Project performance report template pathfinder project "AzoFast"

Executive Summary (1-2 pages)

The final goal of AzoFast is to establish new business opportunities in the area of aquatic farming of *Azolla* species. AzoFast proposes a means to mitigate industrial environmental pressure by connecting major waste streams from the industry, including CO_2 and heat emissions, with aquatic-agricultural farming based on the fresh water fern *Azolla*. Underlying research should finally result in a business plan for the construction of 10 ha pilot plant at Eemshaven. In this pathfinder the following activities are undertaken or will be undertaken: optimizing productivity and biomass quality of Azolla, develop a method for the massive production of sporelings to reliably supply *Azolla* production sites, determine chemical composition of *Azolla* under various conditions and analyse the potential products from Azolla biomass, develop a biorefinery concept for *Azolla*, and finally deliver a business plan for an Azolla pilot plant. The project is now formally 4 months underway, and already some remarkable results have been obtained:

1. Under the environmental conditions used both *Azolla filiculoides* and *Azolla pinnata* show high rates of daily productivity. In time, biomass per ha just before and just after harvest remained constant. Under these conditions, productivity equals the amount harvested per unit of time. Even though we have not established maximum productivity in relation to environmental conditions yet, the average weekly amount harvested was on average approx. 840 kg ha^{-1} dry weight for plants grown at ambient CO_2. Experiments at 800 ppm CO_2 are still running and results indicate an extra stimulation of productivity between 10-20%. When data are extrapolated to glass house conditions and taking into account the integrated amount of light over the period April-October, we can expect yearly productivities varying between 25-35 ton dry weight per ha per year.
2. To optimize *Azolla* productivity, it is important that production sites rapidly reach standing crop sizes required for continued harvest. This is possible if production ponds were inoculated with sufficient synchronously grown sporelings that would cover the pond surface within a week growth. Final goal is to establish a well underpinned method to store and disseminate all *Azolla* species; an invention disclosure form describing the method has already been submitted to Utrecht Holdings B.V. As possibilities for a patent are presently being investigated, the highly exciting results cannot be published in this report.
3. Crude protein content varied between 25-30% of the dry matter, and the amino acid (AA) composition and concentration (g AA g $protein^{-1}$) are such that it fully fulfils the requirement of e.g. Tilapia, hens or pigs. Together with the results on productivity we believe that *Azolla* species may be of high interest for the feed and food industry (high protein yield of 6-7 ton per ha per year). This was confirmed by the interviews held with the feed industry: price per ton dry weight are attractive, and it was also indicated that prices for pure protein after extraction are even more attractive. Experiments are under way to optimize extraction procedures. We cannot give exact figures on the economics yet as the results of the feasibility study (Business Plan *Azolla*, see WP4) are still confidential. Analyses of functional proteins, which may have all kinds of applications in the industry, in *Azolla* biomass are now being performed and will be reported at the end of this pathfinder.
4. Protocols for lipid and polyphenol extraction have been developed. First results indicate relatively high lipid concentrations compared to land plants and varied between 8-11 % of

the dry weight. The *Azolla* lipids mainly consist of fatty acids. First results also show relatively high concentrations of polyphenolics (25-30%). Both the lipids and polyphenolics have a wide array of applications in the industry (food, pharmaceutical, cosmetics, renewable chemicals industry and biofuel industry). Demand by the industry and prospects of *Azolla* as a resource for industrial application will be further investigated.

5. First steps towards the construction of a Azolla biorefinery plant have been made. Preliminary experiments, mainly focussing on protein extraction, have been carried out on the extraction of Azolla with an IL in a microwave reactor, which were successful. First results show a high recovery percentage of proteins originally present. Coming months this will be tested further and expanded to lipids and if possible to polyphenolics (see WP2).
6. Approximately 40 companies were interviewed in autumn 2012 by Bioclear bv (type of companies were e.g. food and feed industry, aquatic farming industry, suppliers of waste heat, CO_2, and nutrients, but also scientific institutes, (non)-governmental organisations etc). Based on this information a first business model was constructed for a large pilot plant. We cannot report on this at the moment as the results are confidentially distributed among stakeholders.
7. In 2012 approx. 43% of the total 200 k€ for this pathfinder was spent on WP1-4. Together with extra cash and in kind contribution we believe that this pathfinder can become a great success, culminating in an Innovation proposal in 2013.

Expected Deliverable (2012)	Status	Comment
Deliverable 5.1.1 - Protocols for optimizing *Azolla* productivity and biomass quality	Harvest potential at two CO_2 concentrations collected; Synchronous in vitro germination of Azolla sporelings demonstrated	Harvest experiments at different temperatures are now under way
Deliverable 5.2.1 - Protocols for lipid and polyphenol extraction from *Azolla* biomass and conversions	Lipids quantified in different species and at different CO_2	Initial data on phenolics yields are impressive
Deliverable 5.3.1 Global *Azolla* Biorefinery concept	In development; first experiments for protein extraction are succesfull	
Deliverable 5.4.1 -Investment costs 10 ha aquatic farming system -Expected revenues -The impact on the environment by studying the control of the plants'	Azolla Pilot business plan established Azolla *in vitro* fertilization and cryopreservation established	Further refinement of Business Plan in 2013

reproduction		

Detailed report (approximately 5-10 pages)

WP1

In this work package, potential growth rate of *Azolla filiculoides* (Af) and *Azolla pinnata* (Ap) were determined using the 'concept of growth' analysis. As potential growth rate (productivity in kg dry weight per ha per day) does not necessarily mean that biomass can be harvested at similar rates, we performed several harvest experiments based both on results from the growth analysis and on theoretical considerations. Harvested biomass under several environmental conditions was used for protein and amino acid analysis (indication of feed/food value; this WP), lipid concentration and composition (indication of fuel value; WP2) and biorefinery analysis (indication of extraction possibilities/methods; WP3). To optimize *Azolla* productivity, it is important that production sites rapidly reach standing crop sizes required for continued harvest. This is possible if production ponds were inoculated with sufficient synchronously grown sporelings that would cover the pond surface within a week growth. Work is ongoing to develop a method for the massive production of sporelings to reliably supply *Azolla* production sites (see WP4).

Growth conditions

Plants were grown in a growth room with the following conditions: average photosynthetic flux density 325 µmol m^{-2} s^{-1} for a 16 h light period. Light was provided by fluorescent tubes and incandescent lamps. Day temperature was set at 23 ^{o}C and night temperature at 20 ^{o}C resulting in an average day-time water and air temperature (1 cm above the canopy) of 24.8 and 24.6 ^{o}C, respectively. Average night-time temperature of water and air were 23.3 and 21.1 ^{o}C, respectively. CO_2 concentration in the growth room was maintained at ambient (350-400ppm) or at 800 ppm. Plants were grown in 30 L containers (Fig. 1) with a surface area of 1505 cm^2 containing a non-limiting nutrient solution. The solution was replaced every week and pH was kept between 6-7 using H_2SO_4.

