PLANT RESEARCH INTERNATIONAL
WAGENINGEN UR

Research Vision Plant Breeding for Biobased Production Chains

Plant breeding explores the biodiversity to uncover the building blocks for shaping a biobased society

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Plant Sciences Group
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Contents

1 Expanded summary

Objective

The Netherlands government aims at acquiring 20% of the Dutch need for raw materials for energy and chemicals from renewable resources in 2020. This ambition has been laid down in seven transition themes, identified in the Innovation Agenda Energy (IAE). Green Raw Materials for Chemical industry is one of the innovation themes of the IAE. One of the activities identified in the Green Raw Materials innovation theme is preparation of the Research Vision Plant Breeding. Objective of this activity is the formulation of a vision on the role of plant breeding in setting up sustainable and promising biobased[1] production chains.

The process

In the period July - September 2009 the Plant Sciences Group of Wageningen UR has - together with Schuttelaar & Partners - held 29 interviews with companies and stakeholders involved in - or possibly interested in - biobased production chains. These interviews helped in identifying the sub-elements of the future biobased raw material supply (Ch5) and to position these in the possible total picture. By further selection from the variety of available puzzle elements, consisting of known stakeholder ambitions (Ch2, Ch6.2), possible biobased crops (Ch6), possible biobased molecules (Ch5) and possible solvable development challenges (Ch6, Ch7), the best possible biobased puzzle has been completed.

Ambitions of stakeholders and the role of plants therein

Chemistry has the ambition to obtain 50% of its raw materials from renewable resources in 2030. The government of the Netherlands has the ambition to reduce CO_2 emission by 30% in 2020. And the Platform Green Raw Materials is expressing the ambition to obtain 1000 PJ (30% of the NL energy consumption) from non-fossil sources in 2050.

Photosynthesis is the only way to absorb CO_2 in a sustainable and usable form. Plant production[2] can make a major contribution to the large-scale exploitation of photosynthesis. Current plant production is mainly aimed at the production of food, animal feed, fibres, and building materials. The development of a new raw material demand, bioenergy carriers and green chemicals, of the same order of magnitude as the demand for, e.g. food, is unique in history. This new demand offers new chances for sustainable economic activities but also holds new breeding challenges.

Successful biobased production starts with control over plant production chains, both in and outside the Netherlands

Plant production is the conversion of CO_2 and sunlight into a plethora of economically useful molecules (see later). An acreage of suitable land (or water) is one of the essential conditions for plant production. This is why in this vision the CO_2 ambition has been translated into acreage and molecules. The total Dutch CO_2 emission is 200 million tonnes/year; about 7 million ha of high-productive crops are required for re-absorbing this amount of CO_2. This is a much larger area than available in the Netherlands. This means that the Netherlands will have to look abroad for complete CO_2 compensation. This requires control over the total production chain, not only for optimum safeguarding of the CO_2 targets but also for the benefit of the Dutch industry sectors involved. The Research Vision Plant Breeding is formulating three ambitions on the basis of these observations:

- designing plant biomass production concepts for at least 2 million ha inside and outside the Netherlands;
- obtaining maximum control over biobased production chains this area by Dutch industry, from agro to chemistry;

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[1] In this Research Vision biobased chains are leading to materials, energy carriers and chemical raw materials; this distinguishes them from production chains leading to food and animal feed.

[2] In this Vision plant production also includes algae, although these are strictly speaking no plants, are fixing CO_2 by photosynthesis, are breedable, and seem promising.

- generation of sufficient added value on this acreage.

Control over production chains for biobased raw materials has particular value for the Dutch economy if this is covering those parts of the production chain that are generating most economic added value. For biobased production this is at the start of the production chain (intellectual property on plant cultivars, seed production) and at the end of the chain (biorefining and processing of raw materials into, in the end, consumer products). This means that control over the production chain can be obtained by developing and implementing unique refinery technology (part of the Dutch Biorefinery Initiative[3]), positioning of refinery facilities on the crossroads of logistic biomass flows, but especially also by producing unique plant propagation material (seeds, cuttings, planting material, seed potatoes) leading to the actual production of biomass at whichever location in the world. This biomass is then preferably processed into raw materials for energy and chemistry use in the Netherlands.

Contours of the vision: the crops

Plant breeding serves to improve the economic added value or security of supply of crop based production chains. Breeding starts with selecting a crop and a development target.

A prerequisite in the identification of promising crop/development road map/product combinations is that Dutch industry must be involved in its development, that the development of the plant production chain should be implemented between now and 15 years be making a substantial contribution to CO_2 mitigation, and finally that the developed road map will with reasonable certainty lead to the generation of economic activities.

Ten crops/organisms have been identified after consideration of these conditions: starch potato, sugar beet, maize, *Miscanthus*, grass, the non-food oil crops *Calendula* and *Crambe*, natural rubber crops (Russian dandelion and Guayule) and microalgae. These crops are potential carriers of the biobased economy because knowledge institutions and industry have substantial knowledge on these crops, they are for a large part already used and processed on a large scale, or are carrying large-scale potential. During the many discussions with the stakeholders in the production chain in recent years these crops were also found to generate most endorsement.

Strictly speaking, microalgae are no plants but they have been included in this vision because they are breedable and potentially promising.

Contours of the vision: the constituents

All crops together are producing a reasonably wide range of valuable constituents: specific starches, C6 sugars, cellulose, hemicellulose (C5 sugars), lignin, protein, oil, pectins, amino acids, organic acids, and finally small amounts of numerous other constituents. Maximum added value is generated by selling these substances in the right markets (Ch6.13). The markets for biobased crops, however, are never only energy and/or chemistry. Protein, e.g., is produced by all crops. Most added value for bulk protein is currently still generated in the animal feed market, with the European market being large enough to absorb the extra protein produced by the biobased crops. The message therefore is that biobased is not a separate market but that all submarkets, food, feed, energy and chemistry need to be developed in coherence.

The above list of raw materials covers 90% (in volume) of the biobased materials that crops can produce. These raw materials therefore form the basis of a biobased economy and this conclusion is directional in breeding. Crops will have to be bred for optimum performance for these new markets.

Breeding targets can be classified into specific and more general challenges. An example of a generic challenge applying for virtually all crops and requiring a multidisciplinary approach is the realisation of better degradability and extraction of plant cell walls into the sub-components cellulose, hemicellulose, and lignin. Such a route requires intensive collaboration between breeding (green biotechnology), white biotechnology, and chemistry. Lignin, e.g., is a potential biofeedstock for aromatics (chemicals), but a

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[3] Annevelink, E., J. Broeze, H. Reith & H. den Uijl (editors) (2009 in final preparation). Dutch Roadmap Biorefinery, Wageningen UR & ECN.

process for economic conversion is currently not yet known. Increasing biomass yield per unit production factor - land, water or nutrients - is another generic challenge.
More specific breeding challenges are identified in the following sections.

Contours of a vision: the business concepts

The heart of this vision is formed by 9 potentially promising crop-development line-product combinations for 10 crops/organisms. In this vision these are called business concepts to indicate that the potential of building economically and ecologically sustainable chains is the determining factor behind the identified development road maps. Some specific breeding challenges are identified for each business concept.

1. Starch potato as production platform for unique starches and protein. Size: 500 000 ha.

The starch potato has a well-developed production chain, mainly based on starch. There are more than three hundred starch-based products with markets in food, animal feed, and industrial applications. The use of degradable plastics and biomaterials of modified starch is one of the success examples. The business concept for potato aims at the creation of added value by utilising all constituents and by increasing the recoverability of those constituents. Breeding and green biotechnology are offering tools for making novel starches with improved properties; this can considerably increase the range of applications, and thus market volume. The main breeding targets are:

- Optimisation of starch properties and production of new high-grade starches for existing and new applications;
- Improvement of the cell wall structure to increase the extractability of starch and protein;
- Increasing the protein content without lowering the starch content;
- Improvement of the properties of pectins to enable applications in medical and industrial products;
- Increasing the content of high-grade components for pharmaceutical applications and nutraceuticals, and improvement of their accumulation by cellular compartmentalisation.

2. Beet as production platform for platform chemicals, sugar and protein. Size: 500 000 ha.

Besides potato, beet is the only other “wet” Northwest European refinery crop. Yield is very high and beet is as such competitive with sugar cane, one of the world’s highest yielding crops. According to a number of chemical companies, beet and cane are obvious crops as source of raw materials for energy and raw materials for chemical industry. Beet, however, is not cost-competitive with sugar cane due to the higher costs of soil, labour and processing. The business concept consists of a number of components. 1) Reduction of the cultivation costs by increasing N use efficiency. 2) Drastic reduction of the capital costs by year-round cultivation and processing, which can double the processing capacity of existing factories. 3) Increase the value yield by extra recovery of proteins from leaf and root. 4) Increase the value yield by extra production of high-grade platform chemicals as building blocks for polymers. 5) Other processing methods enabling complete conversion of all remaining biomass (after recovery of protein and platform chemicals) into ethanol and ethylene. The three main breeding challenges are:

- Introduction of the ability to produce platform chemicals up to a level of about 10% of dry weight;
- Increasing protein content;
- Improvement winter hardiness.

The platform chemicals belong to the group of carboxylic acids and amino acids. The extra gross turnover of the new beet in comparison with the existing beet is estimated at € 1800/ha. This extra turnover and the size of the markets for protein and polymers justify expansion of the acreage from 80 000 to 500 000 ha under control of the Dutch industry. A first expansion step is doubling of the acreage to 160 000 ha, where the extra 80 000 ha biomass can be processed in an existing biorefinery.

3. Miscanthus as a biorefinery crop. Acreage: 1 million ha.

Miscanthus is a perennial crop with a very high biomass yield and is the most close relative to sugar cane. This giant grass is considered as one of the best lignocellulose crops for bio-energy applications in view of the low production costs, low nutrient consumption, the capacity to fix atmospheric N, and a high net energy yield. The business concept for *Miscanthus* includes the development of a refinery crop that can be harvested twice a year, the first harvest for protein and sugar production, the second harvest for lignocellulose biomass production.
The main breeding challenges are:

- Development of a crop with a very high fermentable sugar content by crossing *Miscanthus* with the genetically closely related sugar cane;
- Development of a diploid genotype that can be reproduced via seed;
- Increasing protein content and improvement protein quality;
- Improvement of the cell wall composition to reduce the energy costs for recovery and to improve the fermentability of the lignocellulose biomass.

4. Oil crops as source of oil-based chemicals and protein. Size: two crops on 50 000 ha each.

Most seed oil crops for use in food contain the "standard" C16 and C18 fatty acids. This is the reason why currently only 10% of all plant oil is used for chemical applications.
The oil crops *Calendula* and *Crambe* contain special fatty acids of which 100% can be utilised in industrial products with high added value. *Calendula* oil is very suitable for use as reactive solvent in low-solvent alkyd paints and as wood preservative. *Crambe* oil, with high content of erucic acid, is an excellent raw material for erucamide, an additive for plastics. *Crambe* is also very suitable as production platform for various new oil-based chemicals, such as wax esters for use as high-grade lubricants. The challenge of the business concepts for these crops is the development of cultivars with a high production and quality.
Breeding challenges for *Calendula* are increasing seed production per hectare from 1500 to 3000 kg/ha (this would then be similar to the yield of oil seed rape) and increasing seed oil content from 15 to 25%.
Breeding challenges for *Crambe* are the production of wax esters by introducing fatty alcohol and wax ester genes and a further increase in erucic acid production per hectare through higher seed production, higher oil content, and higher erucic acid content in the oil.

5. Grass as source of protein, fibres, and fermentable sugars. Size 100 000 ha, with the possibility of further growth to an estimated 750 000 ha.

With an acreage of more than 1 million hectare, grassland covers almost half of the existing agricultural acreage in the Netherlands. Grassland is for a large part grazed by cattle; annual average grass production in the Netherlands amounts to about 8 t dry matter per hectare. Grass production can be doubled if grass would be harvested by mowing instead of grazing. In the proposed business concept it will become possible to separate the cut grass via biorefinery into protein, fibre, and fermentable sugars. This would not only make it possible to produce sufficient feed for the existing livestock but would also allow production of better formulated feed for cattle (and even pigs and poultry); this would mean a sustainability step in animal husbandry. The extra grass biomass (8 ton/ha) harvested in the new system can be used as source of valuable industrial raw materials. The challenge is to develop the most suitable grass cultivars for this system and at the same time initiating the development of post-harvest biorefinery techniques. Breeding challenges are:

- Maximum productivity per hectare under the mowing regime;
- Increasing protein content;
- Improvement of the dissolution of cell walls in older and more fibrous grass material by developing genotypes with easier degradable cell walls.

6. European crops as new source of natural rubber

Natural rubber for the production of car tyres, building materials, medical gloves, and other articles is almost completely obtained from the latex of the rubber tree (*Hevea brasiliensis*). Natural rubber is of strategic importance for Europe, in particular for the transport sector. In heavy-transport applications natural rubber cannot fully be replaced by synthetic rubber because the quality is not high enough. This means that there is a need for a different source of natural rubber that can grow in Europe. Two plants are qualifying for this purpose: Guayule (*Parthenium argentatum*), a woody shrub producing rubber in the above-ground parts, and Russian dandelion (*T. koksaghyz*), mainly producing rubber in the tap root. Guayule is especially suitable for cultivation in Mediterranean and desert-type climates, whereas *T. koksaghyz* is very suitable for cultivation in Northwest Europe.

Development of the business concept should lead to European natural rubber production. The added value of the crop should mainly originate from the rubber (average € 1500/ton). An advantage of the Russian dandelion is that a large part of the remaining root biomass consists of inulin, which can be converted into furan-based chemicals with a high conversion efficiency.

The main breeding challenges for Russian dandelion are:

- Increasing root yield to 45 t/ha (fresh);
- Increasing rubber content to 1500 kg DM/ha.

The main breeding challenges for Guayule are:

- Increasing stem and root yield;
- Increasing latex/rubber content to 12% of dry matter yield;
- Improvement breeding methods.

7. Biorefinery of maize straw for feed, biofuels and biochemicals

Maize is one of the world's largest agricultural crops and is an important source of food, animal feed and of raw materials for a large number of industrial applications. The possibilities of the crop as supplier of raw materials for a biobased economy are hardly utilised in the Netherlands. Room for a large expansion of the cultivation of Corncob maize and/or wet grain maize for pig husbandry is expected in the Netherlands. This has the direct environmental advantage that less concentrated feed needs to be imported. Setting up of a biorefinery chain for the remaining maize straw is proposed to enable this development. This development will make a positive contribution to the environment (CO_2 mitigation, more bioenergy) and thus to the sustainability of pig husbandry in the Netherlands.

The main breeding challenges for maize straw are:

- Improvement cell wall composition to improve the digestibility of lignocellulosic biomass for the production of 2nd generation ethanol and other white biotech products;
- Improvement of amount, quality and extractability of proteins;
- Optimisation of starch amount and properties.

8. Microalgae for the production of hydrocarbons (fundamental research)

Development of a valid business concept is a serious challenge. There is in any case no known and profitable business case for the production of biobased raw materials or energy with algae. The potential of algae seems large but sufficient knowledge on sub-areas is lacking. Knowledge on algae mainly covers cultivation and process technology. Knowledge on breeding and biotechnology of algae is virtually non-existent in the Netherlands as well as in Europe.

Algae have a number of advantages, including an efficient photosynthesis (and thus a higher production potential than terrestrial plants), the capacity to produce much protein and much oil, and the possibility to produce biomass at sea or in areas that are unsuitable for plant production. This vision pleads for two development routes.

The first line is setting up genomics and breeding research for a limited group of algae, in particular *Botryococcus* species, of which it is known that they can provide molecules for the production of new bioplastics and high-grade fuels. The main breeding challenges are:

- Unravelling the mechanism for production of hydrocarbons and biopolymers obtained thereof;

- Increasing the growth rate.

This knowledge should form the initiation point for setting up industrial biotechnology with algae aimed at the production of a new generation of bioplastics.

Feasibility of the concepts

At the moment it is not possible to indicate which business concepts have the highest probability of success; this means that a winning choice cannot yet be made. Our best estimate is that the Dutch and European CO_2 mitigation and other sustainability objectives are so ambitious that preferably several business concepts should be deployed simultaneously to meet the sustainability objectives of governments and chemical industry. The industry will then have to adopt a number of these the business concepts. This will only happen if the business concepts yield sufficient added value in the new biobased markets.

The willingness industry has been explored by testing the readiness of the industry to invest in the business concepts. This shows – where it is important to stress that this is a sketch of the situation mid 2009 - that no single business concept is strong enough to develop with sufficient speed without partial governmental support. All stakeholders have the ambition to form biobased production chains but separately they are hesitant to invest in biobased projects which require several partner in the total production chain. Chemical companies are not yet really convinced of the need of now already starting to move their raw material basis from petro to agro. Breeding companies prefer not to invest in the development of crops that can provide the chemical industry with raw materials as long as the chemical industry has not given a clear indication of the nature of the substances they need, or shows no clear interest in the development and investment route.

A government initiative, in combination with the right conditions, is probably sufficient to win parties for the formation of consortia around business concepts (see later). Companies are indicating that they need a better scenario analysis of possible biobased production concepts before making the step to invest in research and development. It should be noted that agro and chemical parties find each other as discussion partners. The "Dutch Biorefinery Cluster" in which parties from Food, Agroprocessing, Chemistry, and Paper & Cardboard are represented is a good example of this development.

In the Research Vision the authors attempt to assess the chance of success of the different business concepts (6.13.5) but the real chance of success is determined by the process after today. This process consists of testing the interest and readiness of the industry to invest in any of the nine business concepts in a scenario which also includes the availability of public means for the development of biobased production concepts that covers the entire production chain.

