



| <b>Algemene gegevens</b>  |  |
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| TKI-projectnummer   | AF-EU-13003  |
| Titel   | SPLASH: Sustainable Polymers from Algae Sugars and Hydrocarbons  |
| Topsector en innovatiethema                                     | A&F  |
| Projectleider (onderzoek)                                       | Dr. Lolke Sijtsma  |
| Werkelijke startdatum   | 1 September 2012   |
| Werkelijke einddatum  | 28 Februari 2017   |
| Korte omschrijving inhoud (bij voorkeur 4 regels, max. half A4) | Wageningen Research is responsible for projectmanagement, sequencing of the algae <i>Botryococcus braunii</i> and, in close cooperation with projectpartners, strain improvement, optimization of algal cultivation and product formation, separation and conversion technologies and development of a pilot facility. |

| <b>uitvoerende partijen</b>  |   |
|------------------------------|---|
| betrokken kennisinstellingen | Wageningen Food & Biobased Research (WFBR)<br>Wageningen Plant Research (WPR)   |
| overige partijen             | <p>Companies:<br/>PAQUES BV, Netherlands (till 1.3.2015), AVANTIUM CHEMICALS BV Netherlands, PNO CONSULTANTS BV Netherlands, NIELS-HENRIK NORSKER, Denmark , VALUE FOR TECHNOLOGY Belgium, ORGANIC WASTE SYSTEMS NV Belgium, LIFEGLIMMER GMBH Germany, NOVA-INSTITUT FUR POLITISCHE UND OKOLOGISCHE INNOVATION GMBH Germany, LANKHORST EURONETE PORTUGAL Portugal, Rhodia Operations, France, Cellulac, Ireland, F&amp;M, Italy</p> <p>Knowledge institutes<br/>WAGENINGEN UNIVERSITEIT, Netherlands, UNIVERSITAET BIELEFELD, Germany, FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V ,Germany, WESTFAELISCHE WILHELMS-UNIVERSITAET MUENSTER, Germany, THE UNIVERSITY OF CAMBRIDGE, United Kingdom, UNIVERSIDAD DE HUELVA, Spain, EGE UNIVERSITESI EGE Turkey</p> |

| <b>Resultaten en deliverables</b>   |   |
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| 1. Welke deliverables zijn opgeleverd, en is dit conform het projectplan? (geef een korte | The aim of the 4.5-year SPLASH project was to develop a new biobased industrial platform using microalgae as a renewable raw material for the sustainable production and recovery of hydrocarbons and (exo)polysaccharides from the species |

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| <p>beschrijving per deliverable uit het projectplan)</p> <p>Deliverables</p> <p>Management deliverables</p> <p>Information on Genome and transcriptome of <i>B. Braunii</i></p> <p>Optimized growth and production of hydrocarbons and polysaccharides</p> <p>Process for continuous product recovery of hydrocarbons and polysaccharides from <i>Botryococcus</i></p> <p>Biosafety</p> <p>Hydrolysis of algae polysaccharides into monomeric sugars</p> <p>Conversion of fucose and rhamnose to 1,4-pentanediol</p> | <p><i>Botryococcus braunii</i> and further conversion to renewable polymers. Main focus points were:</p> <ul style="list-style-type: none"> <li>• Understanding of product formation of two <i>B. braunii</i> strains that produce mainly hydrocarbons or polysaccharides respectively, based on in-depth gene analysis</li> <li>• Development of understanding and procedures for production, in situ extraction and isolation of hydrocarbons and carbohydrates from selected <i>B. braunii</i> strains for further product development</li> <li>• Conversion of hydrocarbons or polysaccharides to products</li> <li>• Process demonstration at pilot scale, Process integration, sustainability assessment and market analysis</li> </ul> <p>All deliverables, as agreed on in the project proposal, have been accepted by the EC.</p> <p>Management of the project</p> <p>Sequence the genome of <i>B. braunii</i> race A, and by combination of transcriptomics, proteomics and metabolomics, together with metabolic modelling, construct metabolic maps for the production of hydrocarbons and cell wall polysaccharides.</p> <p>For the optimisation of growth and production conditions two different approaches will be followed being a statistical/empirical approach that will enable a fast optimization of the medium and conditions and a mechanistic approach, which will lead to a better understanding of <i>B. braunii</i></p> <p>Development of in situ DSP methods</p> <p>A Biosafety Officer will be appointed who will be responsible for drawing up a plan to ensure that all partners comply with the Biosafety regulations required by EU directives 219 &amp; 90, and local Health and Safety regulations</p> <p>The polysaccharide fraction produced in WP3 will be characterized. The polysaccharides will be depolymerized into their constituting monosaccharides by chemo-enzymatic methods</p> <p>Rhamnose and fucose both are excellent starting materials for the subsequent chemo-enzymatic synthesis of 5-methyl furfural. This approach has the potential to lead to both a more environmentally benign as well as cheaper route to 5-methylfurfural</p> |
| <p><b>2.</b> Indien bepaalde deliverables niet gehaald zijn, wat was daarvoor de reden?</p>  |  |
| <p><b>3.</b> Heeft het project onverwachte (neven)uitkomsten opgeleverd, die vooraf niet waren voorzien?</p>   | <p>No</p>  |