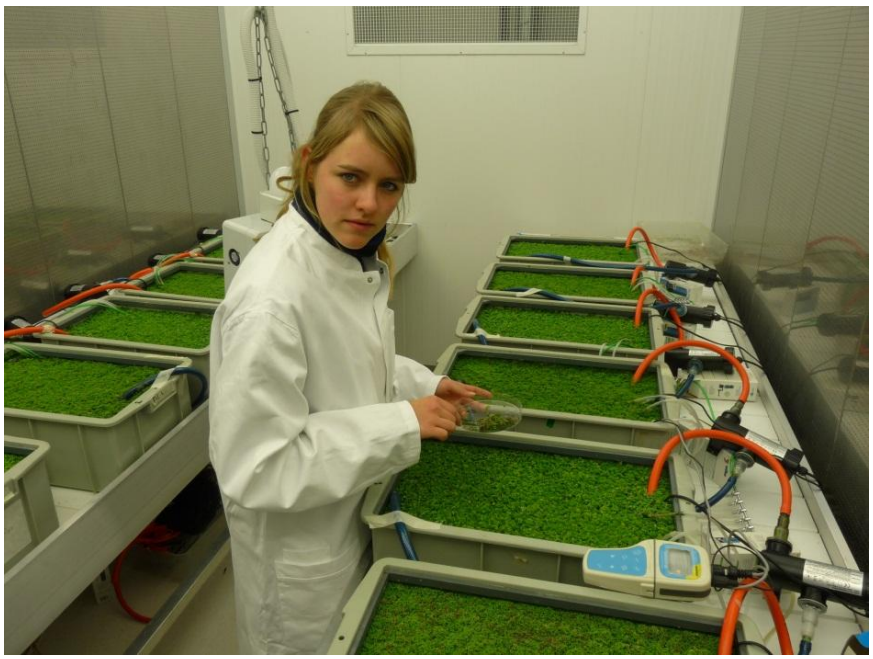


Fig. 1. Laboratory setup with the 30 L containers in which plants were grown

Growth analysis

For this experiment, each container was divided into 15 compartments using a plexiglass construction. In each compartment two plants were inserted. Every four and three days one compartment per species and per container (n=6) was destructively harvested. Leaf area was determined (Leaf Area Meter, LI-3000, Licor) and dry weight was determined on oven-dried plant material (30 ^{o}C or 65 ^{o}C). Relative growth rate (RGR, mg g^{-1} day^{-1}) was determined by linear regression as the slope of the natural logarithm of dry weight versus time. Absolute growth rate (AGR, kg ha^{-1} day^{-1}) in the linear growth phase was determined by the slope of standing crop (SC, kg ha^{-1}) versus time.

Harvest experiment

Plants were grown in the same 30 L containers (n=5/6) without the plexiglass construction as in the growth analysis experiment. Based on theoretical calculations and results from the growth analyses several harvest experiments under similar environmental conditions as described above were performed, i.e. harvesting different surface areas at different time intervals.

Protein and amino acid analysis

Crude protein was analysed using the Dumas method (according to the Official Methods of Analysis of Official Analytical Chemists) by a certified analysis lab, Silliker (Merieux Nutriscience). Plant material was ground in liquid nitrogen and freeze-dried, and used for nitrogen analysis according to the Dumas method. Analysis of amino acids was performed with Biochrom amino acid analyzers using classical ion-exchange liquid chromatography with post-column Ninhydrin derivatisation and photometric detection. Li-Citrate as well as Na-Citrate buffer system elution was used to cover the total Amino Acid Analysis yield. Free and Total (bound) amino acids were analyzed. Plant material was grinded using liquid nitrogen followed by freeze-drying. For total amino acids analysis, the following has been performed: 1) Acid hydrolysis for most of the amino acids, 2) Oxidation followed by acid hydrolysis for cyst(e)ine and methionine, 3) Alkaline hydrolysis for tryptophan. Tryptophan was quantified by reversed phase HPLC

Results

Growth analysis

Only minor differences in growth capacity in both the exponential and linear growth phase were observed between species (Fig. 2). Relative growth rates were 336 and 317 mg g^{-1} day^{-1} for *Af* and *Ap*, respectively. Linear regression of Standing Crop versus time revealed no differences in AGR between the two species (average 119 kg ha^{-1} day^{-1} for *Af* and *Ap*, respectively).

Harvest experiment

Based on theoretical calculations and on results of the growth analysis experiment biomass should be harvested between 500 and 2000 kg ha^{-1}. Furthermore the theoretical analysis reveals that harvested biomass (kg ha^{-1} $week^{-1}$) is independent on harvest interval or amount of surface area harvested (% of surface area; Fig 3. A-C). In the case of a daily productivity of 119 kg per ha, a weekly harvest of approx. 830 kg per ha could be expected. These expectations were confirmed in several harvest experiments (Fig. 4). For example in the ambient CO_2 harvest experiment, each day approx. 14% of the total surface area was harvested. Average weekly harvested biomass for both species was

approx. 840 kg ha^{-1}. First results of the experiments at 800 ppm indicate a stimulation of productivity of approximately 20% (data not shown).