How to proceed after today

With the acquired knowledge and the identified concepts in hand we propose to formulate development routes and to test the true interest of companies. The conceptual space should at the same time be so wide that variants of the presented business concepts are allowed. The following steps are proposed to facilitate this process; these are also supported by most of the stakeholders:

- Continuation of the agro-chemistry consultations in a different form. Cooperation throughout the chain, from plant breeding to chemistry, is essential for shaping sustainable biobased chains for the future. The agro-chemistry consultations, until now held at management level, should be continued at the level of technically more informed individuals and should be given the task to propose chain-wide concepts within six months. Here, interviewees see a facilitating role for the government;
- At the same time there must be the prospect that a number of "winning" concepts can be further developed in public-private partnerships. This can be done in three steps with different financing models:
  - The biobased concepts are assessed for their technical and economic feasibility and potential sustainability gains (for this part e.g. 10% private financing);
  - The main technological challenges must be studied in (pilot) projects to get a feeling of a possible development road map (e.g. 20% private financing);

  - Next, development into public-private projects, in which concepts for commercial semi-finished products is developed further (30% private financing);
- Fundamental research. The technology for alleviating climate change or energy supply challenges is still insufficiently developed or is not economically feasible. Several parties pleaded for maintaining and stimulating fundamental knowledge in the field of metabolism and accumulation of constituents in plants and refining and separation technology;
- Supporting measures. The government can support its CO_2 mitigation policy by discouraging the use of products with an unattractive CO_2 footprint - and of which it is known that a biobased alternative will be available in due course; this would help in establishing a market pull towards the development of biobased products.

How to proceed: development routes that go beyond single business concepts

The most innovative development routes are those routes that are simultaneously addressing several societal issues. There are a number of societal needs that seem to be unrelated but that can also benefit from the biobased production concepts presented in this vision.

The first societal issue, reducing CO_2 emission by supplying sustainably produced raw materials for the biobased market, is addressed in this vision.

The second societal issue is associated with the ambition to make protein production sustainable, also in view of the worldwide increase in demand for meat, fish and other animal protein products. Growth is expected in the demand for high-grade proteins from sustainably produced protein sources, not only for use as feed for livestock and fish, but also for new high-protein food products and ingredients.

The growing demand for fish can, in view of the fact that fish catching has reached its limits, only be met through fish farming. Sustainable growth of fish farming is only possible if this is supplied with high quality raw materials, including easily digestible protein and essential fatty acids (PUFA's). Securing the supply of these raw materials is a third societal challenge.

The fourth challenge is securing phosphate supply in the long term because phosphate is absolutely essential for plant production, for food, feed as well as biobased.

All business concepts in this vision primarily focus on the production of biobased raw materials (societal issue 1), but all concepts are also supplying protein (issue 2). Protein production even is an essential component for economic validity in three concepts: grass biorefinery, potato biorefinery, and microalgae at sea. Supplying raw materials for food, feed and biobased are inseparably interrelated in these concepts. One of the concepts, large-scale cultivation of algae at sea is also expected to make a contribution to the third and fourth societal issue because this concept can at sufficiently large scale supply easily digestible proteins and PUFA's for large-scale fish cultivation and also offers the possibility of phosphate recovery.

Summarising, we conclude that biobased production chains can play a major role in helping to contribute to the four mentioned societal challenges. The business concepts presented in this vision offer ample inspiration for setting up those production chains and for dealing with the four societal issues.

2 Introduction

As expressed in the Innovation Agenda Energy (IAE)[4], the Netherlands strives to obtain 20% of its energy need (energy and chemical raw materials) from renewable resources in 2020. The Innovation Agenda is covering an energy transition over the full width of society for which the policy field has been divided into seven transition themes, of which Green Raw Materials is one. The challenge of the transition theme Green Raw Materials is to arrive at a sustainable production and innovative use of green raw materials for energy, chemicals and bio-materials. One of the seven activities in the innovation theme Green Raw Materials is the preparation of a research vision on the role of plant breeding in the shaping of biobased production chains. Criteria for success of these production chains are that they are making a significant contribution to the CO_2 and energy targets of the Dutch government while at the same time generating substantial added value for the economy of the Netherlands. This not only concerns biomass for generating electricity, heat and fuels but also the supply of chemicals to replace petrochemicals that are now obtained via energy-intensive syntheses.

The Ministry of LNV (Agriculture, Nature and Food Quality, now EA&I, Economic Affairs, Agriculture and Innovation) has commissioned the task of preparing a Research Vision Plant Breeding to Wageningen UR (WUR), for which WUR has called in the assistance of Schuttelaar & Partners. The assignment also included the request to seek connection with other activities within the Innovation Agenda Energy, including the Dutch Biorefinery Initiative (DBI) and the Research Agenda Aquatic Biomass (OAB), when formulating the Research Vision Plant Breeding. The DBI report was published very recently[5]. The OAB, one of the other activities of the IAE has been given shape under direction of the Ministry of Economic Affairs and has also led to a report[6]. Although seaweeds and algae are strictly speaking no plants, cultivation issues and breeding approaches are similar to those of plants. It has been agreed with the principal that breeding issues that arise from the OAB will be dealt with in the Research Vision Plant Breeding.

To achieve accordance with the DBI, the Research Vision Plant Breeding has been analysed against the biorefinery concepts from the DBI roadmap as summarised in the Box below.

From: Roadmap Dutch Biorefinery Initiative[5]:
This roadmap describes a route toward the development of a biobased economy in the Netherlands in 2030. Most promising innovation directions pursue on opportunities that are a good fit to strengths but also to weaknesses. Based on a SWOT analysis, the following promising directions for biorefinery in the Netherlands have been identified:

1. *biorefinery based on domestic Dutch crops, using synergy of existing agro and chemical sectors, including the Dutch plant breeding sector;*
2. *biorefinery of aquatic biomass, using Dutch microbiology, plant breeding and processing knowledge;*
3. *biorefinery of bulk imported biomass and biomass-derived intermediates, using existing logistic and petrochemical infrastructure;*
4. *biorefinery of residues, based on co-operation in production chains and networks, relatively small transport distances and business competences of Dutch entrepreneurs.*

The first direction from the DBI roadmap presents clear questions and chances for amendment of existing NL chains as well as clear challenges for plant breeding.
The second chain, aquatic biomass, has hardly been developed but the potential seems attractive. Breeding work on microalgae and macro seaweed is rudimentary at most.

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[4] Innovatieagenda Energie (Innovation Agenda Energy): http://www.ez.nl/dsresource?objectid=158825&type=PDF

[5] Annevelink, E., J. Broeze, H. Reith & H. den Uijl (2009). Dutch Roadmap Biorefinery, Wageningen UR & ECN: http://www.senternovem.nl/mmfiles/Dutch%20Roadmap%20Biorefinery_tcm24-319385.pdf.

[6] Muylaert K. (2009) Inventarisatie aquatische biomassa (Inventory aquatic biomass). Rapport in opdracht van MinEZ, NL. http://www.senternovem.nl/mmfiles/Inventarisatie%20aquatische%20biomassa%20July%202009_tcm24-312018.pdf

Parallel to the Research Vision Plant Breeding, the IAE activity Aquatic Biomass was given shape under direction of the Ministry of Economic Affairs (EZ). Insofar as the Research Vision Aquatic Biomass contains no breeding questions, these are addresses in the Research Vision Plant Breeding.
The third chain is mainly driven by biomass import. The current Vision is assuming that NL stakeholders can also gain control over import chains. Such control may precisely also be found at the start of the import chain, e.g. by IP (Intellectual Property, such as patents or plant breeders' rights) and reproduction of unique plant propagation material (seeds, cuttings, planting materials, seed potatoes), which - at whichever location in the world - can lead to the actual production of biomass that can then in the Netherlands be processed into raw materials for energy and chemistry. This means that important breeding challenges are also found at the import chains.
The fourth chain mentioned in the DBI mainly demands technology and chain innovations. In the short term breeding is not expected to play a role in optimisation of this chain.

The challenge of this study is the identification of promising biobased chains and conversion of possible optimisation needs from that chain back to required development directions to be followed by plant breeding. The criteria set for promising biobased chains comprise that 1) these are contributing to the CO_2 mitigation target of NL and EU authorities; 2) these are offering chances for new activities by NL companies; 3) NL companies can obtain control over these chains.
In this vision it is assumed that, also when the biomass is grown outside the Netherlands, NL companies can generate economic added value at several points in that chain. Knowledge of the organisation of biomass production chains, availability of unique plant material (protected by breeders' rights or patents) and unique refining technology, with an optimum match between plant material and refining technology, are necessary prerequisites.

3 Working model and reading guide

Up until now, the development of many biobased products started at the end of existing production chains: new applications and markets have been developed on the basis of existing biomass derived commodities e.g., sugar, oil, glycerol and starch.

In contrast, industrial/white biotechnology uses a different innovation model. Microorganisms are indispensable components of a successful business concept in white biotechnology. The development of new biobased production chains in this sector therefore necessarily starts with the development of improved organisms[7].

It is logical to follow a similar model for the development of sustainable biobased production chains for green raw materials: a successful business concept starts with the development of a plant. It is the merit of the Ministry of Agriculture (LNV, now EL&I) that they recognise this and that they see an essential role for breeding and green biotechnology in the development of sustainable and successful biobased production chains. Further information about the added value of plant breeding is given in Ch4.

Interviews have been held with various stakeholders to investigate whether the market is ready for establishing partnerships around promising biobased production chains. These interviews were held to arrive at ideas for promising chains, based on new raw materials, new products, new process technologies or new links between parties in the production chain. The findings of the interviews are presented in Ch5.

The interviews, however, provided insufficient leads for shaping concrete biobased chains with participation by the NL industry. They did offer sufficient inspiration for designing a number of possible business concepts. These biobased business concepts are described in Ch6.

All signals from the market showed that new links between agro and chemistry will only be established if there is a mutual agreement about the nature, security of supply, possibilities for application and market perspective of green-molecules. As long as chemistry, as potential client, does not specify the nature of the desired molecules, agro will not be making investments. A special chapter (Ch7) has therefore been devoted to this bottleneck, the identification of possible substances that can be supplied by the agrosector and that can be adopted by chemistry as renewable raw material.

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[7] Example 1: Dupont with *Escherichia coli* for production of 1.3 propanediol. Example 2: DSM with unnamed organism for production of succinate.

4 Role of plant breeding in biobased production chains

Plant breeding is essential in setting up biobased production chains because the intrinsic properties of biomass are determinative of its economic value and the sustainability of the production chain as a whole. Amount, extractability and purity of the plant constituents, sustainability in biomass cultivation, sustainability in processing of biomass and the assembly of biobased products, the net energy or CO_2 gain of biobased production chains are all strongly dependent on the properties of plant propagation material.

There is a second reason for investing in biobased plant breeding. Biomass needs to be imported to meet the energy targets of the national government. Until now this imported material mainly consists of low-grade biomass such as wood chippings, seed hulls or 1st generation biofuels such as palm oil. This usually means that most of the economic added value in the chains that lead to these products is generated outside the Netherlands. In this Research Vision it is assumed that biomass production chains which allow maximum added value creation for the NL industry are the most interesting ones. As a consequence this report focuses mostly on production chains, including biomass import chains, which at least allow control over the first part of the biomass production chain: breeding, propagation and green biotechnology,.
Control over production chains for biobased raw materials in particular has added value for the NL economy if this covers those parts of the production chain in which most economic added value is generated. For biobased this is at the start of production chain (ownership of elite plant material and seed production) and at the end of the chain (biorefinery and processing of raw materials into consumer products). This means that control over the production chain can be obtained by development and implementation of unique refinery technology (part of the Dutch Biorefinery Initiative[5]), the positioning of refinery facilities at the crossroads of logistic biomass transport routes, but in particular also by producing unique plant propagation material (seeds, cuttings, seed potatoes) which - at whichever location in the world - leads to the actual production of biomass which is then processed into raw material for energy and chemicals in the Netherlands

Breeding starts with the choice of the crop and always aims to improve the economic added value. Three groups of crop properties are leading in a biobased context:
1. maximum production per unit of input: land, water, nutrients (P, N, K) and energy, i.e., kg biomass per m^2 land, per GJ, per kg N, P and K, and per m^3 water;
2. maximum economic value per kg biomass. Associated properties are maximum yield of high-grade constituents (existing substances such as oil, sugar, starch, as well as new constituents), easy (at low energy cost) extractability of constituents and fermentable sugars;
3. maximum tolerance to abiotic (water, nutrients, environment) and biotic stress factors (diseases and insect/rodent damage) because this is indirectly leading to higher yield and quality.

Not all aspects are given equal attention in this Vision but we are particularly focussing on those aspects or properties that have most leverage in the development of promising biobased chains. Improvement of biotic (disease) resistance (point 3) is given no attention at all in this vision because this is already addressed in policy-supporting research or directly by the industry.

Increasing total yield (1) is also given little attention although we recognise that subject 1 is essential in obtaining the energy targets of the government because an increase in the total yield/ha helps in lowering the pressure on limiting resources such as land and water. Also if only yields of food crops would be increased, this would literally create room for the production of biomass for raw materials for energy or chemicals. Although total yield is an important bottleneck in setting up biobased chains, it should be recognised that the mechanism behind crop yield is still insufficiently understood to be able to achieve a rapid yield increase in biobased crops. Breeding companies are now already putting much effort into increasing the yields of crops, including the three major food crops (maize, soy, rice). It is expected that this will in due course result in knowledge that can help in speeding up yield increases of typical biobased crops. The primary focus of this Vision is therefore not the development of generic yield concepts.

This means that we are focusing on subject 2. In Ch6 it is explained that - in order to meet the CO_2 target of the government - it is necessary to obtain control over biomass production from 2-4 million ha of biobased crops. The drive behind such an expansion of the acreage can only be the perspective that such (new) biobased chains are generating at least the same added value as existing food chains. Only then will investments be justified and only then will the NL industry be participating. Current biobased production chains, however, are currently mainly focusing on energy and the main bottleneck in these chains is precisely the limited economic added value. This means that we are particularly focusing on the development of improved biobased crops that can be used for several purposes, such as high-grade constituents for fine chemicals, pharmaceuticals and food, proteins for food and feed, specific constituents that can serve as building blocks for bulk chemicals to replace fossil oil derivatives, and energy. Properties that lead to a better degradation of these constituents and to a more efficient conversion of those crops or parts thereof into bioenergy (biofuel, electricity and heat) are also considered in this Vision.

5 Stakeholders' views on biobased production chains

5.1 Summary

In the period July - September 2009 the Plant Sciences Group of Wageningen UR has - together with Schuttelaar & Partners - held 29 interviews among companies and other stakeholders that are involved in the development of biobased production chains. These interviews were held to find an answer to two questions: 1) how do companies think they can use raw materials for chemicals and energy?, and 2) which could be the resulting development tasks for plant breeding. The interviews also formed the basis for a workshop on 22 September 2009 dealing with the same questions. This chapter presents an outline of the findings from the interviews.

The interviews yielded the picture that companies in plant breeding, agro-processing, industrial biotechnology and chemistry, to a greater or lesser extent sees opportunities for the theme 'biobased economy'. But the interaction between the different parties is still limited; interaction between plant breeding (seed companies) and chemistry is practically non-existent.

On a global scale, a range of business models and production routes seem to be set up for the production of energy carriers, raw materials for chemicals, and materials from biomass. The Dutch Biorefinery Initiative (DBI)[5] identifies four main routes; these have also been discussed with the interviewees. At the short term, little is expected of route 2 (biorefining of aquatic biomass). Support for the other routes of the DBI (biorefinery of crops grown in the Netherlands, biorefinery of imported biomass, and biorefinery of residual biomass streams) is about equal. All parties support the recovery of platform chemicals from plants and the use of lignocellulose as source of fermentable sugars from plants.

The interviewees mention generic breeding challenges, such as yield increase and cultivation under marginal conditions, an important task for plant breeding, directly followed by specific tasks such as improved digestibility of fermentable sugars from lignocellulose and increasing the concentration and purity of specific constituents to improve their market value. The following points were mentioned as main bottlenecks for the biobased economy: security of supply (in time and in required amounts), logistics, development of required refinery technology, and the currently still high costs of plant raw material in comparison with petrochemicals.

Plant breeding is following two main lines: refinery crops and lignocellulose crops. The products of current refinery crops (beet, potato, grain maize) are mainly used in *food* and *feed.* Sales of biomass in biobased chains (read: non-food) requires adjustment of the total chain, especially by the development of concepts that make it possible to obtain extra added value from the biomass to compensate for the usually lower value obtained from non-food (biobased) markets.

Most parties see good prospects for lignocellulose crops (such as *Miscanthus*, energy maize, grasses) in view of the relatively high sustainability over the total production chain, low cultivation costs, and the expectation that they will become a source of cheap fermentable sugars. The recalcitrant lignocellulose biomass also offers chances for the development of new enzymes by industrial biotechnology.

All interviewees consider it important to collaborate with parties in the production chain but at the same time conclude that this collaboration only advances with difficulty. This has to do with the 'chicken-and-egg' situation in which the development of sustainable biobased production seems to be locked into: seed companies and agro-processing want to hear from the chemistry sector which raw materials it needs and chemistry would like to see how the agrosector is solving the bottlenecks in, e.g., global resourcing, security of supply, quality and functionality. All parties do consider this situation as undesirable. A number of proposals are made to overcome this deadlock: (1) Continue agro-chemistry consultations but now at the level of individuals with a more technical background and with the task to arrive at joint concepts. Many of the interviewees see a facilitating role of the government. (2) These biobased concepts must be investigated for their technical and economic feasibility and should then be studied in pilot projects. (3) Insofar as the authorities would be prepared to make funds available, these should be allocated to projects in which the agrosector as well as chemical parties are involved. The IBOS tender model is suggested as an example. (4) Several parties also argued for continuation and stimulation of fundamental knowledge and basic technology. Examples are knowledge of metabolism of plant constituents and refinery and separation technology.

6 Promising biobased production chains

6.1 Introduction

Identification of promising biobased production chains as well as the role of breeding in such chains is the core topic of this report. Promising has been interpreted in two ways: 1. the total volume of all chain concepts should be able to make a significant contribution to the CO_2 mitigation targets of the government; 2. the production concepts are leading to a higher and more sustainable turnover for the NL industry.