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| Zo ja, benoem deze.  |  |
| <p><b>4.</b> Op welke wijze is over het project en de resultaten gecommuniceerd</p>  | <p>Public and potential stakeholders were informed about the outcome and benefits of the project. Conferences, meetings and a summer school were organised in order to inform and train researchers (academic and industrial) in the fields of metabolic modeling and engineering, bioconversion, microalgae cultivation, downstream processing, conversion of biomass (especially hydrocarbons and polysaccharides into chemicals), life cycle assessment and process modeling.</p> |
| <p><b>5.</b> In hoeverre heeft het project bijgedragen aan de ontwikkeling van de betrokken kennisinstelling(en)? (bijv. wetenschappelijk track record, nieuwe technologie, nieuwe samenwerkingen)</p> | <p>The project has largely contributed to enhancement knowledge and tools of WPR in the field of systems biology, genome sequencing and annotation. WFBR build knowledge on algal cultivation, product extraction, conversion of sugars to polymer building blocks and techno economic analysis. The work resulted in a number of presentations and publications (track record)</p>  |
| <p><b>6.</b> Krijgt het project een vervolg in de vorm van een nieuw project of een nieuwe samenwerking? Zo ja, geef een toelichting.</p>  | <p>Although ideas for future cooperation projects have been generated and discussed at the final project meeting, no new projects on this specific topic have been submitted yet. This is partly due to difficulties in stable production of <i>B. braunii</i> at industrial scale.</p> <p>Based on expertise and visibility, however, 2 new projects have been submitted within the blue bioeconomy topic, of which one already is granted (WU coordinator)</p>                     |

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| <p><b>Highlights: geef een korte beschrijving van de belangrijkste resultaten</b> (deze beschrijving wordt als publieke samenvatting op de websites van de TKI's/topsectoren geplaatst)</p>  |
| <p>Around the world steps are being taken to move from today's fossil based economy to a more sustainable economy based on biomass. The 4.5-year European project SPLASH aims to develop a new biobased industrial platform using microalgae as a renewable raw material for the sustainable production and recovery of hydrocarbons and (exo)polysaccharides from the species <i>Botryococcus braunii</i> and further conversion to renewable polymers.</p> <p>After 4,5 years of research we developed tools and technologies needed for the establishment of a new industrial sector: Industrial Biotechnology with algae and/or algal genes for the manufacture of industrial raw materials. We built a foundation for future commercialisation of hydrocarbon production with the <i>B. braunii</i>. We developed knowledge to understand the product formation of two <i>B. braunii</i> strains that produce mainly hydrocarbons or polysaccharides respectively, based on in-depth gene analysis. We improved algae production and were able to recover hydrocarbons and polysaccharides while keeping the algae viable, although further improvements are required. Furthermore, we made substantial progress in the analytics of carbohydrate and hydrocarbon fractions and the conversion of carbohydrates into building blocks and subsequently polymers such as fibres for yarns. As industry, however, needs relatively cheap and pure raw materials, costs for sugars produced by <i>B. braunii</i> are currently far too high. Furthermore, separation of sugars from mixtures remains an important item to be addressed. We developed a pilot facility capable of demonstrating an optimized process for the production and utilisation of both polysaccharides and hydrocarbons and identified potential business cases for <i>B. braunii</i> hydrocarbons based on realistic assumptions.</p> <p>For details I would refer to the cordis website:<br/> <a href="https://cordis.europa.eu/docs/results/311/311956/final1-17-05-23-final-report-splash-2017-summary.pdf">https://cordis.europa.eu/docs/results/311/311956/final1-17-05-23-final-report-splash-2017-summary.pdf</a></p> |

| <b>Aantal opgeleverde producten</b> (geef in een bijlage de titels en/of omschrijving van de producten of een link naar de producten op openbare websites) |           |                        |  |                                       |               |
|--|-----------|------------------------|--|---------------------------------------|---------------|
| Wetenschappelijke artikelen  | Rapporten | Artikelen in vakbladen | Inleidingen/workshops/invited lectures | Aangevraagde octrooien /first filings | Spin-offs (*) |
| 11   |           |                        | 10                                     |                                       |               |

(\*) Hiermee wordt bedoeld: contractonderzoek dat voortkomt uit dit project, aanvullende subsidies die zijn verkregen en spin-off bedrijvigheid.