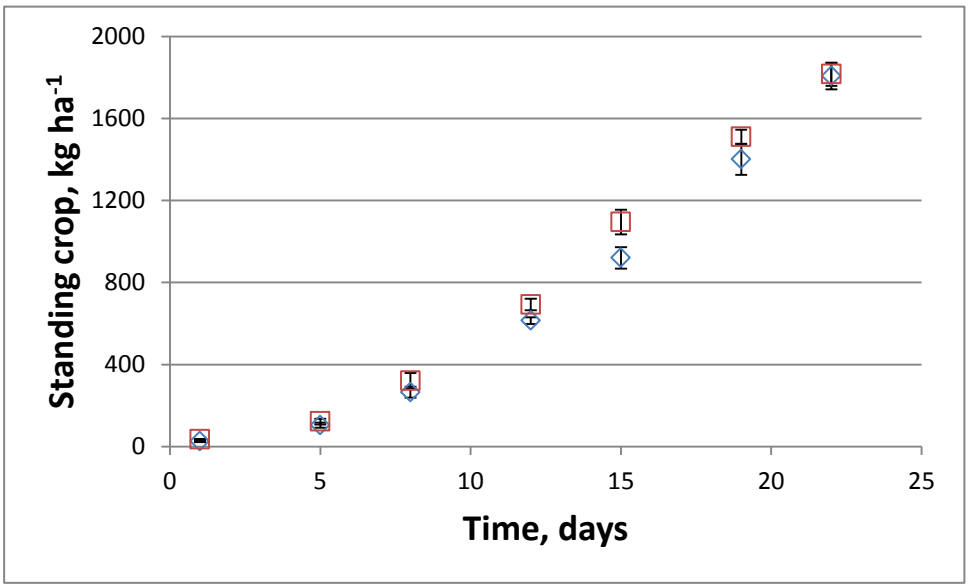
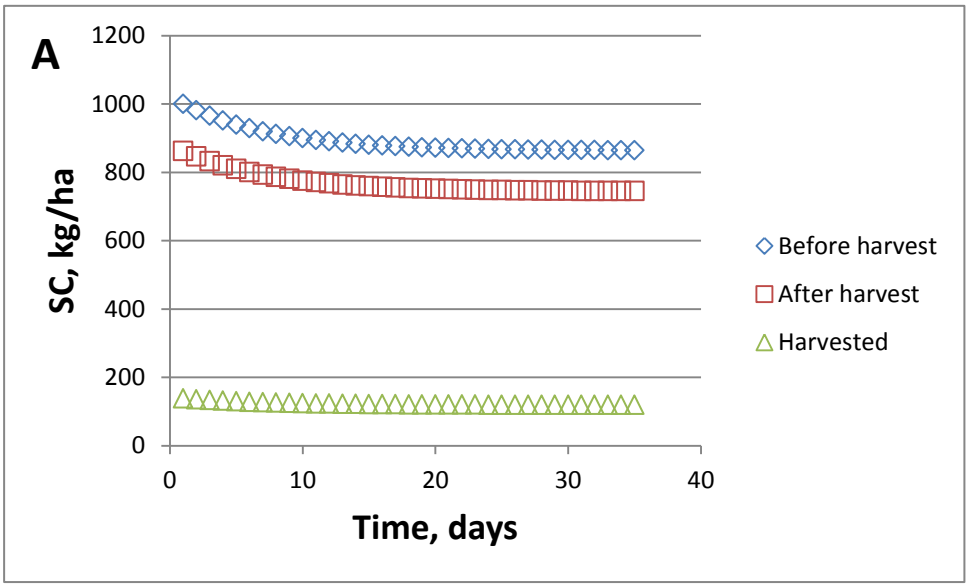
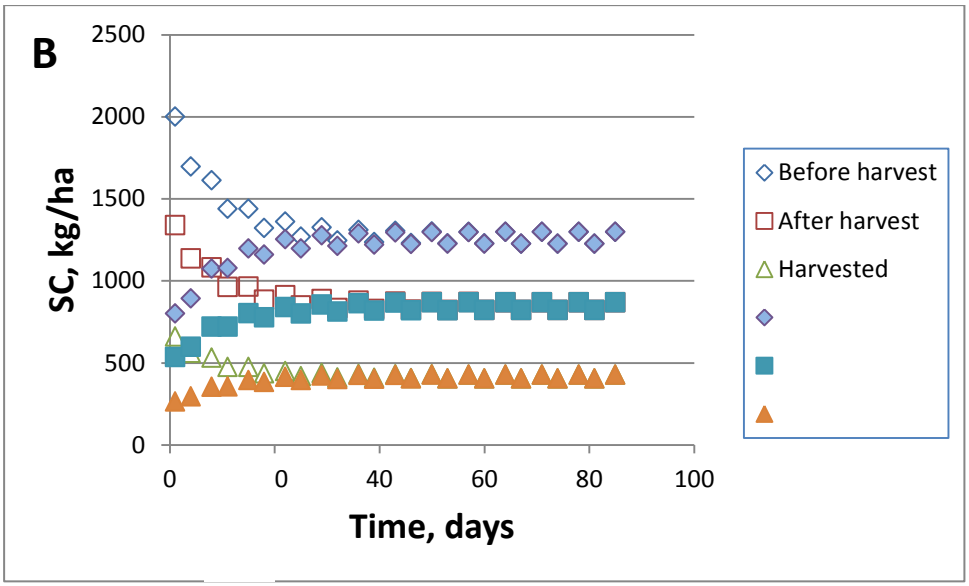


Fig. 2. Growth analysis of Azolla: biomass increase with time for *Azolla filiculoides and Azolla pinnata*

Results obtained until now clearly indicate that *Azolla* species have a high growth potential and that the harvested amount of biomass remains constant with time. When integrated over time, the light intensity used in the growth chamber equals that over the period April-October in glass houses in the Netherlands. All other environmental variables being equal, this would suggest a potential productivity of approx. 25 tons dry weight at ambient CO_2.





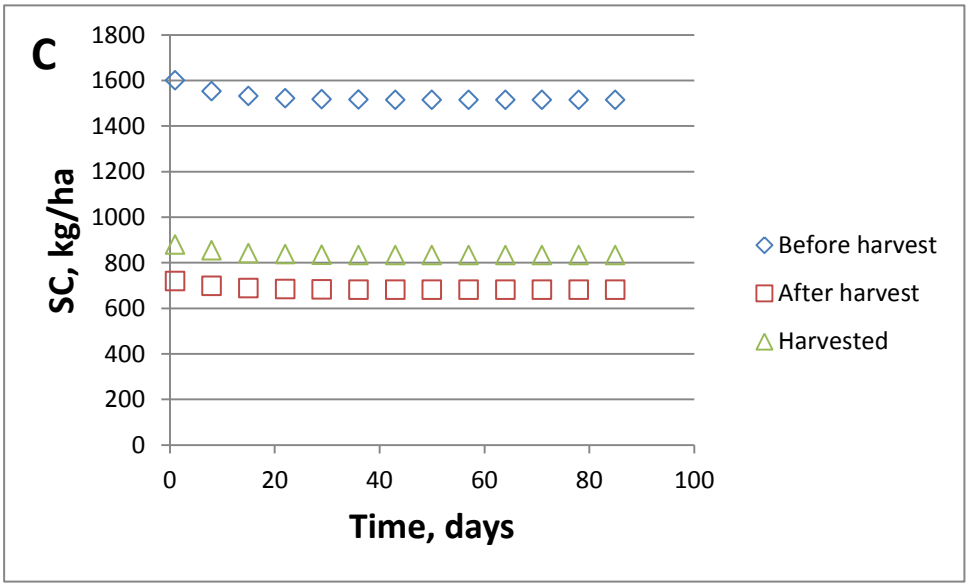


Fig. 3. A) Theoretical analysis of Standing Crop before and after harvest at several start densities and harvest intervals. Standing crop (SC) at t=1 1000 kg ha^{-1} and 14% of total surface area harvested each day, B) SC at t=1 either 2000 or 800 kg ha^{-1} and 33% harvested each 3 and 4 days, C) SC at t=1 1600 kg ha^{-1} and 55% of total surface area harvested once a week. In cases A-C a daily growth rate of 119 kg ha^{-1} was assumed.