The interviews learn that all parties consider sustainable production as an opportunity. Much uncertainty, however, exists about the proper development goal. No sharply defined and still promising biobased production concepts that should be playing a role in biobased production chains emerged during the discussions with the industrial parties. The industry is still considering biobased investments as rather risky. There are a number of reasons for this:

- Biobased chains demand new links between agro parties (breeding and agro-processing) and chemistry (industrial biotechnology and petrochemistry). Links are not yet rapidly formed. Petrochemistry is considering the need to change from petrochemical raw materials to biobased raw materials as less pressing than other challenges such as improvement of the competitive position towards Asia, reduction of costs and increasing the eco-efficiency of production, or simply survival during the financial crisis. Another aspect is that the various chain parties are insufficiently understanding each others language, needs, and production processes;
- The range of promising biobased chemicals is not yet very wide, the performance of biobased products in comparison with petrochemical equivalents is suboptimal, their production demands new technology, and there is doubt about the fact whether the acceptance of new products by the market will be sufficient;
- A second point regarding social acceptance concerns *competing claims*. A solution sometimes suggested for this problem is the setting up of separate chains for food and non-food products and crops. This may be a doubtful solution because competition does not only play at the level of products but also at the level of limited resources such as land, water and nutrients. We just would like to make the point that contrary to complete separation of food and non-food production chains, integration of the production of food, feed, energy and chemicals is a prerequisite to set up economically promising biobased production chains.

Innovations, however, will not be developed at high speed when the risks mentioned above become a leading principle. Fact is that precisely innovations are required to meet the CO_2 and energy targets of governments and society. These targets are, incidentally, not without obligations; they are resulting from international and European agreements. Non-observance of agreements may lead to sanctions and will in due course damage the economy of the Netherlands, and competitiveness of NL industry.

This is the reason for making government targets - rather than the risks – leading in identifying promising chains summed up in the present Vision document: CO_2 mitigation potential, energy saving potential, and the possible economic potential. The task behind this vision is to identify the role of breeding in promising biobased production chains. Crops are the second leading principle in identifying promising biobased chains because breeding particularly concerns crops and crop properties.

This chapter therefore discusses possible biobased crops and an elaboration of biobased business concepts for those crops. Further details about the basic principles that were used are presented in the following section.

6.2 Basic principles in the identification of promising chains

The following basic principles were used to identify promising biobased crops and production chains:

- Calculations in this vision are based on two environmental goals: Goal 1 is achieving 30% reduction of the CO_2 emission in 2020 in comparison with reference year 1990 (government target, Innovation Agenda Energy). This corresponds with a reduction of 96 million t CO_2 in comparison with unchanged policy; 20-40 million should originate from innovations. Goal 2 is the production of biomass with a net fossil energy saving of 1000 PJ (Trend to 2050, Platform Green Raw Materials);
- As regards the 30% CO_2 reduction target: Plant production is the only industrial activity for net and sustainable CO_2 fixation. Production of 1 t biomass requires at least 1.5 t CO_2. An average of 20 t DM biomass per ha is attainable (certainly with beet and *Miscanthus*). At a yield of 20 t these crops are then - gross - fixing 30-35 t CO_2/ha/year. Cultivation and processing, however, lead to CO_2 emission. Assuming an average CO_2 loss of 30-40% this means that the CO_2 reduction target of 40 million t/year can be attained by approximately 2 million ha highly productive crops;
- As regards the 1000 PJ target: A yield of 20 t/ha results in a – gross – fixation of 20 x 18 GJ/t = 360 GJ/ha. The estimated average net energy yield is lower, viz. at 240 GJ/ha, as result of various losses (cultivation, harvesting, refining). This means that the 1000 PJ target requires over 4 million ha and is thus more ambitious than the CO_2 target;
- In addition, meeting these targets should strengthen the economic potential of the NL industry. It should be noted that precise calculations of the economic validity of chain concepts are at the moment hardly possible because assumptions must be made regarding technological breakthroughs between now and 10 years, as well as societal developments as regards biobased crops;
- Biomass chains must in particular meet the energy and chemical needs of the future. Here we are assuming that energy can increasingly be generated locally, e.g., via small-scale methane/ethanol fermentation or by burning locally produced biomass, and that there will be a transition to electrically driven cars;
- Two or four million ha is not available in NL; this means that biomass must also be imported. It is important to acquire maximum control over biomass import chains to gain maximum revenues for the NL economy with biomass import, for which the following instruments are available:
    - By setting up biomass (import) production chains outside NL with NL knowledge and in doing so also ensure biomass production and import under sustainability criteria;
    - By – with NL industry – developing unique plant propagation material for these biomass production chains and to protect that material by plant breeders' rights or other IP, where maximum yield of valuable constituents and the best possible match between plant properties and the refining techniques that are to be developed are leading;
    - By keeping seed production of unique propagation material in NL hands;
    - By developing unique biorefining technology that enables generation of maximum added value from this biomass, more than competing foreign parties could generate from the same biomass.

  This approach would not only give NL industry maximum control over biomass import but it would also position the NL activities in the heart of the parts of the production chain where most added value is created[8].
- The development of promising biobased chains starts with the identification of promising crop/concept combinations. A prerequisite for choosing the right crops is that NL breeding parties can play a role in the development of these crops and that there are opportunities for the development of IP (patents and plant breeders' rights). It is also important that those crops can be

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[8] Successful NW-European examples of this approach are the *Calendula* oil chain (chain knowledge, control and breeders' right at WUR, cultivation in Morocco and Canada, application in NL paint) and the chicory-inulin chain (knowledge and control at the Belgian company Beneo-Orafti, cultivation and part of the processing in Chile, sales of inulin controlled from Belgium)

grown in NL or Northwest Europe because this forms the basis of regional biobased production chains.

- Gaining control over biobased production chains not only requires IP on plant material but this should also be established for solutions and inventions in other parts of the chain (e.g. in biorefinery).
- A number of the crops mentioned below are already grown in NL. Starting point is that the new crop/concept combination offers sufficient added value in comparison with the existing production chain for these crops, which would also result in a considerable increase in the acreage. The criterion is that gross yield per ha, for farmer or processor, should at least be comparable to or higher than the gross yield of the same or a similar crop in the current setting.

6.3 Reformulation of the target

The energy and CO_2 target has been reformulated as follows from of the starting points above*: Give NL industry control over 2 to 4 million ha biomass production.* Here, the focus lies on biomass which contributes to the production of energy carriers and chemical raw materials and at the same time generates a considerable volume of high-grade protein. This is because protein demand and protein costs is expected to increase more strongly than other raw material demands, which may weaken the position of the European animal husbandry sector.

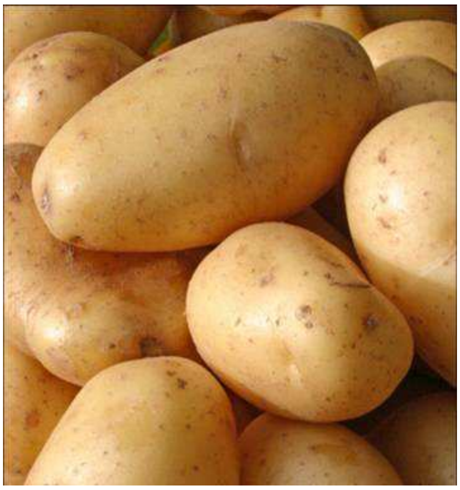
To realise a considerable part of those 4 million ha, nine business concepts have been developed for the following crops

1. Potato: 500 000 ha;
2. Beet: 500 000 ha;
3. *Miscanthus*: 1 million ha;
4. Oil crops (*Calendula* and *Crambe*) for chemical applications: 2 x 50 000 ha;
5. Grass: 100 000 ha (although the corresponding concept can be converted to an estimated 750 000 ha in NL);
6. Rubber-producing plants (Russian dandelion and Guayule) on 100 000 ha;
7. Maize straw for biorefinery on 500 000 ha;
8. Microalgae for chemical applications;
9. Microalgae at sea: 100 000 ha.

These concepts have a joint acreage of 2.9-3.6 million ha.
The business concepts are described in more detail - according to a more or less fixed format - in the following sections. Only the format for microalgae is different because this concept follows a different line of thought and development route than the agricultural crops.

6.4 Concept 1: Potato biorefinery

6.4.1 Concept



Current potato acreage in the Netherlands amounts to 160 000 ha of which 50 000 ha are starch potatoes. There is a shift from the classic applications of starch in food and industrial applications towards new or renewed applications of starch, such as in bio-plastics. Banning of a number of chemicals, however, will make many chemical modifications of starch no longer possible and the production of many starch-based products will become impossible. This means that existing and new biobased applications for starch will in the future demand a range of new and natural starches. And the total demand for different starches will increase as well.

Apart from starch, potato tubers contain other high-value components in relatively high concentrations, such as proteins, amino acids, other sugars (pectins, cellulose, hemicelluloses) and secondary metabolites with possibly heath-promoting effects such as flavonoids, terpenoids and glycoalkaloids. Many of these substances in potato are currently hardly isolated and brought to value.

The potato biorefinery concept entails doubling of the starch potato acreage in the Netherlands to 100 000 ha and an increase of the total European acreage to 500 000 ha. Areas suitable for potato cultivation under control of NL industry are found in Germany, Poland, Ukraine, and Hungary.

6.4.2 Breeding challenges

Emphasis will be on the following research lines, ranked in the order of decreasing importance:

- Optimisation of starch properties for current and new applications, for ‘food’ as well as for ‘non-food’ applications;
- Increasing amount and extractability of proteins;
- Adaptation of the cell wall structure in order to increase the extractability of starch;
- Generating (new) pectins with improved properties and easier extractability;
- Increasing the content of high-value components for pharmaceutical applications and nutraceuticals (flavonoids, terpenoids, glycoalkaloids), and improvement of the purity and recoverability of these substances by specific cellular compartmentalisation.

6.4.3 CO_2 mitigation and energy gain

Net CO_2 mitigation is estimated at 20 t/ha. This means a mitigation of 10 million t CO_2/year from the total acreage of 500 000 ha. The energy gain amounts to 160 GJ/ha/year, a total of 80 PJ/year.

6.4.4 Cost-benefit analysis

Costs: 3.5 million euro in 5 years.
Results: realisation of new varieties that may generate an extra turnover of 2000 €/ha (estimated on basis of the added value of amylopectin) and control over the production chain by producing elite seed potatoes by NL parties. The extra yield of the potato biorefinery concept is: 1 billion euro per year for 500 000 ha. The extra yield is based on the added value of the modified starch; the € yield will be higher in those cases where the pectins, the proteins and other high-value metabolites can be extracted in addition to starch.

6.4.5 Participation by industrial partners

Industrial partners include all sorts of companies, together encompassing the complete production chain, such as breeding companies (HZPC, Agrico, Averis etc.), processing companies (Cosun, AVEBE, Herbstreith & Vos, GE plastics, BASF) and nutraceutical and pharmaceutical companies (Frutarom, Organon and Roche). Possible markets are chemistry (for starch, pectins and hemicellulose-derived C5 sugars), food and feed (starch and proteins) and the health/medical sector (flavonoids, carotenoids, vitamins, vaccins, glycoalkaloids, and free amino acids).

6.5 Concept 2: Sugar beet for production of polymers and platform chemicals

6.5.1 Concept

Sugar beet are grown on 80 000 ha in NL. The production potential of beet is similar to that of sugar cane but the production costs of beet sugar are double those of cane sugar. Objectives of this concept are:

- cost reduction in the total production chain by 50%;
- increasing economic yield by production of high-value constituents and by assuming that the currently developed winter beet has a 30% higher yield than sugar beet;
- gaining control over a production acreage of 500 000 ha by a combination of IP on unique plant material, chain knowledge, and unique refinery technology.

Sub-components of a total concept (can optionally be used in combination) are:

1. Reducing costs by increasing the N-use efficiency (more biomass per kg N);
2. Extending the harvesting and processing period. In the current setting the sugar factories are only used 6 months per year. Winter beet, beet with frost and flowering resistance, can extend the sugar beet campaign by three months and increase the capacity of the factory by 50%. The processing capacity can be further expanded by importing raw semi-finished products, such as concentrated juice or dried pulp. Cultivation can take place in the Southern Hemisphere (Chile, Argentina) and harvest and import can take place from April to August. Control over cultivation in the Southern Hemisphere is possible; this is demonstrated by the cultivation of chicory in Chile under control of the Belgian company Sudzucker-Beneo;
3. Extra processing of foliage to high-grade protein. Foliage yield is about 4.5 t DM/ha. At a protein content of 20% this yields 900 kg protein with a value of €500-600/t;
4. Processing of low-grade pulp to high-grade chemicals (furans);
5. New processing concepts, where granulated sugar is no longer produced but where the total sugar-rich biomass, after recovery of protein or platform chemicals, in its totality is converted into ethanol or ethylene (component 5 can possibly not be combined with 4 because in this concept the hemicellulose fraction of the pulp is fermented into ethanol);
6. Extra production of platform chemicals: high-grade amino acids (e.g. lysine, glutamine acid, asparagine acid) or organic acids (e.g. lactic acid, succinate, itaconic acid) with a value of €800-1500/t.

6.5.2 CO_2 mitigation

- Utilisation of the overcapacity of existing factories in NL would allow processing of an additional 80 000 ha beet. Net energy yield is estimated at 300 GJ/ha (calculation available upon request); this means that with this extra acreage the existing beet processing installations can fixate an extra 24 PJ of energy. CO_2 mitigation at 80 000 ha is estimated at 2.4 million t;
- With control over 500 000 ha (cultivation in NL and abroad, processing partly in NL) total energy yield is 150 PJ and CO_2 fixation amounts to 15 million t/year. Expansion of this concept to 500 000 ha of course requires new factories or re-operationalization of dismantled factories (NL, Poland).

6.5.2 Extra turnover generated by the concept

In 2006 sugar beet cultivation yielded the farmer (gross) about €2700/ha (at 60-65 t/ha). In the new sugar regime this decreases to about €1700/ha. In this concept we are assuming winter beet cultivation (100 t FW/ha) and a gross yield for the farmer of €2600/ha (as of 2010 the minimum price for beet is €26/t fresh). The extra protein, platform chemicals and bioethanol (or ethylene) result in an estimated increase of the gross yield for the agroprocessor by €1800/ha in comparison with processing the current beet into granular sugar. This corresponds with an *extra* turnover of €120 million for 80 000 ha and €0.75 billion for 500 000 ha.

6.5.3 Breeding challenges

1. N-use efficiency (cultivation cost reduction by 5% by 30% lower N application rate);
2. Winter hardiness and flowering resistance (cost reduction of processing by 20% by extending the campaign);
3. Production of platform chemicals;
4. Increasing protein content in tap root.

Breeding companies are already working on challenges 1 and 2.
Research costs for challenges 3 and 4 are estimated at €2.5 million.
A non-breeding challenge is the pre-processing of beet and on-farm storage of thick juice allowing year-round processing by the factory. WUR is already working on this.

6.5.4 Participation industry

Consultations are already being held with a number of partners: SESvanderHave (NL FR, BE), KWS (DE), Cosun (NL), Sabic (NL), DSM (NL) and Itaconix(US), a small company with technology for polymerising itaconic acid. Involving NL chemistry parties in the concept is still subject of discussion.

6.6 Concept 3: Miscanthus biorefinery

6.6.1 Crop

- Very high production potential (30 t DM/ha/year) by C4 type CO_2 fixation, good utilisation growing season. Yields dry lignocellulose with a low mineral content;
- Can be harvested twice a year;
- Sustainable by low mineral input as regards fertilisation, tillage, disease control and harvesting. Efficient water use;
- Can fix atmospheric N in symbiosis with micro-organisms which gives additional sustainability advantages;
- Low cultivation costs resulting from perennial cultivation (10-20 years).

6.6.2 Concept

Miscanthus is currently reproduced vegetatively and fields are planted with rootstock. Our concept aims at a drastic reduction of cultivation costs by enabling seed reproduction. The second aim is the development of unique genetic material with a very high content of fermentable sugars. The third aim is the propagation of seedlings from seed for marketing via a specialised company. This considerably reduces the planting costs in comparison with the costs of rootstock and certainly with those of *in vitro* reproduction.

Miscanthus is used as biorefinery crop with protein, sugar and lignocellulose biomass for energy generation (ethanol or electricity) and chemicals as main products. The crop is harvested twice, with the first harvest in June and the second harvest in winter after leaves have dropped. Protein and sugar are the most valuable products at the first harvest; the remaining biomass can in view of the low lignin content easily be converted into 2nd generation ethanol. The second harvest, dry lignocellulose, can be harvested from November to April and can be used for biomaterials, bulk chemicals (from lignin) and fine chemicals (from hemicellulose) and generation of electricity (residual biomass).

6.6.3 Products from Miscanthus (to be developed in steps)

- Electricity + heat. These products are particularly important for development of the 1st 100 000 ha. The low mineral content makes the biomass of M. extremely suitable for this purpose. This application alone is already cost effective in case of local processing;
- Biomaterials (fibres, building materials, composites);
- Fermentable sugars for bioethanol and BTL;
- Protein;
- Fine chemicals from hemicellulose and bulk chemicals (aromatics) from lignin.

6.6.4 Breeding

- Development of diploid genotypes that can be reproduced via seed (*M. sinensis*);
- Increasing free sugar content by crossing with sugar cane;
- Improving lignocellulose composition to improve the digestibility of fermentable sugars (cheap sugar for 2nd generation ethanol and other white biotech products);
- Improvement of amount, quality, and extractability of proteins.

6.6.5 CO_2 mitigation

- Acreage 1 million ha (a small part of the acreage allocated for energy crops in the EU), of which 50 000 ha in the Netherlands; the rest in other EU countries;
- At a yield of 25 ton DM/ha/year (18MJ/kg) energy yield is 50 GJ/ha/year (*gross*), i.e., 450 PJ/year. The energy input in *Miscanthus* cultivation is very low, 10 GJ/ha/year. With25 t DM/ha/year this means a net energy yield of 440 GJ/ha/year. Lewandowski & Schmidt (2006)[9] show that at the lowest nitrogen rate in their data the net energy yield of Miscanthus is even 590 GJ/ha per year;
- Net CO_2 mitigation is estimated at 41 t/ha (Sims et al., 2006)[10]. Gross CO_2 fixation is 41 million t CO_2/year for the total 1 million ha.