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| Verwacht u nog een octrooiaanvraag naar aanleiding van dit project? | NEE |
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**Bijlage: Titels van de producten of een link naar de producten op een openbare website**

All information on project results, presentations and publications are presented at the SPLASH and cordis websites.

<http://eu-splash.eu/>

[https://cordis.europa.eu/project/rcn/104994\\_en.html](https://cordis.europa.eu/project/rcn/104994_en.html)

[https://cordis.europa.eu/result/rcn/212762\\_en.html](https://cordis.europa.eu/result/rcn/212762_en.html)

<https://cordis.europa.eu/docs/results/311/311956/final1-17-05-23-final-report-splash-2017-summary.pdf>

<https://www.wur.nl/nl/project/SPLASH-duurzame-polymeren-uit-algen.htm>

Akkoord: Hans van der Kolk (Topsectorsecretaris).

## APPENDIX.

### 2015

**Bicas, J.L., Kleinegris, D.M.M., Barbosa, M.J.** (2015) Use of methylene blue uptake for assessing cell viability of microalgae. *Algal Research* 8 (2015) 174–180 (Publication)  
<http://www.sciencedirect.com/science/article/pii/S2211926415000302>

**Broek, L.A.M. van den, J.M. van, Klis, F. van der, Stoutjesdijk, J.H., Boeriu, C., Blaauw, R.** (2015). Exopolysaccharides from *Botryococcus braunii* and the production of bioplastics. In: *Proceedings 4th EPNOE International Polysaccharide Conference: Polysaccharides and polysaccharide-based advanced materials: from science to industry.* p. 136 - 136.

**Broek, L.A.M. van den, Klis, F. van der, Stoutjesdijk, J.H., Boeriu, C.G., Blaauw, R.** (2015). Exopolysaccharides from *Botryococcus braunii* and the production of bioplastics In: *Exopolysaccharides from Botryococcus braunii and the production of bioplastics.* - Warsaw, Poland, 19-22 October 2015.

**Kleinegris, D.M.M** (2015) *AlgaeParc's* EU projects, SPLASH, MIRACLES, FUEL4Me and InteSusal. 8th October 2015, Wageningen, The Netherlands (presentation in the framework of Greentech week)

**Sijtsma, L, A.G. Smith, D.M. Kleinegris, E. de Jong, M. Fenton, P. Willems, D. Vogt, M.J. Barbosa** (2015). *Botryococcus braunii* as production platform for sugars and hydrocarbons to be used as building blocks for polymers: Progress of the EU FP7 project SPLASH. 2nd EABA and EC Algae contractors conference and the 9th international algae congress. Lisbon, 1-3 December 2015

**Sijtsma, L., Barbosa, M.J.** (2015) SPLASH: Sustainable Polymers from Algae Sugars and Hydrocarbons Conference: Making more of Bio-economy results, 6- 7 October 2015, Brussels, Belgium

**Sijtsma, L.** (2015) *Microbes –The New Bioplastic Factories*  
[http://commnet.eu/05\\_News/Microbes-The-New-Bioplastic-Factories.kl](http://commnet.eu/05_News/Microbes-The-New-Bioplastic-Factories.kl)

Vigani, M., Parisi, C., Rodriguez-Cerezo, E., Barbosa, M.J., **Sijtsma, L.**, Ploeg, M., Enzing, C. (2015) Food and feed products from micro-algae: Market opportunities and challenges for the EU. *Trends in Food Science and Technology* 42 (2015)1. - ISSN 0924-2244 - p. 81 - 92. <http://www.sciencedirect.com/science/article/pii/S0924224414002787>

### 2016

**Cubero, R.G., Dorinde Kleinegris, Maria Barbosa** (2016). A better understanding of culture parameters to increase exopolysaccharide production by *Botryococcus braunii* CCALA 778 2016 European Roadmap for an Algae-Based Industry, 6-8 April 2016, Olhão, Portugal.

**Cubero,R.G., Dorinde M.M Kleinegris, Maria Barbosa.** (2016) Indoors culture of *B.braunii* CCALA778 simulating Mediterranean climate conditions. *Algal Biomass, Biofuels & Bioproducts.* San Diego (CA). USA.

**Cubero, R.G., Dorinde M.M. Kleinegris,** Maria Barbosa (2016) Indoors culture of *B.braunii* CCALA778 simulating Mediterranean climate conditions ALGAEUROPE, 13-15 December, Madrid, Spain, Book of abstracts pp177.

**Kleinegris, D.M.M** (2016). Milking of microalgae. Summerschool, Microalgae Biorefinery, 18-20 July 2016, Wageningen, The Netherlands.