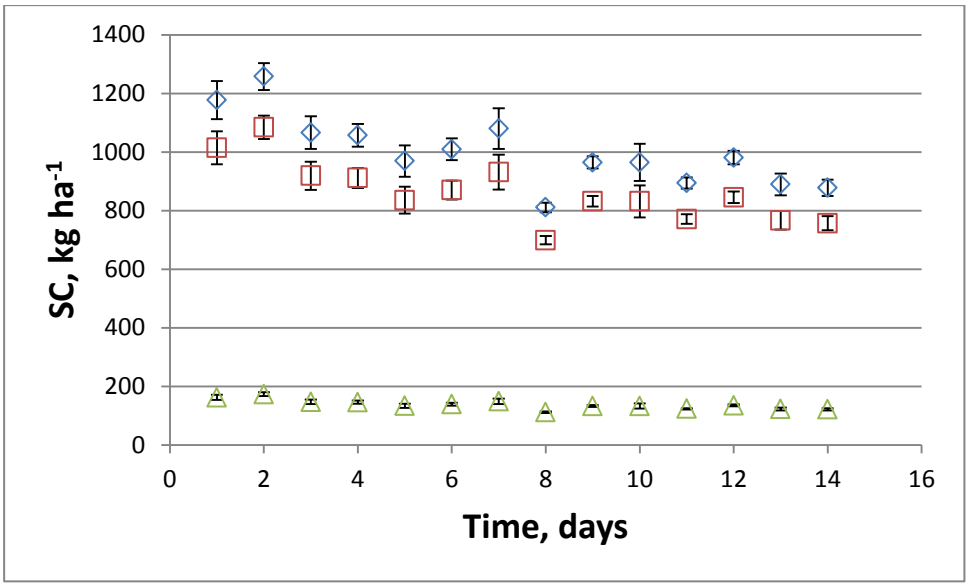


Fig. 4 Example of a harvest experiment. In this case approx. 840 kg ha^{-1} $week^{-1}$ was harvested.

Protein and amino acid composition

Depending on environmental conditions, crude protein concentration varied between 25-30% (data not shown). Furthermore, the amino acid composition of *Azolla* is such that it fulfills the requirement of pigs, hens and tilipia (Fig. 5).

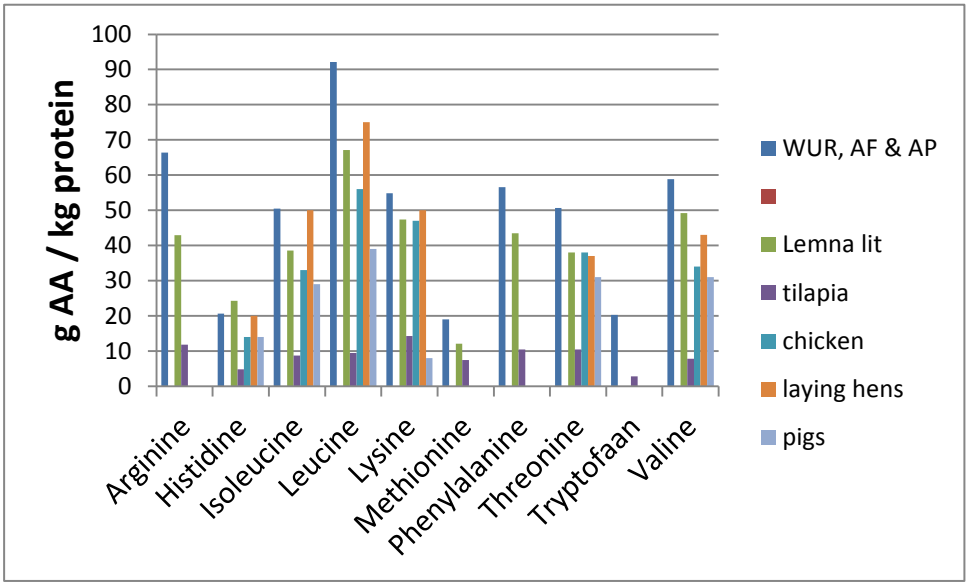


Fig. 5. Average essential amino acid composition for *Azolla filiculoides* and *Azolla pinnata* (WUR, AF & AP) for plants grown at ambient CO_2 (experiment in which 14% of total surface was harvested daily) and Lemna species (based on literature data) and requirement for tilapia, chickens, laying hens and pigs.

The combined first results on productivity and harvest , protein content and amino acid composition indicate that *Azolla* species may be of high interest for the feed and food industry (see also WP 4).

WP2: lipid concentration and composition

Approach

The organic geochemistry group chose to assess the very basis of the refinery of Azolla. We have developed a thorough assessment of the content of the extractable lipids as well as the isolation and quantification of specific compounds within the extractable lipids. These analyses were performed on *Azolla pinata* and *Azolla filliculoides*, grown under ambient conditions (350-400 ppm CO_2). In addition, *Azolla filiculoides* grown under elevated CO_2 concentrations (800 ppm) was analysed (see WP1for further details). Subsamples of the *A. Filiculoides* (800 ppm CO_2) were extracted after a set of pretreatments, to see whether these different pretreatments caused differences in the amount and composition of extractable lipids. Freeze-dried subsamples were cut over a 1 mm sieve and compared with intact *Azolla* plants. In addition, subsamples were either oven-dried at 30 ^{o}C or at 65 ^{o}C. Of the extractable (crude) lipids the yield per gram dried *Azolla* was determined. Furthermore, the extractable lipids were quantified on GC and GC-MS by spiking the samples with an internal standard. Also transmethylation of the lipids using BF_3/methanol was performed to hydrolyse long-chain ester-bound compounds that are otherwise to amenable to GC analyses.

Results

Lipid concentration

In the Table below the yields of the crude lipids are summarized. For the 800 ppm CO_2 grown *Azolla*, yields were hardly affected by the drying method. Interestingly, the *Azolla* grown under ambient conditions produce less Azolla biomass, but yield higher amount of crude lipids. The size refers to the pretreatment of cutting versus uncut Azolla leaves.