6.6.6 Costs and benefits of the concept

Costs: €3.5 million for breeding research for seed reproduction and for quality improvement.
In the cost-benefit analysis we are assuming that all *Miscanthus* will be used for energy. Although energy is the application with the lowest value, in the years ahead it will probably be the main driving force for setting up *Miscanthus* cultivation. In a cultivation concept for later development, Miscanthus is also used for products with a higher value. In this concept *Miscanthus* is harvested twice a year. The (extra) harvest in June means that the crop can also be used for the production of protein and sugar; the calculation below shows that this would result in a higher economic yield of the crop than for energy production alone.
Result for energy applications: estimated gross yield for the farmer is, based on 25 t/ha and a biomass price of €4/GJ (this is 50% of the electricity price: 8.3 €/GJ at €0.03/kWh): 25 t x 18 GJ/t x 4€ /GJ = 1800 €/ha. Cultivation costs are estimated at €2000/ha for the first year and at €700-800/year for the later years. These annual cultivation costs are slightly lower than for maize, which needs to be sown each year. Over a cultivation period of 10 years this results in an average net yield of €880/ha. Net yield is €1240/ha at a yield of 30 t biomass, which is considerably higher than that of wheat cultivation. Net yield is €520/ha at 20 t (see also Uellendahl et al. 2008)[11].
Turnover of the reproduction of plant material amounts to 1000 €/ha (0.02 €/plant). Aim is the supply of plant material for biomass production on 1 million ha in Europe with a growth cycle of 10 years and to let reproduction be carried out under control of the NL industry. Total turnover from plant reproduction alone would then amount to €100 million per year.

6.6.7 Participation by the industry

Companies involved in breeding (KWS, DLF-Trifolium, Limagrain), agroprocessing (Cosun, Herbstreith & Fox), chemistry (Sabic, DSM), and energy (Eneco, Nuon, Shell, Exxon) are interested.

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6.7 Concept 4: Oil crops as biobased raw material for chemistry

6.7.1 Concept

- Production of specialty fatty acids and plant oils for application in chemistry;
- Crops: *Calendula* and *Crambe;*
- Target: 1000 kg oil/ha and 3000 kg protein-containing hulls/ha;
- Market size: 100 000 t oil (attainable in 5-10 years) according to the table below.

***Calendula* oil**:		Total > 20 000 t of which:
	Calendula acid containing oil:	10 000 t
	Reactive thinner from *Calendula* oil:	10 000-20 000 t
	Wood preservative from *Calendula* oil:	10 000-20 000 t
	* value *Calendula* oil:	2.50 euro/kg
	* value derivative from *Calendula* oil:	4.00 euro/kg
***Crambe* oil:**	Erucic acid containing oil:	40 000 t
	New products in *Crambe* oil	
	* wax esters:	20 000 t
	* cheaper calendula acid containing oil:	> 20 000 t

- By-product value (protein-containing seed meal): 0.15 euro/kg or 45 million euro/year.
- Combination of existing products on new markets and new products on existing markets;
- Market development calendula acid applications based on classical breeding, with *Calendula*, market development in the longer term with crop with higher oil yield per ha: *Crambe* with calendula acid and wax esters;
- Total value chain: oil + seed meal = 445 million euro.

6.7.2 CO_2 mitigation

- Direct mitigation by replacement fossil raw material by plant oil: 70 GJ/ha;
- Extra saving because plant oil based chemistry costs less energy per unit end product (fossil often requires 3x as much energy as fixed end product): potentially another 70 GJ/ha/;
- Total for complete concept: 140 GJ/ha x 100 000 ha/year = 14 PJ or 720 000 t CO_2 eq direct and 1.4 million t CO_2 eq/year including energy saving for processing.

6.7.3 Extra turnover by concept

1. Primary production abroad (where farmer receives a competitive price of at least 500 euro/ha);
2. Oil extraction probably abroad (in view of logistic costs seed transport);
3. Processing industry in the Netherlands, marketing from the Netherlands;
4. Total value chain 445 million/year, of which appr. 50% in NL.

6.7.4 Breeding challenges and cost-benefit analysis

Bottleneck: improved oil composition and new plant oils, with aspects such as molecular mutation breeding for lower cost price, better processing quality and change of oil composition.
Costs: 2.5 million euro in 5 years.
Result: new varieties of *Calendula* with higher oil content, better seed shape, and higher calendula acid content (cost price then resulting in very good margins); new varieties of *Crambe* with higher oil yield, better oil composition, new oil qualities and better quality of seed meal (e.g. better feeding value and thus higher value).

6.7.5 Participation by the industry

Calendula Oil BV, Uniqema/Croda, Akzo, DSM Resins; contribution 1.25 million in 5 years (i.e. 250 000 euro/year).

6.8 Concept 5: New grasses for grass biorefinery (GB)

6.8.1 Concept

NL counts 1 million ha grassland which can hardly be used for other crops and which is mainly grazed. The large area means a large CO_2 mitigation potential. The current grassland acreage in NL yields 8 t dry matter/ha/year. Grass biorefinery (GB) would enable doubling of grass production to 16 t dry matter/ha/year, with a totally new production system: no grazing by cattle and fewer harvest moments 3-4 times instead of 5-6 times). The longer inter-harvest periods result in a much higher productivity.

The grass product is refined into: 1) fibres, 2) dry high-grade protein, 3) grass juice with peptides, amino acids and sugars. See the table below for yields in two scenarios (current technology GB and future with improved varieties, agronomy and technology).

Productivity, financial yield, CO_2 emission reduction, per year and per 100 000 hectare

Scenarios	Grass yield, t/ha	Gross yield of milk and biorefinery products, €/ha	Extra gross per 100 000 ha (million €)	CO_2-eq emission reduction (Mt)	Profitable investment in GB
(0) Only dairy/meat	8	3900	0	0	Margin under pressure
(1) Current technology grass biorefinery	10	4200	30	0.3	Margin at processing and investment costs below 140 euro/t
(2) GB future (better varieties, better agronomy and better processing)	16	6224	230	1.2	Margin at processing and investment costs below 288 euro/t

In the concept the complete 16 t/ha grass is in the end processed by GB. On average, half of the biomass (8 t/ha) is used to feed the existing NL dairy herd and the other half remains as extra raw materials for the biobased economy (fibres for paper, cardboard or isolation material, protein for high-grade food and feed ingredients or for industrial applications, grass juice for white biotechnology, lignocellulose for biogas or products such as ethanol).

6.8.2 Breeding challenge

New grass varieties with the following properties are needed to meet the requirements of this new concept:

- higher production per hectare and higher persistence when cut instead of grazed;
- higher protein content than now attainable with fewer harvest moments (heavy cuts). One solution direction envisages ‘stay green’ grasses which, unlike current varieties, are not degrading the protein in the lower leaves, which results in more protein being harvested;
- better fibres with heavy cuts.

6.8.3 CO_2 mitigation

Target is to start with 100 000 ha, with an extra CO_2 mitigation potential of 1.2 million t CO_2. Finally, 750 000 ha grassland in NL can be processed under this concept with a total CO_2 mitigation potential of 9 million t CO_2/year: 4.5% of the total NL CO_2 emission. Further CO_2 emission reduction is possible if part of the nitrogen requirement would be covered via nitrogen fixation by legumes (white and red clover) in new grass/clover combinations.

6.8.4 Extra turnover by concept

Extra turnover in biobased raw materials per 100 000 ha: 30 million euro per year in scenario 1 (see table) and 230 million euro in scenario 2; in maximum scenario in NL (750 000 ha) this is 225 million euro per year in scenario 1 and 1.8 billion euro in scenario 2 (with improved varieties and improved technology).

6.8.5 Cost-benefit analysis

Costs: 2.5 million euro in 5 years (for breeding research, a separate business case is available for investments in the biorefinery facilities themselves).
Result: new grass varieties that could result in 30 to 230 million extra value per 100.000 ha.

6.8.6 Participation by the industry

Courage, biorefinery consortium in Friesland, PROGRAS consortium (with AVEBE as one of the participants), DLF-Trifolium, Provinces Gelderland and Brabant (already working on GB).

6.9 Concept 6: European crops as new source of natural rubber

6.9.1 Concept

Natural rubber for the production of car tyres, building materials, medicinal gloves and other articles (a total of 40 000 applications) is virtually for 100% produced from latex from the rubber tree (*Hevea brasiliensis*). This tree is mainly grown in Southeast Asia. The genetic diversity (for e.g. disease resistance) is very narrow; this makes cultivation vulnerable to diseases or even total destruction. In the original production country, Brazil, *Hevea brasiliensis* has been struck by crop failures caused by *Microcyclus ulei* infestation (South American leaf blight). This disease made it impossible for Brazil to develop into a major rubber producer, despite attempts by Goodyear, Firestone Rubber Companies and Henry Ford early in the previous century. Natural rubber is strategically important, especially for the transport sector. The reason is that natural rubber cannot completely be replaced by synthetic rubber because its quality is too low for heavy applications. In addition, a strong increase in natural rubber demand and price is expected as result of a rapidly increasing car use in China and India. This means that there is a demand for other sources of natural rubber that can grow in Europe. Two plants may serve this purpose: Guayule (*Parthenium argentatum*), also grown on a small scale in the US (Yulex), although production is mainly focused on low allergenic nature rubber products. Some variety research and technology development has been carried out on Guayule. The second crop is the Russian dandelion (*Taraxacum koksaghyz*) which is indigenous in the low mountain ranges of Southeast Kazakhstan. In WW II Russia used this rubber for the production of tyres for army vehicles. Guayule is especially suitable for cultivation in Mediterranean and desert-like climates whereas *T. koksaghyz* is extremely suitable for cultivation in Northwest Europe. Besides rubber, the tap roots of *T. koksaghyz* contain a considerable amount of inulin. Inulin can be used in food, as is the case with inulin gained from the dandelion-related chicory. Inulin is also a source of fructose, which can with a high conversion efficiency be converted into furan-based chemicals.

A second product of guayule is a resin-type of product with applications in chemistry and the paint and ink industry. Another property of both crops is that a considerable amount of valuable biomass remains after extraction of the most valuable components, which may give the biorefinery concept additional value.

6.9.2 CO_2 mitigation

The yield of optimised rubber crops is estimated at appr. 45 t fresh weight per ha (11-12 t DM/ha). CO_2 fixation is estimated at appr. 18 t/ha at this biomass yield. At a total estimated acreage of 100 000 ha, of which 10 000 ha in NL and 90 000 ha in other countries this means a CO_2 mitigation potential of 1.8 million t CO_2/year. European consumption amounts to appr. 1.5 million t, which corresponds with 1 million ha of this optimised dandelion. Use of the remainder of the biomass for ethanol production would result in appr. 3.5 million t bioethanol, equivalent to the energy content of 1.5% of the total European petrol consumption.

6.9.3 Turnover from concept

The world market for natural rubber is 10 million t (2008). Over the past 10 years the value of natural rubber ranged from €1-2/kg (we are assuming an average of €1500 per t). Europe is using appr. 1.5 million t natural rubber (2008). Based on the perspective that the Russian dandelion can be developed into a crop with a production potential comparable to that of chicory (now average 45 t fresh weight roots/ha), and that the rubber yield can reach 1500 kg/ha, potential gross yield is €2250/ha. At this root yield the crop would yield 7 t inulin and 3 t DM as other biomass (pulp), where the inulin can be used for the production of furan chemicals or 1st generation bioethanol, and the remaining biomass for biogas or 2nd generation bioethanol. The value of the inulin is €100/t when converted into energy or chemical raw material. The value of the pulp is estimated at €80/t DM. This takes the total gross yield of the Russian dandelion to €3250 (as comparison: current gross yield of beet is appr. €5000/ha). There are no reasons to assume that dandelion would in due course not be able to obtain a yield similar to, e.g., sugar beet, which would take gross yield to €4300. Russian dandelion is a good example of a potential biobased crop. In comparison with sugar beet, Russian dandelion produces a natural chemical raw material (natural rubber) with a high value (>1€/kg) and represents a major strategic interest. A considerable part of the remaining dandelion biomass consists of inulin which can easily be fermented into ethanol or is extremely suitable for the production of furan-based chemicals. This means that the potential of this crop is high although there still is a long breeding road to go.

Guayule can as perennial crop be grown with annual yields up to 15 t DM per hectare. If breeding could raise the rubber content to 12%, this would result in a rubber yield of 1.8 t/ha/year (in comparison with current estimates of 1 t/ha) and 1 t/ha/year resin. Gross production value of the rubber then becomes €2700/ha, that of the resin €1000/ha (€1000/t) and that of the other biomass €500/ha (€40/t), together a total of €4200/ha. The Netherlands can play a major role in the development of propagation material of guayule and can thus acquire a key role in the chain control of this alternative rubber source. The Netherlands can also provide separation and biorefinery technology for the processing of guayule raw material and play a role in the further production of rubber products.

6.9.4 Breeding challenges

T. koksaghyz

- *T. koksaghyz* is currently a wild crop. The quality of the rubber is just as good as that of the rubber tree but rubber yield, now estimated at 3-600 kg/ha, need considerable improvement, for which two routes can be followed:
  - Increasing root yield (from “little finger” thickness to “fist” thickness),
  - Increasing rubber yield (from 3-4% to 12% of DM yield),
- Cultivation in Northwest Europe also requires breeding in of herbicide resistance to meet competition with indigenous dandelion (*T. officinale*) and other weeds and to keep cultivation costs low;

- *T. koksaghyz* is a diploid, self-incompatible plant species of which a wide genetic variation is found in the area of origin. These are virtually all hybrids, originating from genetically strongly differing parents. The self-incompatibility will make it a challenge to obtain homogeneous populations of this plant species. Various breeding strategies are available, such as vegetative reproduction or crossing in the possibility to circumvent the self-incompatibility and thus enable reproduction via seed.

Guayule
An extensive collection of guayule lines has been tested in the United States. Many of these are via USDA available for further breeding of varieties suited to European conditions.
Thirty limes are currently being tested in Europe (in France in cooperation with CIRAD and in Spain under responsibility of Plant Research International).
Breeding challenges:

- Increasing stem and root yield;
- Increasing latex/rubber content (from the current 5-7% to 12 % of DM yield);
- Improving breeding methods (more control over sexual or asexual reproduction; development of accelerated selection methods with molecular markers).

6.9.5 Participation by the industry

Rubber production in Russian dandelion and guayule is developed in the 4-year 7th Framework Project EU Pearls (http://www.eu-pearls.eu/UK/). The project is running until May 2012. A number of Dutch partners are involved in this project, including Keygene, Vredestein and Stramproy Contracting. And there are ongoing discussions with a number of new companies and investors that are interested in collaboration.

6.10 Concept 7: Biorefinery of maize straw for feed, biofuels and bio-chemicals

6.10.1 Concept

The maize acreage in the Netherlands covers about 250 000 ha, of which the largest part is used for silage maize cultivation. The crop is almost completely used as cattle feed. A small part is used as pig feed where only the cob fraction is used. The remaining part, the straw fraction, is hardly suitable as feed. The idea therefore is to set up a biorefinery chain for this fraction for the production of energy and biochemicals.

A very large amount of concentrated, usually imported, feed is currently being used for feeding pigs. The sustainability of pig husbandry in the Netherlands can be considerably improved by using locally produced feed. Alternative NL produced products are wet grain maize and Corncob mix (CCM); products consisting of grain and cob fractions of a maize crop, respectively.
In this case we are proposing a dual purpose approach for maize: the cob fraction is used as high-energy feed in pig husbandry and the straw fraction is used as raw material for protein and fermentable sugars. Biorefinery is separating the straw fraction into a pressed juice fraction containing proteins and soluble sugars, and a pressed cake fraction with mainly cell walls.
The proposed biorefinery concept for maize for NL entails increasing the maize acreage by 100 000 ha and for Europe an increase of the total acreage by 500 000 ha.

6.10.2 Products from maize straw

- Bioethanol and biogas;
- Protein;
- Biomaterials (fibres, building materials, composites);
- Fine chemicals from hemicellulose and bulk chemicals (aromatics) from lignin.

6.10.3 Breeding challenges

- Improvement of the lignocellulose composition to improve the digestibility of fermentable sugars for the supply of cheap sugars for the production of 2nd generation ethanol and other industrial biotechnology products;
- Improvement of amount, quality and extractability of proteins;
- Optimisation of yield and quality of starches.

6.10.4 CO_2 mitigation

- With a yield of 16 t DM/ha/year (18MJ/kg) gross energy yield is 300 GJ/ha/year (*gross*), i.e., an extra 30 PJ/year in NL and 150 PJ/year in Europe.
- Net CO_2 mitigation is estimated at 27 t/ha. Total gross CO_2 fixation is 2.7 million t CO_2/year for the extra 100 000 ha in NL and 13.5 million t CO_2/year for the total 500 000 ha in Europe.

6.10.5 Costs and benefits of the concept

Costs: €3 million euro in 5 years for breeding research to optimise dual purpose maize for production of CCM maize or wet grain maize and straw for biorefinery.
Result: Identification of varieties that are suitable for dual purpose applications and as starting material for further breeding.

6.10.6 Participation by the industry

Breeding companies (Limagrain, KWS, DLF-Trifolium,) and companies working in fields such as agroprocessing (Genencor, Novozymes, Imenz, Cosun, Herbstreith & Fox), chemistry (Sabic, DSM) and energy supply (Eneco, Nuon, Shell, Exxon) are among the interested parties.

6.11 Concept 8: Microalgae for chemistry

6.11.1 Introduction

Successful biobased production chains starting with algae do not yet exist. A number of unique properties generate positive feelings about algae but a number of quite serious challenges is to be taken as well. Breeding of algae, e.g., has hardly been developed. Breeding always starts by choosing a potentially successful production concept, a suitable organism, and a development goal, but for algae these choices have until now insufficiently been made. This is why we are - for the development of the business concepts for microalgae[12] - paying some more attention to providing background information than we did for the crop-based business concepts.