**Sijtsma, L.,** A.G. Smith, D.M. Kleinegris, E. de Jong, M. Fenton, P. Willems, D. Vogt, M.J. Barbosa. (2016) Sustainable Polymers from Algae Sugars and Hydrocarbons (SPLASH). Poster presentation, Workshop Algae & Seaweed: February 18th 2016, Wageningen, The Netherlands

**Sijtsma, L.,** A.G. Smith, D.M. Kleinegris, E. de Jong, M. Fenton, P. Willems, D. Vogt, M.J. Barbosa. (2016). SPLASH: Sustainable polymers from algae sugars and hydrocarbons. Presentation: European roadmap for an algae-based industry, 6-8 April, Olhão, Portugal. [http://eualgaeroadmapconference.eu/fileadmin/intesusal\\_docs/Documents/Industry\\_Seminars/Presentations\\_2016/Welcome/08.Lolke\\_2016-04-05\\_Olhao\\_SPLASH\\_LS.pdf](http://eualgaeroadmapconference.eu/fileadmin/intesusal_docs/Documents/Industry_Seminars/Presentations_2016/Welcome/08.Lolke_2016-04-05_Olhao_SPLASH_LS.pdf)

**Sijtsma, L.,** A.G. Smith, D.M. Kleinegris, E. de Jong, M. Fenton, P. Willems, D. Vogt, M.J. Barbosa. (2016). SPLASH: Sustainable polymers from algae sugars and hydrocarbons. Abstract: European roadmap for an algae-based industry, 6-8 April, Olhão, Portugal.

**Sijtsma, L.,** A.G. Smith, D.M. Kleinegris, E. de Jong, R.J. van Putten, J. Carrigan, P. Willems, D. Vogt, M.J. Barbosa (2016). Abstract: Sustainable polymers from algae sugars and hydrocarbons (SPLASH): Lessons learned. ALGAEUROPE, 13-15 December, Madrid, Spain, Book of abstracts, pp89.

**Sijtsma, L.,** A.G. Smith, D.M. Kleinegris, E. de Jong, R.J. van Putten, J. Carrigan, P. Willems, D. Vogt, M.J. Barbosa (2016). Presentation: Sustainable polymers from algae sugars and hydrocarbons (SPLASH): Lessons learned. Presentation, ALGAEUROPE, 13-15 December, Madrid, Spain, <http://algaecongress.com/2016-conference-program/>

**Van der Klis, F., van den Broek, L., Blaauw, R., Knoop, R., Bitter, H.** (2016). Structure-property relations of biobased polyesters from 1,4-butanediol-analogues and biobased diacids. Oral presentation. CHAINS 2016, the Dutch chemistry conference on 6-8, December 2016. Veldhoven, The Netherlands.

**Van den Broek, L.A.M.** Stoutjesdijk J.H., Gelo-Pujic M., Norsker N.H., **Togtema A.**(2016). Properties of extracellular polysaccharides from *Botryococcus braunii*. Abstract: European roadmap for an algae-based industry, 6-8 April, Olhão, Portugal.

**Van den Broek, L.A.M.** (2016). Analytical characterization techniques. Summerschool, Microalgae Biorefinery, 18-20 July 2016, Wageningen, The Netherlands.

## 2017

Carreres BM, de Jaeger L, Springer J, Barbosa MJ, Breuer G, van den End EJ, Kleinegris DMM, Schäffers I, Wolbert EJ, Zhang H, Lamers PP, Draaisma RB, Martins Dos Santos VA. (2017). Genome Announc. 2017 Jan 19;5(3). <https://www.ncbi.nlm.nih.gov/pubmed/28104651>

Gouveia JD, Ruiz J, van den Broek LAM, Hesselink T, Peters S, Kleinegris DMM, Smith AG, van der Veen D, Barbosa MJ, Wijffels RH (2017). *Botryococcus braunii* strains compared for biomass productivity, hydrocarbon and carbohydrate content. J Biotechnol. 2017 20;248:77-86. (doi: 10.1016/j.jbiotec.2017.03.008)

Yildiz-Ozturk,E., Esra Ilhan-Ayisigi, Arnoud Togtema, Joao Gouveia, Ozlem Yesil-Celiktas (2017). Effects of hydrostatic pressure and supercritical carbon dioxide on the viability of *Botryococcus braunii* algae cells. *Bioresource Technology*.  
<https://doi.org/10.1016/j.biortech.2018.02.041>

García-Cubero, R. Teles Dominguez Cabanelas Sijtsma L., Kleinegris DMM., Barbosa M.J.(2017) Production of exopolysaccharide by *Botryococcus braunii* CCALA 778 under laboratory simulated Mediterranean climate conditions. *Algal Research* 29, 330-336

Cubero, R., Weiliang Wang, Judit Martín, Elisabeth Bermejo, Lolke Sijtsma, Arnoud Togtema, Maria Barbosa, Dorinde M.M. Kleinegris. Do's and don'ts while milking exopolysaccharide from *Botryococcus braunii* CCALA778. Submitted for publication