Species	CO_2 concentration [ppm]	Drying method	Size	Yield [% /dry weight]
A.Filiculoides	800	30 °C	1 mm	7,70
A.Filiculoides	800	65°C	1 mm	7,94
A.Filiculoides	800	Freeze-dried	Intact	8,11
A.Filiculoides	800	Freeze-dried	1 mm	8,13
A.Filiculoides	400	Freeze-dried	1 mm	10,55
A.Pinnata	400	Freeze-dried	1 mm	9,30

Composition and yields of the various lipid classes are presented in Fig. 6.

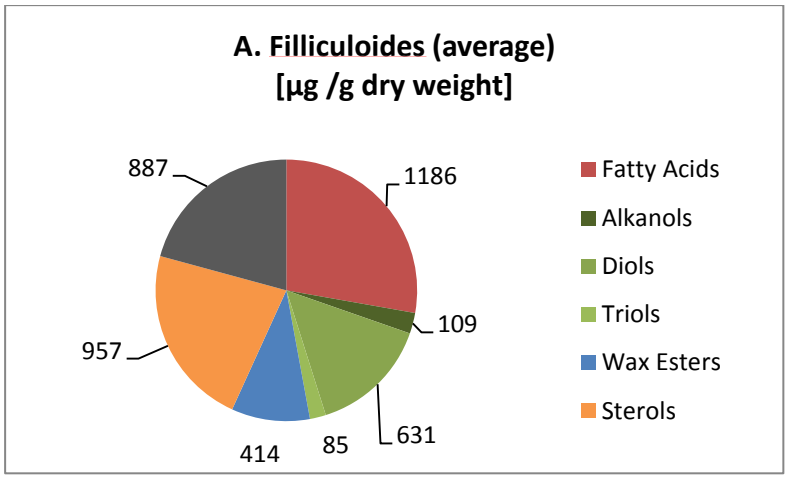


Fig. 6 Composition and yields of the various lipid classes

Composition and amounts of the various lipids analysed after hydrolysis and methylation are shown in Fig. 7. The hydrolysis procedure yields a large amount of fatty acids. However, these data are based on only two samples, and more data will be produced to assess this process more accurately.

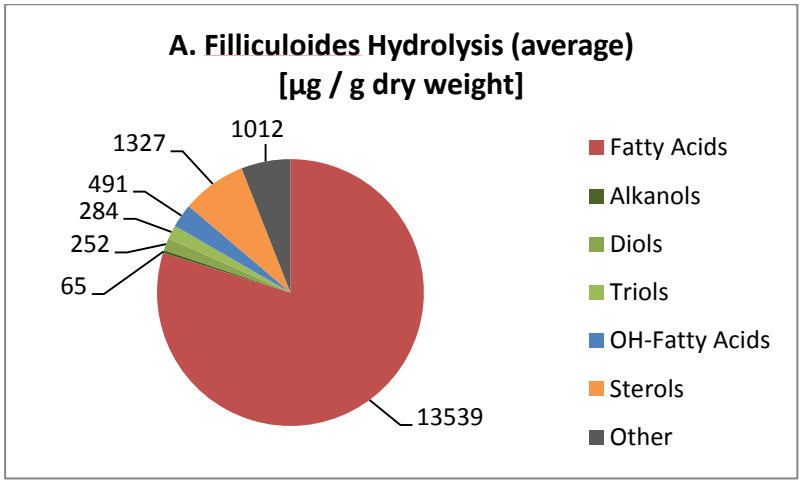
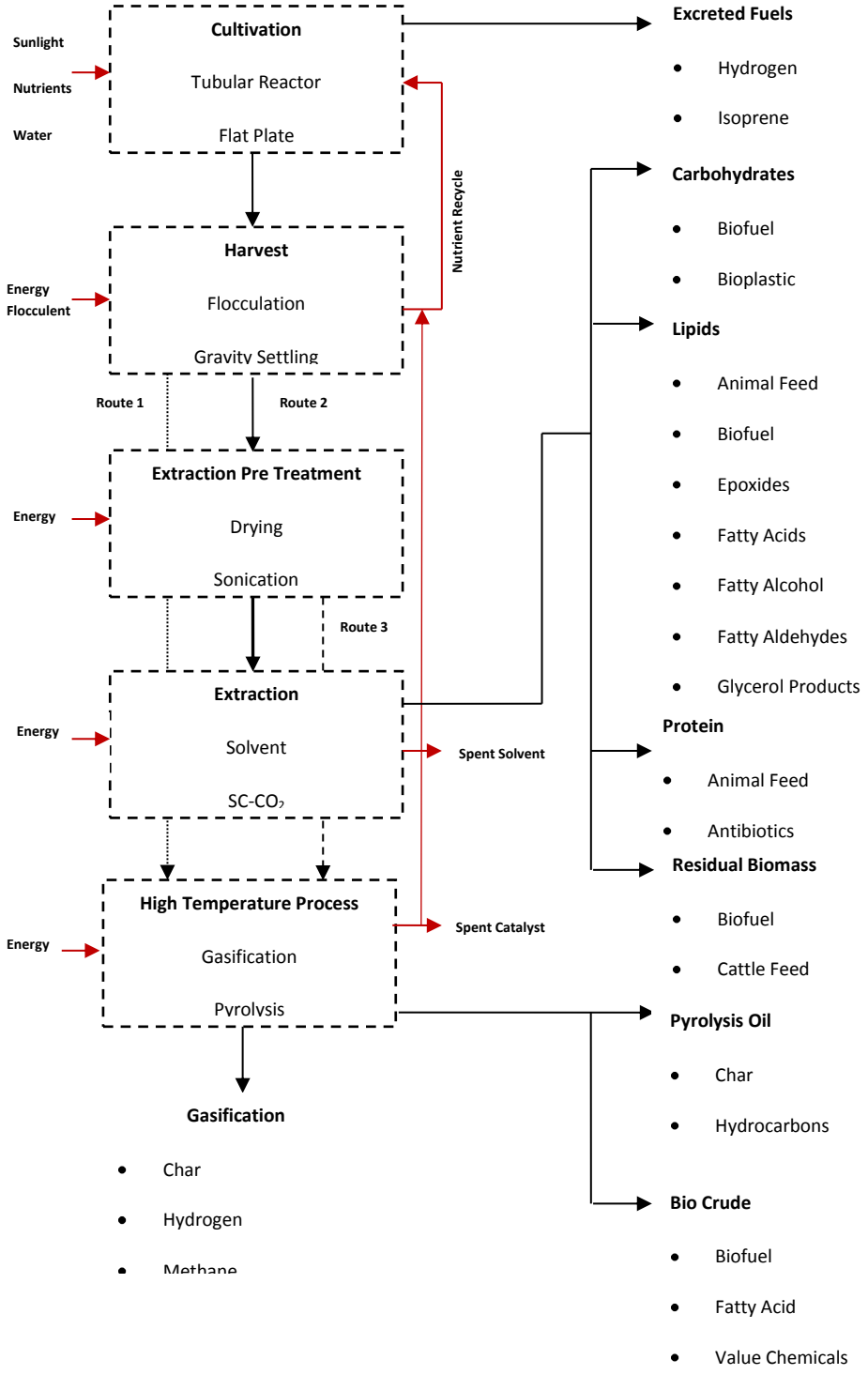


Fig. 7 Composition and amounts of lipids analysed after hydrolysis and methylation

With these analyses, we have more robust data in hand to assess the quality and absolute quantity of the extractable lipids. This yields more substantial information for potential products, to make a good business case for an innovation proposal.