The group of algae includes tens of thousands of species and several cultivation techniques are possible, including open *vs.* closed, autotrophic *vs.* heterotrophic, artificial light *vs.* sunlight. At the moment it is difficult to make a balanced choice for an algae-cultivation system-product combination: there are too many production options with a number of those options still in the research stage. Until now only production concepts aimed at high-value constituents, such as food supplements, health ingredients and biotechnology products seem commercially valid. Until now none of the options seems to yield an economically sustainable biobased production concept. This is caused by the fact that micro-algae cultivation is still using wild, unmodified algae and that the costs of cultivation, harvesting and processing of algae are higher than for terrestrial plants. Measured against the progress that has until now been achieved in the breeding of agricultural and horticultural crops, the unlocked potential for improvement in yield and quality of algae must be considerable.

Recent publications[13, 15, 14, 15, 16, 17] present the view that the potential of algae as supplier of raw materials for fuel and chemistry is mainly based on four characteristics of algae: 1) their potentially high productivity, resulting from, e.g., higher photosynthesis efficiency and a higher harvest-index[18]; 2) the capacity to produce certain molecules (oil, protein, carbohydrates) as main component; 3) the capacity to recover valuable nutrients (P and N); 4) the capacity to produce biomass where terrestrial plants cannot grow (well). This last aspect offers possibilities for biobased production systems that are not competing with food production for limiting resources such as land and water.
The high expectations regarding algae are partly based on estimates of production levels resulting from conversion of production figures in a laboratory environment to a ‘field’ situation. In this vision we are adopting the conservative estimate that the maximum production potential of algae is comparable to, or at most 1.5-2 times higher than, that of the best producing terrestrial plants (sugar beet, sugar cane and maize). We also assume that algae should be used to do what they are best at: production of oil, carbohydrates and protein.

Two components are essential for successful production concepts for microalgae: 1) the choice of the right species, and 2) the choice of the right cultivation concept. The most important criteria for selecting a promising *algae species*, is the capacity of the algae to produce the right constituents (oil or bulk chemicals) and the capacity of NL knowledge parties to rapidly increase the knowledge and IP level. The main criterion for selecting a good *cultivation concept* is the possibility of cultivation in NL –

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[12] Macro-seaweed is not taken into consideration in this report. The reason is that macro-seaweed is only producing small amounts of oil, which makes a link with the need for raw materials for energy and chemistry difficult. Large-scale fermentation of macro-seaweed is of course possible but the options for production of seaweed for this application are sufficiently addressed by Florentius et al.16.

[13] Muylaert K. (2009) Inventory aquatic biomass. Report for MinEZ, NL

[14] Van Iersel S, et al. (2009). Algae-based biofuels, a review of challenges and opportunities for developing countries. Ecofys, GBEP and FAO report.

[15] Wijffels R (2007). Potential of Sponges and microalgae for marine biotechnology. Trends in Biotechnol. 26: 27-32

[16] Florentinus A, Hamelinck C, de Lint S, Van Iersel S (2008). Worldwide potential of aquatic biomass. Ecofys report for VROM

[17] From: Catie Ryan (2009). Cultivating Clean Energy, The Promise of Algae Biofuels, Terrapin Bright Green, LLC http://www.nrdc.org/energy/files/cultivating.pdf

[18] The proportion of the biomass that can actually be harvested.

preferably large-scale cultivation in view of the energy and CO_2 targets -, controllability of the cultivation, and the chance of a cultivation concept that yields maximum value at minimal cultivation costs. Two concepts are chosen in this business case for algae. One concept is aimed at the development of a microalga for production of high-grade fuels and bioplastics (6.11.2). This should in due course yield a production concept comparable to current industrial biotechnology: high-technology, closed and relatively large-scale production of raw materials for energy (ethanol) and chemistry (1,3-propanediol, lactic acid, etc.). The second concept (6.12) is aimed at the development of a concept for open cultivation designed for very large-scale CO_2 fixation and production of raw materials for energy, chemistry, food and feed.

6.11.2 Concept microalgae for chemistry

The main criterion for selecting a suitable alga is the constituent. The constituent should be present as main product and it must be possible to use the constituent as raw material for energy or chemistry. And there must be a good chance that NL can rapidly build up a unique knowledge position and there must also be a good chance for developing IP.

As regards constituents, algae can be divided into three groups: algae producing oil, carbohydrates (e.g. starch), or hydrocarbons[19]. Most promising in these groups are the hydrocarbon-producing microalgae, because hydrocarbons give the best link with high-grade fuels (e.g. jet fuels) and bulk chemicals. The most important representative of this group is *Botryococcus braunii.* This alga was referred to as 'promising' in a recent report by Ecofys for the FAO[14].

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[19] Hydrocarbons are molecules mainly consisting of the elements C and H. Crude oil and petrol, e.g., mainly consist of hydrocarbons.

Box 1. Hydrocarbon producing *Botryococcus braunii*

Botryococcus braunii is one of the most intriguing algal species, because *Botryococcus* is one of the very few living organisms that can produce hydrocarbons (C_nH_{2n}). B. braunii can accumulate hydrocarbons up to 15 to 75% of its DW, which is conspicuously higher than that commonly observed in other unicellular algae. Next to B. braunii, the only other hydrocarbon rich algae known at the present time is the halophilic species *Dunaliella salina*, which accumulates catotenoids (used as colourants). *B. braunii* is widespread in freshwater and brackish lakes and quite resistant to stress conditions.

The hydrocarbons produced by *B. braunii* are largely (up to 95%) located in the outer wall of the cell, which cell wall matrix act as a sponge for the accumulation of hydrocarbons. These hydrocarbons can easily be extracted from the algal cell wall by hexane extraction, without impairing cell viability, either after several extractions. Since the *B. braunii* hydrocarbons are highly reduced compounds comprising only carbon and hydrogen as elements, these hexane soluble hydrocarbons are very well suited to be converted into useful fuels such as gasoline by catalytic cracking. Therefore *B. braunii* is identified as interesting but still untapped resource for production of hydrocarbons. Successful use of this organism as an alternative source of energy depends on its growth rate, hydrocarbon productivity and their fuel efficiency.

An intriguing trait of *B. braunii* is that in natural environments the alga can bloom, thus forming hydrocarbon rich slabs covering lakes and lakeshores. However, none of the strains tested so far, have shown this ability under cultivated conditions. Growth is generally very slow, at maximum 1 division in 3 days, while other rapid growing green alga can multiply 3-4 times a day. Therefore increasing B. braunii growth rate in terms of biomass yields is a potential research goal.

B. braunii is classified into A, B and L races depending on the type of hydrocarbons synthesized. Race-A produces C23–C33 odd numbered n-alkadienes, mono-, tri-, tetra-, and pentaenes, which are derived from fatty acids. These linear olefins can constitute up to 61% of the dry cell mass of the green active state colonies. The L race produces a single tetraterpene hydrocarbon known as lycopadiene ($C_{40}H_{78}$) and it constitutes up to 2–8% of the dry biomass. The B race produces polyunsaturated and branched C30–C37 terpenoid hydrocarbons referred to as polymethylated botryococcenes. These compounds are promising as a renewable energy source as they accumulate to very high levels (26–86% on dry weight) in B. braunii. Different hydrocarbons, for example the three types of hydrocarbons present as main component in the three different B braunii species, can be used for different applications, not only as pure fuel but also as fuel extender, or as building block for polymers. Having different strains each producing different hydrocarbons to high purity would be highly preferable to prevent down stream purification and allow controlled polymerization into a range of polymers.

Text from a European research proposal submitted for the Joined Biorefinery Call 2009, but not honoured.

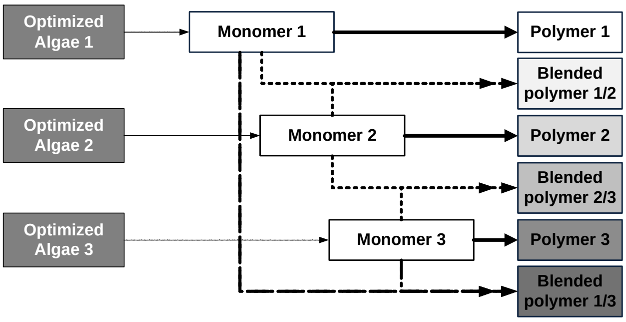
Botryococcus is representing a unique group of microalgae that can accumulate hydrocarbons, under some conditions up to 80% of their dry weight. In the literature it is suggested that this alga has provided the hydrocarbons for the deposition and formation of crude oil in a number of oil fields.

The genus *Botryococcus* has three variants or species: A, B and L-race. These variants are producing varioushydrocarbons (see box).
All three subspecies have in common that the synthesis of the hydrocarbons (monomers) is taking place in the algal cell and that these are excreted after synthesis. Beyond the cell the hydrocarbons can

polymerise into a spongy bioplastic cell wall. New monomeric hydrocarbons can again be accumulated in the spongy cell wall. The hydrocarbons are therefore largely found outside the algae in the cell wall. This then offers possibilities for "milking": a method for extracting/harvesting constituents without harvesting the living algae.

The observation that the cell wall is a natural plastic offers leads for the development of a concept in which the *Botryococcus* alga can be used for the production of monomers for polymeric synthetics. In this concept different *Botryococcus* algae are selected or bred that are separately capable to synthesise one of the reactive monomers. After extraction and refining, these monomers can be used for the production of various polymers. E.g., variety 1 produces monomer type 1, variety 2 produces monomer type 2. Extraction of these separate algae leads to different monomers. A range of new biopolymers can be produced by mixing in various combinations; see Figure 1.

Figure 1. Concept for the production of a range of unique polymers by using one or more of the three different monomers from three separate *Botryococcus* producing platforms.



6.11.3 Breeding challenges

First objective is the development of various strains that can produce the different hydrocarbon monomers with a reasonable degree of purity. This then allows development of a new generation biopolymers with properties that are widely differing from the existing biopolymers (e.g. based on starch or lactic acid). The best cultivation concept for these algae and these molecules probably is controlled cultivation in closed fermenters (similar to bioethanol production with yeast). Second objective is increasing the growth rate of this microalga.

Knowledge about the genetics and breeding of this alga is required to make this production concept possible. This knowledge is currently totally absent. Acquiring insight into the metabolism of the various hydrocarbons, linking metabolism and genetics, and the development of breeding methods, including a transformation method, are therefore essential and fundamental knowledge lines.

6.11.4 Participation by the industry

The interest of NL companies is still hard to estimate. Polymer chemistry and industrial biotechnology may be interested parties.

6.12 Concept 9: Microalgae production at sea

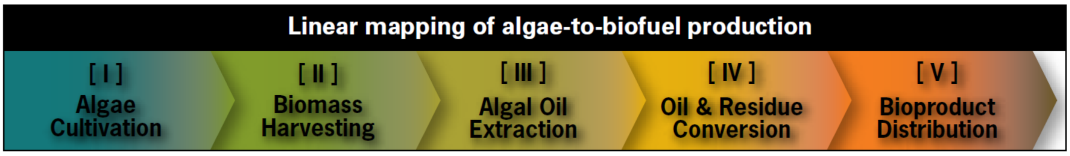
6.12.1 Concept

The objective is to arrive at a production concept that enables large-scale industrial CO_2 fixation. The main criteria for the development of such a production concept are the estimated contributions to the energy and CO_2 commitments of the NL government, the possibility of using the concept as export product, control over and controllability of algae cultivation, and the chance that the cultivation concept yields maximum value against minimal cultivation costs.

To set a ballpark, a scenario is assumed in which 30 million t CO_2, corresponding with the CO_2 production of Rotterdam harbour, must be fixed. A production of 25 t dry algal biomass per ha (with an oil content of 30%) is fixing about 45 t CO_2 per ha. Fixation of 30 million t CO_2 by algae requires 670 000 ha (6700 km^2) algae.

This calculation shows that:

- if the objective would be to make a significant contribution to the NL CO_2 mitigation targets, attention should focus on the development of production systems beyond agricultural soils. And keeping the required area as small as possible requires realisation of a maximum algae production per surface unit. This in any case means that the algae cultivation system should be optimally fed with CO_2 and nutrients, just as in agriculture and horticulture;
- large cultivation areas are involved; this means – as in agriculture – designing and establishing a completely new industry based on the most commonly found algae products: oil, protein and cell walls (dependent on the alga: cellulose or silicates);
- innovative solutions are required in all sorts of areas, such as cultivation, processing and marketing of algae. An example of an algae-biofuel chain is presented in the figure below[20]. When a more or less open cultivation system is assumed, which is unavoidable at such a volume, the largest costs are made for harvesting [II], concentrating and dewatering of algae [between II-III], and oil [III] and protein extraction [IV] from the algae;



- this means that the development of a production concept should primarily be aimed at solving the largest problem: high costs of harvesting the algae and the extraction of its products oil and protein.

6.12.2 A possible solution and chances for knowledge development

A possible concept in which the largest cost factors in algae cultivation, harvesting and concentration, can be strongly reduced has recently been identified by some employees of WUR. The concept is relatively simple and can be applied in a large number of cultivation environments (fresh-salt, moderate-tropical). This is the reason for first investigating whether the concept can be patented, and if this would indeed be the case actually submitting a patent application. A NL production system for large-scale microalgae cultivation can then be developed around this concept.

6.12.3 Step-by-step plan

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[20] From: Catie Ryan (2009). Cultivating Clean Energy, The Promise of Algae Biofuels, Terrapin Bright Green, LLC http://www.nrdc.org/energy/files/cultivating.pdf

1. Patent search (costs WUR, early 2010);
2. Patent application (costs WUR, first quarter 2010);
3. Check concept with stakeholders (action LNV, EZ and WUR);
4. Apply for financing;
5. Form project team and further theoretical development of the concept, including required investments, micro- and macro-economic analysis;
6. Small-scale demo in facilities under construction (e.g. AlgaeParc);
7. Demo on large(r) scale (tens of hectares).

6.12.4 Participation by the industry

The concept offers possibilities for extremely large-scale production of high-grade oil (for fuel, as raw material for chemistry and/or for fish farming) and high-grade protein (for feed and food). Recovery of minerals (e.g. phosphate) for the production of fertilisers is another market. Analogous to the existing agroproduction systematics, where flows of residual plant material form the basis of large-scale animal production in NL, the residual flows of algal cultivation can form the basis of large-scale fish farming, in which the nutrients are recycled in the algal production system.

All knowledge elements for designing and implementing such a system are available in NL, including knowledge of offshore infrastructure, water management, nutrient recycling, fish farming, and algae cultivation. The concept is suitable for worldwide application in fresh and salt water and is therefore extremely suitable as knowledge export product.

6.13 Markets for the products from the mentioned business concepts

6.13.1 Sugar and carbohydrate polymers

Implementation of the business concepts would mean a considerable increase of the crop acreage under control of NL industry. This increase can only be achieved if there is also a market for the biomass products from these crops. The volumes of raw materials that can be produced by the business concepts, at 100% realisation, are presented in Table 1 and are discussed below in relation to the expected size of the market.

A total of 56 million t biomass is harvested from about 2.9 million ha biomass (Table 1), with an energy content of about 1050 PJ (without taking cultivation costs and losses into account).

All cultivation of biomass together yields about 17 million t cellulose per year (Table 1), or after hydrolysis, an almost equal amount of fermentable sugar. This would be enough to produce 4.5 million t petrol equivalent, about the volume of the NL petrol demand (4.2 million t in 2006). Cellulose can also be used for paper, cardboard and chemistry (cellulose-based synthetic materials).

Total hemicellulose production amounts to more than 10 million t, of which the largest part is yielded by *Miscanthus* and maize (Table 1). About 70% of the hemicellulose consists of xylose, one of the platform chemicals in Table 4 and raw material for the production of furans. The market for furan-based chemicals does not yet exist but may grow to an estimated 1 million t, certainly if furans can also be further developed into high-grade motor fuels[21]. After hydrolysis into fructose, inulin can also easily be converted into furans. Xylose can also serve as raw material for ethylene glycol, with a world market volume of 18 million t[22] and an annual growth (before the crisis) of 4-5%; this corresponds with an annual increase in demand of 800 000 t. The total estimated production of 7.2 (0.7 x 10.3) million t xylose (Table 1), can therefore in principle be absorbed by the chemicals market. The total amount of *Miscanthus* and maize biomass, however, can also serve as raw material for methane fermentation (heat and electricity) and/or ethanol fermentation (biofuel), of which the markets are virtually infinitely large.

Beet yields 5.5 million t sugar (Table 1), from which 2.7 million t ethanol or 1.5 million t bio-ethylene can be produced. The world market for polyethylene is 80 million t, this means that this amount of beet-ethanol for ethylene can easily be absorbed by the market. World bioethanol production volume is now 35 million t, but demand is in fact almost infinitely large.

The mentioned biobased business concepts can – in potential – produce sufficient pectin for the world market, with beet and potato as largest suppliers (Table 1). Pectins are not only used in a large number of food products (as gelatinising product, as fat replacement, for texture improvement) but also in pharma and technical applications. The world market volume is estimated at 45 000 t/year[23] and shows an annual growth of 4.5-5.5%[24].

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[21] http://www.avantium.com/news-events/press-releases/2007-2/avantium-steps-ahead-with-its-biofuels-program/

[22] http://www.sriconsulting.com/WP/Public/Reports/eg/

[23] Willats, W.G.T., Knox, J.P., Mikkelsen, J.D. (2006). Pectin: New insights into an old polymer are starting to gel. Trends in Food Science and Technology 17: 97-104

[24] http://www.icis.com/Articles/2003/07/11/502950/ups-and-downs-in-the-tangled-hydrocolloids-market.html

6.13.2 Protein

Protein is an important product of the biobased chains, a total of 3.9 million t (Table 1). The existing markets for plant protein, mainly from soy and wheat, are food and feed. Whether the market can absorb this extra protein production depends on the application. Total NL consumption of soy proteins, mostly for feed, amounts to 1.4 million t (2.8 million t soy meal with 50% protein). Soy protein import (as beans and hulls) is a factor three higher, appr. 4.2 million t. European soy meal consumption is appr. 35 million t[25], of which 17.5 million t protein.
Part of the protein is produced by algae: 0.5 million t. The protein from the algae cultivation concept (6.12) has a very high quality and is - except for food - extremely suitable for fish cultivation. NL fish catchings amount to appr. 470 000 t fresh[26]. The average protein content of fish is 20%. Assuming a protein/protein conversion factor from feed to fish of 2, means that about 190 000 t protein is required to grow this volume of caught fish. It must therefore be concluded that the total protein production of all business concepts together is too large for the NL market and must therefore also be sold outside NL, as is already te case with part of the protein imported today.

