communication from the Commission - Towards a circular economy: A zero waste programme for Europe. European Commission, 398, 1–14.

Gutberlet, J., Bramryd, T., & Johansson, M. (2020). Expansion of the waste-based commodity frontier: Insights from Sweden and Brazil. Sustainability (Switzerland), 12(7). https://doi.org/10.3390/su12072628

H2020 VOLATILE. (2020). Biowaste derived volatile fatty acid platform for biopolymers, bioactive compounds and chemical building blocks. Retrieved from http://www.volatile-h2020.eu/

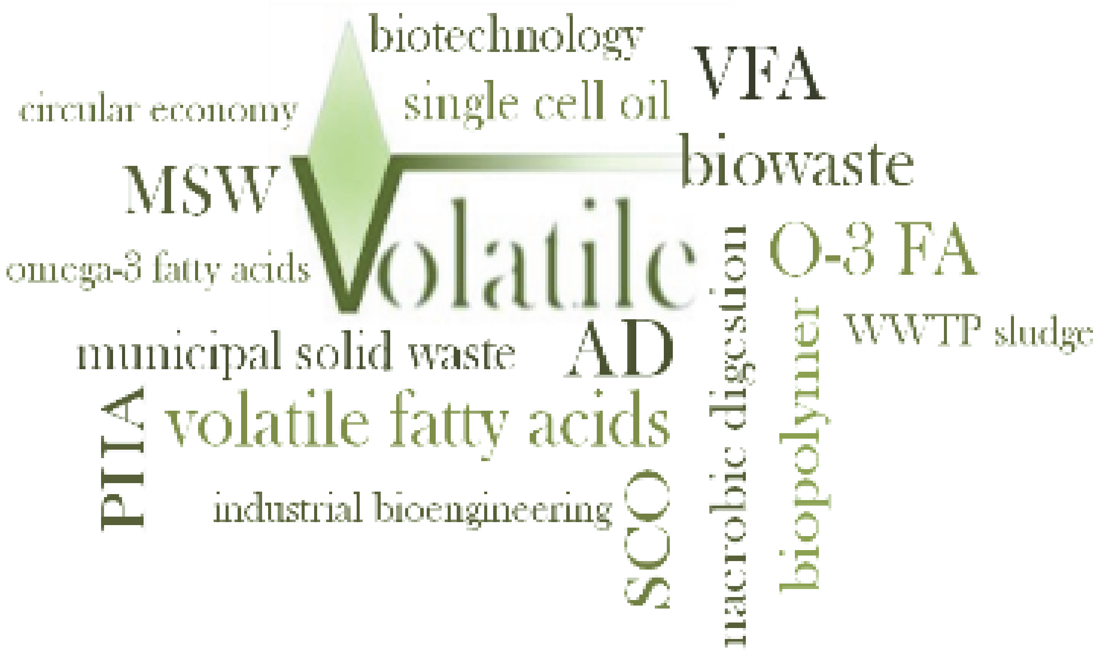
Holland, J. H. (2014). Complexity: A Very Short Introduction. Complexity: A Very Short Introduction. https://doi.org/10.1093/actrade/9780199662548.001.0001

Korhonen, J., Honkasalo, A., & Seppälä, J. (2018). Circular Economy: The Concept and its Limitations. Ecological Economics, 143(January), 37–46. https://doi.org/10.1016/j.ecolecon.2017.06.041

Moreau, V., Sahakian, M., van Griethuysen, P., & Vuille, F. (2017). Coming Full Circle: Why Social and Institutional Dimensions Matter for the Circular Economy. Journal of Industrial Ecology, 21(3), 497–506. https://doi.org/10.1111/jiec.12598

Pan, S. Y., Du, M. A., Huang, I. Te, Liu, I. H., Chang, E. E., & Chiang, P. C. (2015). Strategies on implementation of waste-to-energy (WTE) supply chain for circular economy system: A review. Journal of Cleaner Production, 108, 409–421. https://doi.org/10.1016/j.jclepro.2015.06.124

Wilensky, Uri ; Rand, W. (2015). What Is Agent-Based Modeling? An Introduction to Agent-Based Modeling Modeling Natural, Social, and Engineered Complex Systems with NetLogo.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 720777