WP 3: biorefinery Azolla

Figure 8 shows a concept, developed at Imperial College London, of an algal bio refinery. This was subsequently proposed as a model for the development of an Azolla bio refinery. Since Azolla is a multicellular plant, its harvesting from its aquatic environment would be relatively straightforward. From the analytical information received the protein content of dried Azolla averages at around 300 g per kg. The extraction of protein is therefore likely to be the most useful component of Azolla. However proteins can be difficult to extract, necessitating controlled temperatures and pH values to avoid the irreversible denaturation of their tertiary structures.


Sunlight
Nutrients
Water
Cultivation
Tubular Reactor
Flat Plate
Energy
Flocculent
Harvest
Flocculation
Gravity Settling
Route 1
Route 2
Energy
Extraction Pre Treatment
Drying
Sonication
Route 3
Energy
Extraction
Solvent
SC-CO2
Spent Solvent
Energy
High Temperature Process
Gasification
Pyrolysis
Spent Catalyst
Nutrient Recycle
Gasification
Char
Hydrogen
Methane
Excreted Fuels
Hydrogen
Isoprene
Carbohydrates
Biofuel
Bioplastic
Lipids
Animal Feed
Biofuel
Epoxides
Fatty Acids
Fatty Alcohol
Fatty Aldehydes
Glycerol Products
Protein
Animal Feed
Antibiotics
Residual Biomass
Biofuel
Cattle Feed
Pyrolysis Oil
Char
Hydrocarbons
Bio Crude
Biofuel
Fatty Acid
Value Chemicals

One solution would be to extract commercially important bio chemicals from Azolla *via* supercritical CO_2 extraction. This is an established technology which is depicted in Figure 8 and which will be investigated alongside other more novel methods. A different potential solution would be to extract bio chemicals using ionic liquids(ILs). Ionic liquids can be "tuned" in an almost infinite number of ways to provide designer solvents. Although such solvents can be expensive, they are relatively robust, so they can be recycled. Moreover it is feasible to fractionate biomass into compounds while simultaneously carrying out "one pot" chemical transformations. Imperial College is at the forefront of research in ionic liquids as green alternatives to traditional organic solvents. Figure 9 shows an outline of an Azolla based hypothetical bio refinery, which is at this stage based mainly on data gathered from the scientific and patent literature, some of which is referenced below.

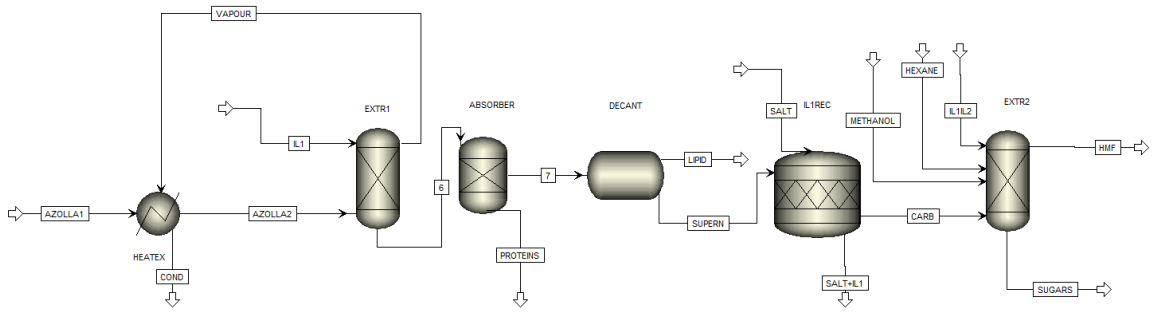


Fig. 9 Concept Azolla bio-refinery Note: (i) IL1 is recycled from the mixture with a kosmotrophic salt or methanol. (ii)The mixture IL1+IL2 does not need any recycle. (iii) All the chemicals (IL1, IL2, Hexane, Methanol) are characterised by a make-up equal to 1% of their federate.

In Figure 9 Azolla is harvested and dried until the moisture content is reduced to < 10 wt-% in a heat exchanger. This Azolla is then lysed in an ionic liquid (IL1) at ca. 100°C and the proteins as well as cell debris settle and are filtered off (sent to further processing). The energy input and processing time to lyse Azolla could be reduced using microwave radioation. Furthermore, if waste heat from the industry could be used costs are further reduced. The lipid content of the Azolla would be immisible in IL1 and therefore could be decanted readily. These lipids could be converted to biodiesel (or serve as building block for other industrial applications) with supecritical methanol without the need for a catalyst. In the final stage IL1 could be mixed with methanol and then salted out with a kosmotrophic salt (very inexpensive) in oder to remove the carboydrate fraction (as solution in methanol). This carbohydrate solution is mixed with fresch IL1 to effect the partitioning of the carbohydrate back into IL1. IL1 solution is then mixed with acidic IL2 (to facilitate hydrolysis) and methanol/hexane (to extract products such as HMF, which is a kinetically stable product in the presence of an IL2 such as $C_4H_8SO_3HmimHSO_4$). Other monosaccharides may also be formed, depending on the nature of IL1 and IL2. HMF is a readily extractable example. Preliminary experiments, mainly focussing on protein extraction, have been carried out on the extraction of Azolla with an IL in a microwave reactor, which were successful. First results show a high recovery percentage of proteins originally present. Coming months this be tested further and expanded to lipids and if possible to polyphenolics (see WP2). First costs estimates (€ per ton of weight going into the reactor). These data will be fine-tuned and linked to the economic model for the installation and exploitation of the Azolla pilot plant and finally to the revenues (price received for each of the components leaving the biorefinery plant).