6.13.3 Oil for energy and chemicals

Vegetable oil production from all business case together amounts to 0.9 million t, roughly equally divided over chemical and biodiesel applications (Table 1). The current NL petrodiesel consumption is 6.3 million t; the 0.5 million t algae diesel can therefore easily be absorbed by the NL biodiesel demand.

The oil-based chemicals originate from the two oilseed crops *Crambe* and *Calendula* and can be sold in several markets: paints and coatings, wood preservatives, additive for synthetics, and high-grade lubricants. The estimated sales volumes for the various markets are presented in the table below. This table shows that the estimated product volume of the two oil crops can be absorbed by the market.

Market estimates of oil components of biobased oil crops

Market	Oil crop	Current demand (world)	Market demand new product	Realistic market demand
Paint/coatings: reactive thinner	Calendula	10 million t paint 2.5 million t alkyd paint 500 000-750 000 t alkyd resin	100% market share with 20% reactive thinner = 100 000-150 000 t	10% market share: 10 000-15 000 t
Wood modification	Calendula	EU: 6 million t wood now preserved with toxic chemicals NL: 100 000 ton thermally preserved wood	At 100% application: Worldwide tens of millions of tonnes EU: 1.2 million t NL: 20 000 t	10% market share in EU of current wood preservative market: 120 000 t
Erucic acid	Crambe	Mainly erucamide and behenic acid derivatives	100% replacement technically and economically possible: 100 000 t	50% substitution: 50 000 t
Wax ester	Crambe	High performance lubricants Specialty wax esters with, e.g., reactive conjugated double bonds.	Specialty products now only represent5% of the total lubricant market Complementary to market for Calendula oil	10% substitution of current specialty lubricant market (200 000 t) Doubling of the market for calendula oil by lowering cost price (+ 15 000 t)
TOTAL				400 000 t

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[25] MVO Task force Sustainable Soy. Sustainable soy production. What is the Dutch industry doing? http://www.taskforcesustainablesoy.org/

[26] http://ec.europa.eu/fisheries/publications/fishyearbook2007.pdf

6.13.4 Water-soluble chemicals

There are three business concepts (potato, beet and maize) for the production of water-soluble platform chemicals. The joint production volume of chemicals by these crops is 1.5 million t. Examples of platform chemicals that can be produced by potato and beet are various amino acids and organic acids (Table 4). An example of amino acid is lysine, which can serve as raw material for nylon-6 via ε-caprolactam. The annual production of ε-caprolactam is 3 million t[27]. An example of an organic acid is itaconic acid. Current production volume is between 5-10 000 t/year (at €2.5/kg). New markets are opening at lower production costs, including the market for superabsorbants (100 000 t/year, production now based on petrochemical polyacrylates) and methylmetacrylate-based products. Annual methylmethacrylate production amounts to more than 2.4 million t[28]. The market can easily absorb the volume of plant-produced platform chemicals, provided that a number of different platform chemicals, with different markets, is chosen.

6.13.5 Natural rubber

An acreage of 100 000 ha is assumed in the business concept for natural rubber, which can yield about 150 000 t rubber. Total European rubber import is 1.5 million t, ten times the amount that can be produced on this acreage.

6.14 Chance of success

The objective of this vision document is the identification of promising biobased production chains and identification of the role of breeding in such chains. At the beginning of this chapter "promising" has been defined as follows: 1. the total of production chain concepts should be able to make a substantial contribution to the CO_2 mitigation target of the government; 2. the production concepts are leading to a higher and more sustainable turnover by the NL industry.

All business concepts mentioned in this chapter do potentially meet these criteria but the chains still need to be developed and set up. This means that the chance of success is especially determined by the business perspective seen by chain parties for these chains and by their willingness to co-invest in development routes. These and other questions related to the chance of success are listed and scored in Table 2. A second success factor, the possibility of IP development, is considerable for all concepts. IP concerns unique plant material that can be protected by patent right or plant breeders' rights but also patents on technology. The availability of imported plant material, e.g. crops with a higher content of biobased constituents or with an improved cell wall degradability is also offering possibilities for developing unique refinery technology on which IP can be established as well.
Finally, the success of a number of concepts is depending on the future development of the societal perception of GM plants for biobased applications.
A number of business concepts demand a GM approach. Other concepts can be realised with classical breeding.

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[27] ICIS 2009. http://www.icis.com/v2/chemicals/9075184/caprolactam/pricing.html
[28] http://chemsystems.com/reports/search/docs/abstracts/0405-2_abs.pdf

7 Promising biobased chemicals from plants and microalgae

7.1 Introduction

All market parties in the biobased production chain indicate that direct production of chemicals by plants is a promising route. The possible clients for such plant chemicals, the chemical industry, however, still find it difficult to say which chemicals they would wish to buy from the agrosector. The agrosector will not be investing in the development of plants with a high level of recoverable constituents without knowing which substances are concerned, what the market potential is, and how sales are guaranteed. Although establishment of the link between chemistry and breeding in NL is still difficult, the future perspective is promising in view of the movements in the worldwide market. Worldwide, there are quite a number of examples of alliances between agro and chemistry that are aiming at the development of new markets for biobased chemicals (see box).

The last five years have seen all sorts of movements in the market which illustrate that biobased production of chemicals is a valid economic activity. In the US, mainly companies with an original basis in agro-processing as well as chemistry, such as Dupont, Cargill, ADM and Dow, are setting up routes for the large-scale production of platform chemicals (lactic acid, succinate, 1,3-propanediol, polyhydroxybutrate): in the order of 10-100 000 t/year, or sometimes more.
Another trend is that Industrial Biotechnology, through its involvement in the development of new enzymes for biomass degradation and for facilitating biorefining (DSM, Genencor, Novozyme), seems to move somewhat towards Agroprocessing. Agroprocessing research is investigating routes for carrying out simple chemical syntheses themselves to increase the value and applicability of plant constituents. Agroprocessing thus seems to move towards chemistry (cooperation Cosun and Avantium[21]). More and more partnerships are established between agro and chemical parties. This gives Agroprocessing access to new markets and chemistry a secure supply of raw materials. Examples of partnerships are: Dupont & Tate & Lyle (1.3 propanediol), DSM & Roquette (succinate), BASF and CTC (ethanol and ethylene), CSM and BASF (succinate).

The plant constituents with a potential for chemistry are presented in this chapter. First, a long list with all possible substances is drawn up (Table 3). The list is then restricted to relatively high-value constituents that can directly be produced by plants (Table 4).

A number of questions need to be answered for the identification of promising chains for the production of raw materials for chemistry from biomass:

1. Which substances are drawing interest from chemistry?
2. Which substances can be supplied by plants and the agrosector?
3. Which parties can play a role in setting up such production chains?
4. Which are the corresponding crops and breeding challenges?

These questions are elaborated in the following sections.

7.2 Which substances are drawing interest from chemistry?

A number of approaches can be followed to answer the first question. A first approach is to prepare a list of all chemicals that are, e.g., produced in the world, Europe or Rotterdam. Based on these petrochemical molecules, plant or natural molecules can be identified with the closest resemblance to the chemical structure. This is followed by a search for a synthesis route for converting the biological molecule into the desired, now still petrochemical, molecule in the smallest number of steps. This approach is followed by Sanders and Scott[29, 30] and learns that, e.g., a number of amino acids and organic acids can potentially serve as raw material for existing or new chemical chains, such as solvents, synthetic materials, building chemicals, etc.

A second approach has been followed by the National Renewable Energy Laboratory (US). This approach is mainly based on glucose as basic raw material because glucose is a cheap and well-defined raw material. An iterative process, which included the complexity of required transformations, the possible market volume, the possibility of producing a range of derivatives and intermediates, resulted in identification of a group of 30 molecules of which twelve were selected as promising. A number of companies have carried out this exercise from their own strengths and interests and arrive at their own variants of this list. Furthermore, LNV (Ministry of Agriculture, now Economic Affairs, Agriculture and Innovation) has asked TNO to conduct similar analyses, focusing on the NL situation.

A third approach is to make an inventory of the chains for the production of chemicals already set up by parties in biobased production chains; a clear economic perspective appears to exist for molecules in such chains.

The molecules resulting from these three approaches are presented in Table 3.

7.3 Which substances can be supplied by plants and the agrosector?

The challenge in this section is to identify substances that may give a clear added value in (existing) biorefinery chains. These are substances/molecules that may be recovered as co-product besides sugar, starch, oil or protein. The substances are recovered from the watery (residual) flow, concentrated and purified, and can be applied as platform chemical. Although petrochemistry finds it difficult to define a true winner platform chemical, discussions with the stakeholders resulted in identification of a number of generic principles that would need to be met by a potential winner:

1. The market value of the molecule is between € 1000-2000/t. At a lower value, petrochemical production of the molecule is preferred. The minimum value, however, is not sharp and even subject to erosion as appears from the initiative of a number of chemical companies (Dow, Braskem, BASF) for the production of (poly)ethylene from cane sugar. Within chemistry, ethylene is the product with the largest sales volume and the lowest value (price ex Rotterdam around €900/t);
2. Substances costing more than €2000/t are better produced by industrial biotechnology. This limit is neither sharp; all sorts of substances with a market value > €2000/t are still obtained from plants because microbial synthesis too complex or more expensive than extraction from plants. The volumes, however, are small, no more than a few tonnes per year;
3. At a market value of €1000 processing costs are not higher than €400-500/t. To avoid processing costs running up to high, the content of the molecule should preferably not be lower than 1 t/ha (all rough estimates based on intuition). Inherent to the last requirement is that the molecules must be able to accumulate in plants without damaging the physiology of plants. This eliminates molecules such as ethanol and butanol (Table 3) because these are at low concentrations toxic to

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[29] J. Sanders, E. Scott and J v Haveren (2006). Rotterdam – a port in a biobased economy (WUR report)

[30] E. Scott. F Peter, J Sanders (2007). Biomass in the manufacture of industrial products, the use of proteins and amino acids.

plants. Molecules such as glucaric, levulinic and furandicarboxylic acid are also eliminated because the last step in the synthesis is chemical and can therefore not take place in living plant organisms;

4. The market volume of the molecule is (can grow) to over 25-50 000 t/year to justify extra capital costs for processing;
5. The molecule can also be sold in the food or feed market. Petrochemistry has difficulty with the thought of being dependent on the agrosector for their chemical supply. If the molecule can first be sold in other markets, such as in food, feed and pharma, where it can grow, these markets serve as proof of concept for supply security to petrochemistry. This principle has, incidentally, been mentioned by all parties and this roundabout principle does not work for all molecules.

When the list of Table 3 is screened according to these criteria, the molecules of Table 4 are remaining. Condition 5 is applicable for a limited number of molecules; only for citric acid (food) and a number of amino acids, including lysine (feed).

The molecules that can be supplied by the agrosector to chemistry are mainly molecules that are now already produced by industrial biotechnology. Direct production by plants brings agroprocessing (refinery from plants) in competition with industrial biotechnology (synthesis from plant sugars by microbes). The most cost-effective and sustainable production method will in the end survive. It is still too early to establish which route will become most cost-effective for these molecules, direct production by plants is especially expected to have advantages at sales volumes exceeding 25-50 000 tonnes.

7.4 Which crops and which corresponding breeding and research challenges?

The most important questions that apply for all molecules are: 1) how do I reach the highest possible level of a particular substance in the plant; 2) how do I obtain molecules with the best possible purity with the lowest amount of process energy. The challenges are found at three levels:

a. fundamental strategic research into accumulation mechanisms,
b. classical breeding, including selection, (eco)TILLING, crossing, etc.,
c. molecular breeding, application of existing or new fundamental strategic knowledge on designing and making new GM plants with the desired property.

The approaches to be followed for which molecule are summarised in Table 5.

7.5 Which parties/markets can play a role in setting up production chains for platform chemicals?

Clarity is required about the market for the mentioned molecules or about who is prepared to buy the plant-produced substances. In due course, the chemical industry is the most likely candidate for the chemicals mentioned in Table 4. The last column of Table 4 mentions the parties that can possibly be interested in the development of plant production routes and markets. It is, however, still unclear for all substances whether the chemical industry is interested in a production route that starts in agriculture, and this makes chemistry as market the most uncertain chain in setting up biobased production chains.

Setting up of an intermediate party that guarantees a minimum purchasing volume and price for a number of platform chemicals for a number of years is another way to “guarantee” sales of platform chemicals. Such a guarantee may take away the major doubt of the seed and agro-processing companies about investing in an innovation route. Such an intermediate party could be a government, trading company or investor. The challenge for such a party would have to be the selling of platform chemicals, regardless of the market, although development of the market for chemistry would also have to be an ambition, possibly for an ever increasing part of the sales volume.

Depending on the substance mentioned Table 4, development of the market may show a changing picture, as slightly elaborated below.

Organic acids (carboxylic acids) form one of the most promising groups of platform chemicals, with polymer chemistry as most important, but not only, market. Agrobiotech and chemistry parties involved in the development of initiatives to transform or expand production of organic acids (lactic acid, succinate and 3-OH propionate) are DSM, BASF, Cargill and smaller companies. In this field we also find alliances between agro and chemistry, with BASF/CSM and DSM/Roquette (both succinate) as examples. The worldwide production of lactic acid, currently the organic acid with the largest production volume, is expanding steadily. Various parties (non-disclosed) have research activities in the field of itaconic acid. The NL IP position is rather strong. The IP for itaconic acid production in microorganisms is held by TNO and the IP for production in plants is held by WUR. Seed companies and agroprocessing companies have shown an interest in production in plants.

For a number of amino acids (lysine, glutamic acid, proline) bulk chemistry is a possible market but this is not sure because the chemical industry has not yet adopted these molecules as renewable chemical raw material. According to the concept of 7.3 (point 5) the step towards bulk chemistry can possibly be made via other markets, including fine chemistry, pharma, food or feed. Feed grade lysine has a worldwide production volume of appr. 800 000 t, and the market volume is increasing, especially as result of the growing meat production in China, India and Brazil with a few per cent per year (30 000 t/year). The NL knowledge and IP position in the field of overproduction of lysine in plants, considered in a worldwide perspective, is rather strong. Seed companies and agro-processing companies are also showing an interest in production in plants.

The polymers PHA and cyanophycin should mainly find their market in chemistry. The NL knowledge position as regards PHA is weak. IP on genes and production routes and knowledge of PHA production in plants is mainly present at Metabolix (USA). Under a Metabolix licence, the also American company ADM is setting up the commercial production of PHA in microorganisms. Production in plants (tobacco and switchgrass) are being developed by Metabolix itself and permission for the first field experiments has recently been granted. This does, incidentally, not turn the production of PHA in typically West-European crops into an impassable road. Depending on the licensing conditions and the development of market volume, production of PHA in potato (tubers) and beet (foliage) can be an attractive option.
The situation for cyanophycin is similar. University Rostoc (De) has knowledge about cyanophycin production in plants. *Undisclosed parties* (breeding and chemistry) outside NL are cautiously interested in cyanophycin production.

Section 6.13.3 contains a fairly detailed review of the market for specific vegetable oils.

The market seems to show substantial interest in hydrocarbons and fuels from algae. A remarkably high number of (small) companies are involved in the development of high-grade fuels for, e.g., airplanes, including Solazyme, Sapphire Energy, Solix, Algenol, Petrosun Biofuels, Synthetic Genomics, Martek, etc. Petrochemical companies such as Exxon Mobile ($600 million), BP ($10 million) and Dow Chemical ($25 million) are making considerable investments in the development and production of algal fuels. And airplane developing companies and airlines such as JAL, Boeing, Continental Airlines are very interested in the development of biofuels from algae.

Modified starches are sold in a very large number of markets that will be similar to the existing markets for existing starches and well-known to the NL parties mentioned in Table 4, last column. Production by potato is a promising development route in view of the NL knowledge and possible NL partnerships. NL has a good knowledge position in modified starches and good possibilities for IP development.

Pectins form an existing market and most pectins are recovered from residues of citrus juice and apple juice production. The market value of pectin is $11-13/kg. The worldwide market volume is 45 000 t/year and is growing by 5-6% per year which leaves room for new production sources. Pectins are, also in ten years time, still mainly sold in food, feed and pharma markets. Production by potato and beet are promising development routes in view of NL knowledge and NL partnerships although the sales volumes are limited (see also 6.13.5).

7.6 Summary and conclusions

Almost all parties that can play a role in setting up biobased chains have indicated that production of chemicals by plants is a desirable development option. At the same time, the chemical industry cannot yet pinpoint the chemicals concerned. Looking from two perspectives, from market developments and from the capacity of the plant, resulted in identification of a restricted set of platform chemicals and crops that are potentially promising. Sugar/fodder beet, potato and – in due course – possibly also the Russian dandelion are crops that are extremely suitable for the production of water-soluble platform chemicals. The most promising molecules are organic acids (such as succinic acid, itaconic acid), amino acids (such as lysine, aspartate), modified starches and natural rubber, also in view of the availability of knowledge and IP at NL companies and knowledge institutions.

The NL knowledge position for the biopolymers PHA and cyanophycin is weak. Production of these substances is assumed to be attainable and promising but further development is expected to be carries out by non-NL parties.

Production of oil-based chemicals on basis of calendula acid, erucic acid and wax esters is promising, with *Crambe* and *Calendula* as most promising crops. The NL knowledge position is strong and participation of NL industry is reasonably certain. A GM approach is required for the production of wax esters by the non-food oil crop *Crambe*.

Development of a rubber-producing crop is promising; the development route is long but the required basic knowledge is available at NL parties.

Production of hydrocarbons by microalgae is promising as well. There are two possible markets: biofuels and bio-plastics. Since a few years substantial capital have been invested in the development of biofuels from algae, especially in the US. The development of bioplastics from specific algal hydrocarbons is a new research area with provide ample opportunities for the development of unique knowledge and IP by NL parties.