WP 4: Business plan Azolla pilot

- Investment costs 10 ha aquatic farming system and revenues **(Business plan Azolla pilot)**

Approximately 40 companies were interviewed in autumn 2012 by Bioclear bv (type of companies were e.g. food and feed industry, aquatic farming industry, suppliers of waste heat, CO_2, and nutrients, but also scientific institutes, (non)-governmental organisations etc). Based on their information an economic model was applied to get insight in the investment costs, operational costs and revenues for a 10 ha pilot plant at Eemshaven. The model was applied for either an algae pilot farm and for an aquatic floating plants plant (*Azolla* and *Lemna*), and the combination of them. At present the results are still confidential so cannot be presented in this report, but from these first preliminary results it was concluded that a combined pilot plant for algae, *Azolla* and *Lemna* has a high potential. In the next months, the consortium will further work on this business case for an aquatic farming pilot system at large scale. First results also show that based on information shown in WP1-3 in combination with the economical model, *Azolla* biomass may have a considerable value and potential.

- Impact on the environment by studying the control of the plants' reproduction.

The reproduction of *Azolla* is crucial for a serious business case as it is important to control dissemination of *Azolla* strains in the wild but also dissemination to production sites. In addition control over *Azolla* reproduction is important for storage of improved lines or wild types particularly suited to biomass production. This autumn protocols were designed and successfully tested for the reproduction of *Azolla in vitro* (Fig. 10) and for cryopreservation of *Azolla* wild types. We conclude that sporophytes of all species tested until now are unable to survive freezing cycles typically found in Dutch winters suggesting that if non-sporulating species are used for production these will not disseminate. Final goal is to establish a well underpinned method to store and disseminate all *Azolla* species; an invention disclosure form describing the method has already been submitted to Utrecht Holdings B.V. As possibilities for a patent are presently being investigated, the highly exciting results cannnot be published in this report.

Fig. 10. Stages during sexual reproduction of *Azolla filliculoides*. A, Sporophyte; B, Macrosporocarp and microsporocarp on the sporophyte; C, Macrospore; D) Microspore with massulae; E) Fertilized macrospore; F) Germinating macrospore; G) Sporeling with macrospore capsule still attached; H) Sporeling with root and I) Sporeling with first leaves at the surface.

Progress towards KPIs and deliverables – by work package (WP[1])

Expected KPI[2]	Status	Comment
55 ton CO2e /ha yr reduced	In development	WP 1-4
2 Concrete tangible products	In development	WP 1-3, concepts for biorefinery & reproduction Azolla plants; still confidential
Prototypes developed and built	In development	Still confidential, part of the innovation proposal, WP1-4
4 market analyses	In development	Still confidential; business case, WP4
4 Benchmarking methods & standards	In development	Still confidential, WP4
1 report on best practices and policy recommendations	0%	End of project
1 new innovation project	In development	This summer
3 scholars involved in the project	66%	
2 fellows involved in the project	100%	
2 professional training sessions	50%	
2 workshops with business generation taskforce	100%	Two meetings were organised autumn 2012 for stakeholders
1 workshop organised	100%	15 febr 2013
30 participants at workshops and conferences	0%	
1 project member spending x months at other CLC/RIC partner	?	Hard to tell, there is a regular exchange of ideas and visits between Utrecht & Wageningen (an estimated average of 4 half days per month)
3 peer reviewed publications	20%	First paper is now almost ready and will be submitted end of March 2013
1 media appearence	0%	Working on a press release and newspaper advert

For all WPs please explain the performance by focussing on the following points:

[1] If, for some reason, you feel that a performance report by work package is not the right structure, please consult with your local Innovation lead on your alternative suggestions.

[2] It would be great if you could also scan the KCAs (complementary activities) of your project in order to surface KCAs generated by these as well since they can also be reported to the EIT. If this is the case, please list these KPIs in a separate table.

- Summarize whether the work package is on track (or not) and if necessary justify the situation: *the consortium only started recently (September 2012). All workpackages are on track.*
- Estimates of the costs by WP if possible (it can be rough): *all WP's link to each other, so we averaged total sum 2012 over the WP1-4, i.e. approx. 19 k€ per WP.*

Expected Deliverable (2012)	Status	Comment
Deliverable 5.1.1 - Protocols for optimizing *Azolla* productivity and biomass quality	Harvest potential at two CO_2 concentrations collected; Synchroneous in vitro germination of Azolla sporelings demonstrated	
Deliverable 5.2.1 - Protocols for lipid and polyphenol extraction from Azolla biomass and conversions	Lipids quantified in different species and at different CO2	Initial data on phenolics yields are impressive
Deliverable 5.3.1 Global Azolla Biorefinery concept	First conceptual design Azolla biorefinery established; first extraction procedures developed and tested	
Deliverable 5.4.1 -Investment costs 10 ha aquatic farming system -Expected revenues -The impact on the environment by studying the control of the plants' reproduction	Azolla Pilot business plan established Azolla *in vitro* fertilization and cryopreservation established	

Overall Project management and justification of costs

- Is the project on track overall? Please provide the status and justification for:
  - Timeline: What is the end date expected? *August / September 2013 for the AzoFast pathfinder project*
  - Budget: Current burn rate (% of budget to date), over/under spend: *100% over 2012 as agreed*
- Please give an update on the project management / governance structure. Is this perceived as adequate? *Project management of AzoFast is in hands of Dr. A. van der Werf and Dr. P.K. Bijl; at present this combination works very well. Project progress and flow is evaluated during the first consortium Workshop 15 Febr 2013*

- Please identify major obstacles if any (inactive partner, technical issues etc.) and the action planned to tackle it: *None*
- What is the status of the Consortium Agreement? *approved*
- Are there other KPIs to be achieved from a more synergistic perspective, i.e. not clearly attributable to a specific WP but due to g the project as a whole? *We expect them in due time*
- Do you have the right partners on board to really achieve the KPI and exploit it in the market in the future? *Yes, including industrial partners. LOI's still need to be signed*
- How have you engaged and established a clear relation to the demand side to check whether there is a continued real demand for your outputs? *Yes, see WP4*