An important generic challenge (generic because the challenge applies for almost all crops and is demanding a multidisciplinary approach as well) is realisation of a better degradation of plant cell walls into its subcomponents, cellulose, hemicellulose and lignin, and the conversion of these molecules into chemicals (hemicellulose into furans, lignin into aromatics). Lignin and hemicellulose are “unavoidable” products of all biobased crops (the average content is appr. 15% and 25%, respectively. This makes solution of this challenge an unavoidable target of the ambition to build sustainable and economically valuable biobased chains. The necessary development route must be supported by knowledge from breeding (green biotechnology), white biotechnology and chemistry. The next step, the conversion of these cell wall components into raw materials for energy and chemistry, is also offering opportunities for NL research institutes and private companies.

Annex 1. List of interviewees

Depth interviews
Peter Bruinenberg (AVEBE), 8 July 2009
Gert de Raaff, Gerald van Engelen (Cosun), 9 July 2009
Casper Vroemen, Francis Stalder (Genencor), 10 July 2009
Léon Broers (KWS), 13 July 2009
Marcel Wubbolts and Ynte Hoekstra (DSM), 21 July 2009
Jacques Joosten (Regiegroep Chemie), 21 July 2009
Ton Runneboom (Platform Green Raw materials), 1 September 2009

Telephone interviews industry
Leon van Beuningen (Limagrain), 22 July 2009
Frank Kuipers (Sabic), 29 July 2009
Dick den Ouden (Avantium), 5 August 2009
Klaas van der Woude (SESVanderHave), 13 August 2009
Jos Keurentjes (AkzoNobel), 13 August 2009
Chris Kruze (Solvay Pharmaceuticals), 21 August 2009
Anne van Gastel (BASF), 25 August 2009
Piet van der Linde (D1Oils), 27 August 2009
Malcolm Osseweijer (Croda), 1 September 2009
Michael Weitz (Choren), 1 September 2009
Mark van der Mee (Sabic Innovative Plastics), 16 September 2009

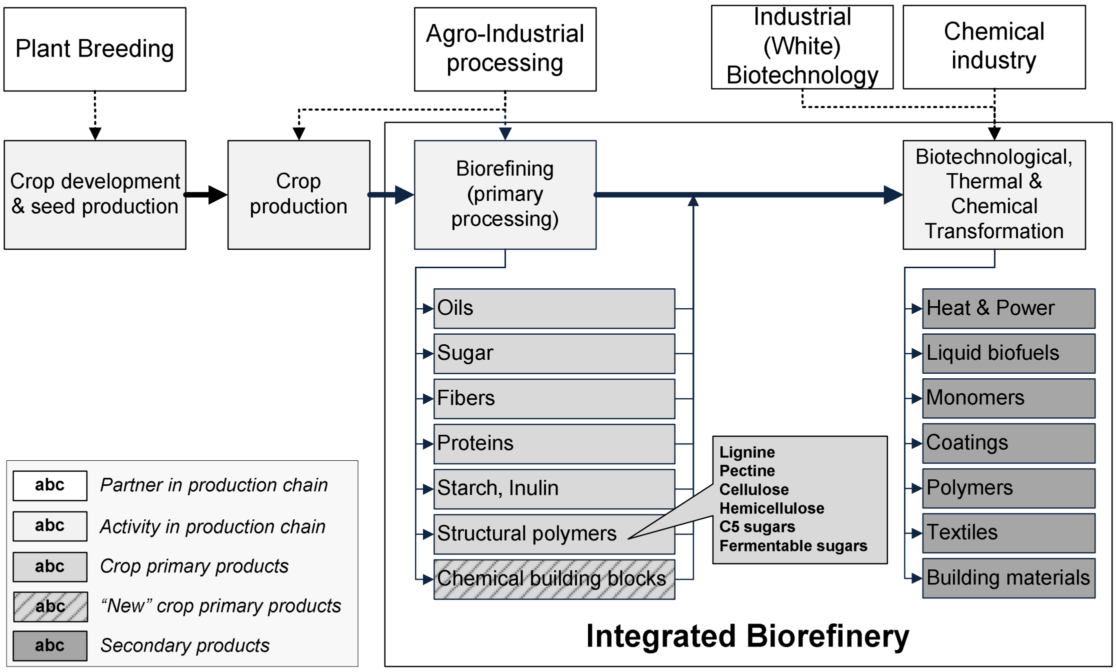
Telephone interviews other stakeholders
Aad van Elsen (Plantum NL), 23 July 2009
Johan Sanders (WUR), 27 July 2009
Peter Nossin (DPI), 29 July 2009
Mariët vd Werf (TNO), 3 August 2009
Felix Luitwieler (Min. VROM), 5 August 2009
Jan Wisse (Niaba), 5 August 2009
Fons Voragen (Carbohydrate Competence Center), 17 August 2009
André Faaij (Copernicus Instituut), 19 August 2009
Herman den Uil (ECN), 26 August 2009
Rob van Haren (Kiemkracht), 8 September 2009
Luuk van der Wielen (B-Basic), 28 September 2009

Failed interviews
The following organisations have been approached for an interview but were not prepared or not able to cooperate: Cargill, Dow Chemical, Stichting Natuur and Milieu, Shell.

Annex 2. Procedure interviews

The conversations were shaped as semi-structured interviews. When the appointment for the interview was made, a background document about biobased economy was sent to enable preparation on the subject. This document contained an outline of all possible biobased chains as inspiration.

Figure 2. Outline of possible biobased chains. Presented are the chain parties involved, the role of these parties in the chain, existing and new raw materials (primary products), existing and new biobased products (secondary products). In the Netherlands agroprocessing and chemical processing are still separate activities but these may in the future be integrated into Integrated Biorefinery.



An interview protocol - with a checklist of questions and subjects to be discussed - was used during the actual interview. The questionnaires were suited to the type of organisation (breeding, agroprocessing, industrial biotechnology, chemistry or other stakeholders). The questionnaire used for the telephone interviews with the chemical companies is presented below as an example:

1. What is the view of your company on the biobased economy? How would you define biobased economy?
2. Do you share the ambition of the Steering Committee Chemistry to halve the use of fossil raw materials in 25 years? If not, why not? If so, which contribution do you think your company can make to achieve this?
3. Which challenges and chances in the field of biobased do you see for the short term (appr. 1-3 years), for the medium term (appr. 3-10 years), and for the long term (10-25 years)?
4. There are different routes for the recovery of raw materials for the biobased economy from plants, ranging from direct production of high-grade substances in plants recovered from the plant via refining to the recovery of high concentrations of platform chemicals that are subsequently via chemical processing steps converted into the desired substances.
5. In which production route(s) are you most interested (in view of your type of product or your production facilities)?
6. Which specific role do you see for plant breeding?
7. Which role do you see for GMOs?

8. Are you interested in setting up new collaboration projects with parties from the agricultural sector (primary production, trade) aimed at the supply of "biobased" raw materials and in setting up production chains?
9. Do you have contacts with plant breeding companies about your needs in the field of raw materials for the biobased economy? Are you interested in collaboration projects with breeding companies in this field?
10. Are you collaborating with universities or other knowledge institutions around this subject? If so, can you tell a bit more? Are you interested in (more) collaboration projects with knowledge institutions?

Annex 3. Report workshop

Report of the workshop 'Plant breeding in the biobased economy', Tuesday 22 September 2009, Tollens Fabriek, Rotterdam.

Aim of the workshop

Key issue in the workshop was identification of promising chains for 'biobased' raw materials for chemistry and which new plant requirements would be arising from such chains. The NL government as well as the chemical industry have expressed an ambition to increasingly obtain raw materials for chemistry from renewable raw materials. Meeting this ambition requires the establishment of new production chains in which plant breeders are developing crops for new, industrial purposes and the chemical industry is obtaining its raw materials from agricultural products. In the weeks prior to the workshop about 30 interviews had been held with companies and other stakeholders in the biobased economy.

Promising chains

The workshop was opened by Roel Bol, programme director biobased economy at the Ministry of Agriculture (LNV, now EA&I, Economic Affairs, Agriculture & Innovation). He stressed the significance and urgency of a transition to an economy with a stronger basis in what nature has to offer.

Andries Koops (WUR) then outlined seven promising chains and presented four of these in more detail. With "promising" he meant that they are contributing to the CO_2 targets of the government as well as those of the Netherlands economy. The Netherlands acreage is not large enough to meet the CO_2 targets. A large part of the 'biobased' crops will therefore have to be grown outside the Netherlands. Through intellectual property on plant varieties and on biorefining technology these cultivations can still contribute to the Netherlands economy, regardless of their location. The four chains were those of potato as producer of modified starch, protein, and high-grade substances for pharmaceutical applications; sugar beet as supplier of protein and platform chemicals; *Miscanthus* for, e.g., energy production; and oil crops such as *Calendula* and *Crambe* for the production of oils for industrial applications. After this introduction the participants split into two groups for further discussions about the possibilities for and restrictions on the biobased economy on the basis of two business concepts from an agroprocessing perspective.

Opening van the workshop door Roel Bol

Case 1: Starch potato of AVEBE as supplier of raw material

In one group Peter Bruinenberg (AVEBE) held a short introduction about the raw materials that can be obtained from starch potatoes. The discussion that followed showed a lot of support for the proposition that it is practical to start with existing chains, such as those of starch potatoes or sugar beet, but the development of new chains was considered important as well. It was also mentioned that plants themselves suffer little from (new) polymers which means that accumulation in the crop is quite well possible. This requires a link with the knowledge and needs of the chemical industry in the Netherlands, Belgium and North Rhine-Westphalia. There was also a plea to especially use valuable molecules nature already has on offer, such as fibres.

Case 2: 'Unbeatable beet' of Royal Cosun
In the other group Ad de Laat (Royal Cosun) held a talk about the 'unbeatable beet'. He showed that the sugar beet, as regards energy efficiency as well as applications, offers a good basis for the formation of biobased chains. The sugar beet can in good years and with good crop management yield more than 110 t roots/ha with a dry matter yield of more than 33 t/ha (4.5 t dry matter of leaf mass not included). He also sketched possible innovations in breeding and refinery, including the 'winter beet' with a longer field period and 30% more yield; a beet – besides sugar - accumulating new valuable constituents/chemicals in the root; protein recovery from foliage; recovery of C5 and C6 sugars from the pulp followed by conversion into high-grade chemicals. Beet contain per hectare 40-50 m^3 water; this makes then extremely suitable as production platform for water-soluble chemicals.
One of the remarks during the discussion that followed was that the biobased economy could also be approached from the question whether certain substances could possibly simpler or cheaper be recovered from plants than from petrochemicals.


Discusions during the break

Discussion
Three subjects were discussed in both parallel sessions. A remark in both sessions about the 'food versus fuel' debate was that farmers would precisely benefit from the fact that a crop would have several markets. A strong separation between food and non-food would be a disadvantage in making cultivation of a crop attractive and profitable for a farmer. This is why a company like D1Oils is now working on making a typical biobased crop as Jatropha suitable as raw material for animal feed. GMOs were found to be a hot issue in both groups: a majority of the participants saw a role by GMOs in a successful biobased economy and considers European regulations as a restriction on the development of the biobased economy. Research financing was the third subject in both groups. Society should make funds available for such an important transition in our economy. This, e.g., concerns knowledge of metabolic routes in plants. Such research would have to be carried out in a consortium such as a top institute, in which industry, knowledge institutions and governments are jointly working on answering strategic questions. The Netherlands can in such a collaboration deploy two strong sectors (chemistry and breeding) to utilise new chances. An important characteristic of the research projects in such a consortium is that all links in the chain are participating. This ensures that the projects are based on a realistic demand from the market.

Conclusions
After the parallel discussions, reported back to the plenary session by Ton den Nijs and Richard Visser (both WUR), Hans Dons (Bioseeds BV) made some critical comments. He wondered whether the presented promising chains were business cases or research proposals. Successful chains also require economic input and knowledge of the market. The chairman of the day, Ronald Hiel (Schuttelaar & Partners), dealt with the observations of Hans Dons by passing his questions on to the audience. According to the audience business cases can only be developed by bringing together all links in the chain, from breeder to chemistry, and to let them jointly develop a portfolio of technically and economically attainable and sustainable production chains. There is a role for the government, e.g. in the form of a stimulation fund, to stimulate parties to cooperate and invest in joint research.

Tables

Table 1. Overview of the volumes of raw materials (t dry matter) that can be supplied by the total of all business concepts. The figures are a very rough indication and are only meant to weigh the extra production volumes of the different raw materials against the volume of the different markets given in the bottom row: NL, European (EU) and world (W). The acreages mentioned in the third column correspond with the ambitions of the business cases (6.3), but may also be larger in size. The figures in the table are rounded to 0.1 million t/year (Mt/yr)

Crop	Bio-mass	Total area	Total biomass	Sugar	Cellulose (ferment. sugar)	Inulin	Hemi-cellulose	Lignin	Pectin	Starch	Protein	Oil for energy	Oil for chem.	H_2O-sol. Chem's	Rubber
	(t DS ha/yr)	(1000 ha)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)	(Mt/yr)
Potato	13	500	6.5		0.3		0.2		0.1	5.0	0.4			0.4	
Beet	22	500	11	5.5	3		0.9		0.2		0.4			0.6	
Miscanthus	25	1000	25		10		8.0	5			1.2				
Oil crops	10	100	1		0.3		0.2	0.2			0.2		0.1		
Grass (biorefinery)	16	100	1.6		0.5	0.4	0.3	0.1			0.3				
Rubber crops	11-15	100	1.1			0.7	0.2								0.2
Maize	16	500	8	0.2	3		2	2			0.5			0.5	
Microalgae[31]	18[32]	100	1.8		0.4		0.2				0.5	0.4	0.4		
Sum		2900	56	5.7	17.4	1.1	11.8	7.3	0.3	5.0	3.9	0.4	0.5	1.5	0.2
Size existing market				∞[33] (bioethanol; W) 80 (polyethylene; W)		1 (furans in EU) 18 (ethyleneglycol; W) ∞[33] (bioethanol; W)			0.045 (W)	2.5 (W)	1.4 (NL) 17 (EU)	6.3 (diesel in NL)	260 (polymers; W)		1.5 (EU)

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[31] Only weighing the business concept Microalgae production at sea

[32] This area concerns (semi)open or seminatural systems. Biomass production of such a production system is estimated at 18 t/ha corresponding with the estimated biomass production of river estuaries (http://en.wikipedia.org/wiki/Trophic_dynamics).

[33] Very large market

Table 2. Chance of successful implementation of the business concepts of this research vision, measured against a number of criteria.

Crop	Total area (1000 ha)	Total net CO_2 mitigation potential (Mt/yr)	Potential gross added value (AV) (€/ha)			Chance of involvement chain parties				Main breeding challenges	Necessary approach regarding No 1 and 2 challenges	
			Existing chain	New chain	Extra AV	Breeding	AgroProc.	Ind. Biotech[34].	Chemiistry		GM	Non-GM
Potato	500	10	2780 (starch)	4780	2000	10-50%	10-50%	<10%	<10%	1. New starches, 2. Improved extractability 3. Higher protein content 4. Platform chemicals	x x x x	x x x x
Beet	500	15	3420 (sugar)	5230	1800	10-50%	>50%	<10%	10-50%	1. Platform chemicals, 2. Protein content	x x	x x
Miscanthus	1000	41	-	1800		10-50%	<10%	10-50%[34]	<10%	1. Seed reproduction 2. Increase fermentable sugar content 3. Optimise cell wall composition	 x x	x x x
Oil crops	100	1.4	-	4450		>50%	>50%	<10%	>50%	1. Oil composition and content . 2. Wax esters	x[35]	x
Grass	100	1.2	4000 (milk)	6200	2200	10-20%	>50%	<10%	<10%	1. Higher production at new cutting regime 2. Higher protein content 3. Better fibres		x x x
Rubber crops	100	1.8	-	3300-4200		10-50%	10-50%	<10%	10-50%	1. Increased biomass yield 2. Increased latex/rubber content 3. Better breeding methods		x x x
Maize	500	13.5	2500 (animal feed)	4500	2000	10-50%	10-50%	10-50%	10-50%	1. Increase fermentable sugar content 2. Optimise cell wall composition 3. Higher protein content 4. New starches	x x x x	x x x x
Microalgae	100	3.2	-	4700		<10%	<10%	<10%	<10%	1. Basic knowledge genomics and methods for algal biotechnology 2. Cultivation at sea and other open water surfaces	x[36]	 x

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[34] Industrial biotechnology as sector is especially envisaged around enzymatic digestion of cell walls of *Miscanthus*. The other cases do not need Industrial Biotechnology as condition for success.

[35] Only for *Crambe*

[36] Only for business concept 6, in case the concept evolves into closed production on industrial biotechnology scale

Table 3. Overview of platform chemicals (PC) that can be used as building block for chemical syntheses. Column 1 refers to molecules that could be derived from the raw plant materials presented in column 3, using the processing method indicated in column 4. Examples of platform chemical uses such as in polymers, solvents or chemical intermediates are described in column 5. Some platform chemicals are already produced on commercial scale, or a production facility is in the process of being started up (columns 7, 8).