Communication

- Strategy or plan
    - Do you have a communication plan/strategy? *No, just recently started, but soon we will work on this*
        - Attached plan and updates (excel spreadsheet should do)
    - Have you established contact with your local Climate-KIC communications manager? *Yes*
    - Have you discussed options / needs for communicating on local and European scale? *Not yet, in pipe-line*
- Achievements
    - What has been communicated so far, to whom, when, and how (channels) ?
      Submission to the NRC Jaarprijs in 2012-Team Schluepmann- Utrecht University.
      Just recently discussed possibilities for an advertisement on the front page of 2 of the largest news papers in the Netherlands, action week 2 February.
    - Where have you presented your project this year? Did you also present the KIC on certain occasions?
      Within Wageningen UR two presentations, one for the Business Unit Agrosystems Research, and one for the Wageningen UR working group Biobased Economy; within Utrecht University one presentation for the Biology Department.
    - Are you using KIC communications material in your communications? *NA yet*
- Potential short- to mid-term News Stories:
    - Are there achievements that could be communicated as success story? *Still confidential (see WP4)*
    - Which success stories do you anticipate in 2013 so as to prepare for communicating them? *Still confidential (see WP4)*
    - Target date and channel for communicating these? *Still confidential (see WP4)*
    -

Project ecosystem

- Could you please update the list of KCAs (from your original project document) contributing to your project in 2012 (as reported in Capplan) and briefly explain the relation? (a figure could sometimes help)

- Could you please provide a synthetic view on how your project and its ecosystem relate to initiatives/projects outside the KIC (in the various CLC/RIC countries or globally)?
- Did the KIC project generate new initiatives/funding? Or do you anticipate such new initiates in 2013?

Integration with Education:

1. For all students involved in your project please complete the following table[3]:

Student name	**Degree type** (Masters / PhD)	**PhDs** – Registered University + funding source. **Masters** – Registered University and masters programme title	**Activity description** (PhD title, internship title, master thesis etc.)	**Duration of activity** (start – end date)	**Climate-KIC activities completed by the student** (theJourney, SPARK!, greenhouse etc)
Paul Brouwer	Masters	Utrecht University-Energy Science, 50% at Org. Geochemistry and 50% at Biology	Master thesis Title: Considering Azolla domestication for biomass production Invention Disclosure form submitted at Utrecht Holdings B.V.	1 Nov 2012-1 April 2013	WP2, WP4 (Planned attendance at SPARK! Utrecht 21 feb. 2013)
Thibault Krommenhoeck	Masters	Utrecht University-Business and Sciences (100% at Biology)	Master thesis Title: Considering Azolla domestication	1 Nov 2011- 31 June 2012	

2. Education activities delivered through your project: Please give details of your engagement (if any) course content provision (the Journey, SPARK, professional education etc), provision of internships for Climate-KIC students, or others.

The Master's student Thibault Krommenhoeck received training in plant physiology, cryopreservation techniques and molecular biology as well as scientific writing and oral communication.

The Master's student Paul Brouwer received a training into organic geochemical processing of plant material, including powdering, extraction, processing and analysing using GC, GC-MS and Pyrolysis. Further, the student was trained in scientific writing, as well as oral presentation. Paul Brouwer further was trained in plant physiology and molecular biology as well as in cryopreservation

[3] Please indicate the information included in the table. If you have several students, you can of course annex this table in landscape format. If you have few students you might look for an alternative format.

techniques. He further received consultation at Utrecht Holdings B.V. supported by the Utrecht University Sustainability programme.

3. Please tell us your thoughts on the added value of (increased) integration of Innovation or Pathfinder projects with Education.

Integration with Entrepreneurship and Business development:

We have engaged in including the Utrecht Sustainability Institute in the consortium, and Institute that is experienced in sustainable development and the 'sustainable market'. The immense network of companies that are potentially interested in climate mitigation with Azolla and/or the potential added business opportunities that may emerge from that will help us in future development of the project.

- Have you clarified the business model of your products/services? *Work in progress*
- How far do you intend to be on the route to market for your products by the end of the project or of 2013: *it should result in commitment by industrial partners for the implementation in a pilot plant at Eemshaven; Invention Disclosure form submitted at Utrecht Holdings B.V. should result in a patent and possibly in a spin-out company*
- Have you engaged with business coaches?
    - If yes, with whom (name, organization), what was the focus and what are the first results / lessons learnt? How could this be improved going forward? *Thomas Koenig business developer from Utrecht Holdings B.V., lesson learnt: patents will only be supported by the holdings if they are likely to returns on investments in the very near future.e. within the 30 first months of the patent application.*
    - If not yet, do you foresee any business coaching needs with which Climate-KIC could support you in 2013? If yes, in what areas?
- Integration of SMEs/start-ups: Have you had contact with Climate-KIC SMEs or start-ups to see how they could be integrated in your project? Do you see any opportunities for this, e.g. by using SME vouchers? *One of the master's students may start its own company in the nearby future, this depending on the outcome of this pathfinder and expected Innovation proposal*
- Integration with CMA: Have you explored relations to one of the Climate Market Accelerator pilots?

Request for changes in the project plan

Please list here if you wish to, for example:

- Reallocate approved budgets between tasks and partners
- Involve new partners
- Increase the project budget (with a clear explanation how this would lead to additional KPIs)

Annex 1 – Updated KPI table

Please update the attached KPI table in collaboration with your local Innovation lead (please paste it into the report if the format/size allows). Please make sure this excel table indicates both:

At this stage the contribution to mitigation and adaptation will be difficult to assess. However, please indicate by what mechanisms and criteria your project will be assessed in this regard. If easy to do, please attempt to provide a rough estimate of your project's expected climate mitigation or adaptation benefits.

Annex 2 – Proofs for KPIs and deliverables

This Annex should contain a list of all proofs for achieved KPIs and deliverables and include the document names. The related documents should be named accordingly and be submitted separately so that they can be stored in our data repository system. *Will be done in 2013, project just recently started*

Please bear in mind that the exact process for registering KPIs and the format is still under development. We are thus open to suggestions. Please find below explanations regarding our current approach.

Confidentiality

Regarding confidentiality, please indicate:

- which ones could be made public
- which can be made available to KIC partners on request
- which can only be seen by KIC management staff (in particular the Executive Team, the Innovation Team and the platform facilitator)
- which ones cannot (or only be partially be shared with EIT)

Format

- Written deliverables / presentations should be supplied in their original format (including copies of scientific publications)
- Contracts or similar proofs (e.g. for knowledge transfer agreements, products/services launched):
    - Non-confidential proofs: We put entire contract (where sensitive information is blackened out) on the repository
    - Confidential proofs:
        - The partner provides a letter to the Innovation team explaining the deal with a minimum number of non-confidential information. The letter is stored on the repository.
        - The partner agrees that – if desired by EIT or the KIC – more information must be made available based on a signed NDA for a selected group of management staff

Annex 3 – Updated mailing list

Please fill in the attached excel file so that we have an updated mailing list of all relevant project partners. This is important to us so that we can provide information on the KIC to all involved participants, some of which currently do not know enough about the KIC.