1 Platform chemicals (PC)	**2 Recommended as "top 10" by:**	**3 Raw plant material for PC**	**4 Production method of PC**	**5 Examples of main use for PC**	**6 Industry parties involved in R&D**	**7 Industry parties involved in (near) commercial production**[37]	**8 Production volume 2009 (x 1000 ton)**
Alcohols							
Ethanol	TNO[38] WUR[29,30]	Sugar, starch, lignocellulose	Fermentation (from sugars)	Fuel	Many		35000
Ethanol			Chemical (from ethanol)	(Bio)ethylene and (Bio)polyethylene (plastics)		Braskem, several others	
Ethanol							
Butanol	DSM[39]	Sugar	Fermentation	Fuel, solvent, commodity chemical		BP + Dupont	
1,4-Butanediol	TNO	Sugar or succinic acid	Fermentation or chemical		Genomatics		
1,3-Propanediol		Sugar or glycerol	Fermentation	Co-monomer in polyesters		Dupont + Tate & Lyle	
1,2-Propanediol (propylene glycol)	See glycerol	Sugar of glycerol	Fermentation	Resins, lubricant, solvent de-icing			
3-hydroxybutyrolactone	NREL[40]	Sugar	Chemical	Tetrahydrofurans, new polymers			
Sugars or sugar-based							
Sorbitol	TNO, NREL	Glucose; natural product	Chemical; refining	- Isosorbid → Ethylene glycol - Propylene glycol → Polyesters,			
C5 sugars e.g. xylose, arabinose		Hemicellulose,	Refining and hydrolysis	Furfural/Tetrahydrofuran			
C5 sugars via xylitol, arabinitol	NREL	Hemicellulose	Refining/ hydrolysis; chemical	Ethylene glycol, propylene glycol, Lactic acid → Polyesters			
C6 sugars (fructose, glucose)		Starch, sugar, (ligno)cellulose		Hydroxymethylfurfural	Avantium		
Sugar Polymers							
Modified and thermoplastic starches	TNO	Starch	Enzymatic, thermal, chemical	Biodegradable polymers, binders, coatings, packaging		Novamont Plantic Technologys Ltd, Cereplast	
Carboxylic acids							
Citric acid	DSM	Sugar	Fermentation			DSM and several	1000 (mainly

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[37] Building of factory announced in last years (but later postponed because of financial risks)

[38] Groenestijn (2008), Biobased Economy – exploring the opportunities for the Netherlands. TNO report for LNV: http://www.innotact.nl/fileadmin/user_upload/Publicaties2008/Bijlage_1.pdf

[39] Personal comm. Peter Nossin, DSM

[40] Werpy, T and G. Petersen (2004). Top Value Added Chemicals from Biomass Volume I – Results of Screening for Potential Candidates from Sugars and Synthesis Gas National Renewable Energy Laboratory, Available: www.eere.energy.gov .

						others	food)
Itaconic acid	DSM, NREL, TNO, UCIP[41]	Sugar	Fermentation	Metacrylic acid Polymers, superabsorbants	Itaconix	Several small Chinese suppliers	~10
(Poly)lactic acid (2-Hydroxy-propionic acid)		Sugar (to lactic acid)	Fermentation, chemical	Biodegradable plastics		Cargill/NatureWorks-LCC; several others	140 (Cargill)
Glucaric acid	NREL			Monomer in nylons (replace adipic acid)			
3-Hydroxy-propionic acid	NREL	Sugar	Fermentation followed by chemical	Acrylic acid and AA based polymers (plastics, fibres, coatings, superabsorbants	Cargill + Novozymes		
Levulinic acid	DSM, NREL	Sugar (C5 and C6)	Chemical				
2,5 Furandicarboxylic acid	NREL	Sugar	Chemical	Monomer in polyesters (replace terephthalic acid)			
1,4 succinic acid (or fumaric or malic acid)	DSM, NREL, TNO, UCIP	Sugar	Fermentation	Several chemicals, e.g. de-icers and new polymers, including polyesters polyamides		DSM/Roquette, Bio amber, Myriant, CSM and BASF	~ 10
Vegetable oil based							
Unsaturated fatty acids		Soy or any other oils	Refining & hydrolysis	(bio)olefins, polypropylene and polyethylene		Cargill	
Unsaturated fatty acids		Soy or any other oils	Refining & hydrolysis	Polyurethane foams		Cargill	
Hydroxy fatty acids (ricinoleic acid)		Castor oil	Refining & hydrolysis	Sebacic acid → polyamides		Arkema, BASF, Dupont	
Glycerol	DSM, NREL, WUR	All vegetable oils	Refining & hydrolysis	Epichlorhydrin → epoxy resins		Solvay, ADM, DOW	
Glycerol		All vegetable oils	Refining & hydrolysis	Methanol		BioMethanol Chemie The Netherlands	
Glycerol		All vegetable oils	Refining & hydrolysis	Propylene glycol (1,2-propane diol) → Resins, lubricants, paints, detergents, antifreeze		ADM. DOW, Huntsman, Ashland + Cargill	65
Calendic acid		Calendula	Refining	Reactive solvent in paints/coatings		Uniqema, AKZO, DSM	1-2
Erucic acid		Crambe or rape seed oils	Refining	Erucamide		Several	100
Amino acids derived							
Arginine	WUR	Proteins; isolated compound; sugar	Refining & hydrolysis; fermentation;	Butanediol + Urea			
Aspartic acid	NREL, WUR	Proteins; isolated compound; sugar	Refining & hydrolysis; fermentation	Various amino-diacids, polyaspartartes (substitute polyacrylic and polycarboxylates)			
Glutamic acid	NREL, WUR	Proteins; isolated compound; sugar	Refining & hydrolysis;	Polyglutamic acid, 5-amino-1-butanol			1000 (mainly food)

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[41] Undisclosed Chemical Industry Party

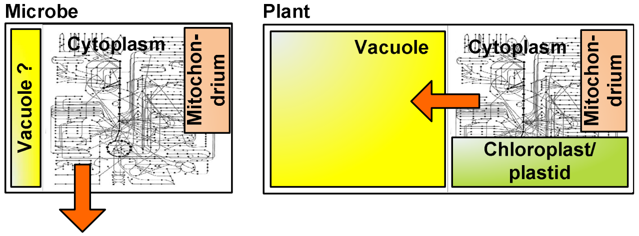
			fermentation				
Serine	WUR	Proteins; isolated compound; sugar	Refining & hydrolysis; fermentation	Ethanolamine			
Proline	WUR	Proteins; isolated compound; sugar	Refining & hydrolysis; fermentation	Pyrrolydine			
Lysine	DSM, WUR	Proteins; isolated compound; sugar	Refining & hydrolysis; fermentation	Caprolactam → nylon 6			700 (mainly feed)
Cyanophycin	UCIP	Sugar	Fermentation				
Terpenes and hydrocarbons							
Rubber	Strategic importance	Plant natural product	Refining, chemical	Tyres, polymers		Goodyear, Pirelli, Michelin, etc	Million
Monoterpenes		Plant natural product	Refining, chemical	Pharmaceutical stereoisomers		Many parties (in relatively small volumes)	10
Isoprene		Glucose, Plant natural product	Fermentation, chemical	Synthetic rubbers	Genencor + Goodyear		?
Tri- and tetraterpenoids		Algal natural product	Refining and polmerisation	New bioplastics			
Polyhydroxyalkanoates	CIP	Glucose	Fermentation	Biodegradable polymers		ADM + Metabolix	50
Phenolics							
Phenol, aromatics		Lignocellulose pulp from wood, grasses, refined crops Lignin	Chemical, thermal, enzymatic	Several aromatic compounds (e.g. phenol)		Borregaard	?

Table 4. Platform chemicals that can directly be produced by crop plants or algae.

Platform chemicals	**Natural compound in plants?**	**Production possible in plants?**	**Possible Dutch Industry parties**
Carboxylic acids			
Citric acid	Present in all plants (potato, sugar beet) sometimes in high concentrations (citrus, acid fruits). Possibly already feasible to isolate from potato, particularly if concentration could be increased. Can possibly be realised by TILLING (breeding technology)	Increase citric acid concentration proven in transgenic plants, though not to very high level	AVEBE, DSM
Itaconic acid	Not a natural compound in plants	Can very well be synthesised in plants; proven in potato so far without adverse effects Suitable production platform crops are water-rich storage organs, e.g. potato, cassava or sweet potato tubers, stem of sugar cane, tap roots of sugar beet	Several partners across total production chain, based in NL, Germany and USA
3-Hydroxy-propionic acid	Not a plant compound, but can probably be synthesised in plants	Not tried	
Lactic acid	Traces	Overproduction not tried	Purac, CSM
Succinic acid (or fumaric or malic acid)	Present in all plants in relatively low concentrations	Overproduction not tried	DSM
Vegetable oil based			
Calendic acid	Present in Calendula seed oil in high concentrations		Uniqema, AKZO, DSM
Erucic acid	Present in Crambe seed oil in high concentrations		
Amino acids derived			
Arginine	Most abundant amino acid in plant proteins		
Aspartic acid			
Glutamic acid	Abundant amino acids in plant proteins, especially in gluten		
Lysine	Generally low levels. Increase in lysine level can be realised by TILLING (breeding technology)	Overproduction proven in transgenic maize, potato (WUR), soy, tobacco. IP with AVEBE and WUR	Partners in Breeding and Agroprocessing (so far not in chemistry)
Proline	Very high concentration in pollen, and under stress conditions in plants	Overproduction proven in transgenic plants. Proline may provide protection against abiotic stresses, especially drought	
Serine	Low levels		
Cyanophycine (aspartic acid backbone + arginine side chains)	No	Overproduction to up to 6% of DW proven in potato, tobacco.	
Terpenes			
Rubber	Plant natural product, rubber tree is the only source. Two alternative sources next to rubber tree are under investigation: Guayule and Russian Dandelion (FW 7 project EU Pearls, coordinator WUR).	Major challenges for EU-based rubber production are domestication of Dandelion and Guayule, and increase in rubber yield (t/ha)	Keygene, Vredestein
Monoterpenes	Natural product in many plant species, large variation in structures	Market volume of most abundantly used monoterpene (limonene from citrus fruit) is 10 000 t	

Isoprene	Plant natural product, emitted	Volatile compound, Overproduction probably possible in oilseed crops, not in other crops	Genencor
Polyhydroxyalkanoates	No	Overproduction, up to 4% of DW, proven in plants	
Carbohydrate polymers			
Modified starches	Starch is present in all plants and some plants are able to store it in a granular form ex. Potato, cereals, cassava, etc	Yes, has been done by AVEBE, BASF and at Wageningen UR.	AVEBE, COSUN, BASF
Improved Pectins	Pectins are polysaccharides present in the primary cell walls of all plants.	Yes, by Wageningen UR and already used in a pilot experiment to coat medical devices.	HZPC, AVIKO, Herbestein & Fox,

Table 5. Research and breeding challenges for the production of platform chemicals in plants and the most suitable crop for each platform chemical.

Molecule or group	**Challenges**	**Crop**
Organic acids	Organic acids (OA) are produced in various compartments of the plant cell (mitochondrion) and are stored (the vacuole), or are then distributing themselves according to physical/chemical laws over the different cell compartments (vacuole, cytoplasm, mitochondrion and cytoplasm). The challenge is to store substances that can disrupt those cytoplasmic processes, and the OG are certainly among those, in the vacuole (red arrow). A microorganism dissolves this to excrete substances. .  The vacuole is the largest compartment in most plant cells (up to 90% of the cell volume) and thus extremely suitable for storage of valuable constituents. The metabolic processes that are realising transport from one compartment to the other (red arrow) are crucial in the realisation of this storage. The secret behind storage of OA by plants up to economically attractive levels lies in the understanding and smart application of these processes. This process is reasonably understood for sucrose (beet sugar), much less so for organic acids. Investment in this point in fundamental research and application of this knowledge in molecular breeding is important. This is especially true for crops 1 and 2 in the adjacent column. For one of the OA, itaconic acid, this route has already led to a transgenic potato that accumulates itaconic acid up to 2% of tuber dry weight. The next target crop is sugar beet. Classical breeding is in particular recommended for increasing OA in grass (3).	"Wet" crops with a large vacuole volume. Examples: tubers and roots. Leaf cells also have a large vacuole volume but the strong day/night rhythm of leaves could make good control over the storage of OG in leaves difficult. Tubers and roots are a more likely end station in storage. Ranking, based on the arguments above, is as follows: 1. Sugar beet root (classical breeding and GM) 2. Potato tuber (classical breeding and GM) 3. Grass (classical breeding and TILLING) 4. Maize (classical breeding and GM)
Amino acids	Almost the same considerations as for OA. The research task particularly lies in the application of existing fundamental knowledge in molecular breeding. This is especially true for crops 1, 2 and 4 in the adjacent column. WUR PRI is currently attempting to grasp the mechanism behind storage in the vacuole of lysine (potato). Next step is the application of acquired knowledge in sugar or fodder beet. Classical breeding is in particular recommended for grass (3).	"Wet" crops with a large vacuole volume. Examples: tubers and roots. Leaf cells also have a large vacuole volume but the strong day/night rhythm of leaves could make good control over the storage of OG in leaves difficult. Tubers and roots are a more likely end station in storage. Ranking, based on the arguments above, is as follows: 1. Sugar beet root (classical breeding and GM) 2. Potato tuber (classical breeding and GM) 3. Grass (classical breeding) 4. Maize (classical breeding and GM)
Amino acids/ proteins	Proteins are built from 20 amino acids. This means that proteins can also be a source of amino acids, certainly if some amino acids are overrepresented such as glutamine acid in wheat gluten. The expected growth in the worldwide demand for proteins also makes proteins an important ingredient for food and feed. It is important that various sources are formed alongside each other because there is a particular need for a range of proteins with different functionalities (fibre proteins, taste proteins, fat-binding, water-	Best short-term crop choice: 1. Potato tuber (classical breeding) 2. Beet foliage (classical breeding) In the longer term. The existence of various sources alongside each

	binding, proteins with specific amino acid composition). This also needs a range of crops, seed, leaf, and tuber/root crops. Leaf protein will usually more or less have the same functionality, regardless whether it originates from beet or grass. Larger functional differences are to be expected for proteins in seeds and tubers/roots The research challenge for beet and potato particularly lies in understanding the mechanism behind protein accumulation in tuber and root, and the subsequent application of existing fundamental knowledge in molecular breeding of potato and beet and legumes. The leaf crops beet, grass, *Miscanthus*, maize (in view of its yield of 4.5 t leaf DM/ha beet foliage is included as leaf crop) have sufficient genetic variation to realise a doubling of the protein yield by means of classical breeding.	other is important, including: 1. Grass (classical breeding) 2. Fodder beet root (classical breeding and GM) 3. Seeds of one or several of the legumes, including pea, lupine or field bean (classical breeding and GM) 4. Leaves/stems of Miscanthus or maize (classical breeding and GM) 5. Algae (classical breeding)
Hydrocarbons	Three varieties of the alga *Botryocoocus braunni* are producing or alkenes, terpenes of carotenes. These hydrocarbons are polymerising into a sort of bioplastic In the cell wall of this alga. This development route aims at setting up industrial production of carbohydrate monomers with the objective to make a new generation of biopolymers. Necessary knowledge activities for setting up a *B. braunii* based production platform are: 1) Genome sequencing of the three varieties; 2) Unravelling the synthesis of the monomers and the polymers in the three varieties and identification of the corresponding genes; 3) Setting up a transformation system for this alga; 4) Development of breeding tools. This research is of a fundamental nature.	Best choice: 1. The alga *Botryococcus braunii*
Polymers (Cyanophycin and polyhydroxyalkanoates)	A reasonable amount of knowledge is present in the international research arena for the production and accumulation of these molecules. This is mainly a matter of applying this knowledge (the right genes and vectors for genetic transformation) in a plant of choice, or making GM plants that accumulate these substances. The nature of the synthesis means that plants/parts of plants that are rich in plastids/chloroplasts are most suitable because this compartment gives the highest yield. Second criterion is that preferably refinery crops should be involved because extraction and purification of the mentioned substances requires high-grade technology.	Best crop choice in order of chance of success: 1. Potato tuber (GM) 2. Beet foliage (GM) 3. Energy grasses such as maize and *Miscanthus* (GM)
Pectins	Pectins are part of the cell wall and end up in the pulp fraction that remains after extraction of starch, oil, protein, sugar, chemicals, etc. The side chains of the different pectins (Homogalacturonan, rhamnogalacturonan) are determining the (use) properties of pectins. Modification of these side chains makes it possible to make tailor-made pectins with desired properties.	Best crop choice in order of chance of success: 1. Potato tuber (classical breeding and GM) 2. Beet root (classical breeding and GM)
Starch (and modified starches	WUR has a lot of knowledge about modified starches . The natural location for starch synthesis and storage is the amyloplast, which makes potato the most suitable crop for the EU and cassava for the tropics.	Best crop choice: 1. Potato tuber (classical breeding and GM) 2. Maize (classical breeding and GM)
Lignin/cellulose/ hemicellulose	The three components are found in all crops as part of cell walls. The genetic variation in the composition of cell walls in plants, including the interrelation between the three main components lignin, cellulose and hemicellulose is considerable. This offers possibilities for optimisation via breeding. Breeding should aim at steering cell wall synthesis to desired lignin and hemicellulose variants and at the selection of genotypes with easily decomposable cell walls. Coordinated R&D is required in the field of breeding, industrial biotechnology (enzymes for cell wall digestion) and chemical synthesis to arrive at sustainable processes for conversion of cellulose, lignin and hemicellulose in platform chemicals and energy carriers.	The three components are found in all crops. There is not so much a demand for more as for a better composition (e.g. other hemicellulose) and better digestion into individual components. The best crop choice for better digestion of hemicellulose and transformation into furan-based chemicals is: 1. Miscanthus (classical breeding and GM) 2. Potato (classical breeding and GM) 3. Sugar beet (classical breeding and GM). 4. Maize (classical breeding and GM) Best crop choice for the digestion of biomass into cellulose, hemicellulose and lignin are the perennial grasses, in view of the low cultivation costs and the high biomass yield. Best choice in EU context is: 1. *Miscanthus* (classical breeding)

		2. Maize (classical breeding and GM)
Rubber	Rubber is a natural component of the rubber tree. High-quality rubber is also found in two other crops, which have the advantage that they can be cultivated in Europe: Guayule and Russian dandelion. The biggest challenge lies in increasing total biomass (root) yield and rubber content. Current yield of existing commercial root crops such as sugar beet and chicory illustrate the great advances that have been made in root yield as well as the potential for non-domesticated crops. Retrieval of the breeding history of beet and chicory, e.g., by genome sequencing of historic germplasm, could identify the genes that have been essential for root development and can trace heritability. identification of related genes in, e.g., Russian dandelion and their location on the genome of this plant allows more specific and faster breeding to increase the root yield of this as yet non-domesticated crop. Rubber content is mainly associated with the extent to which specialised latex- producing cells come to development. Increasing the frequency of these cells demands fundamental knowledge about the mechanism behind the transition of meristem (stem) cells to latex producing cells.	Best crop choice for EU 1. Guayule and Russian dandelion (both classical breeding and GM)