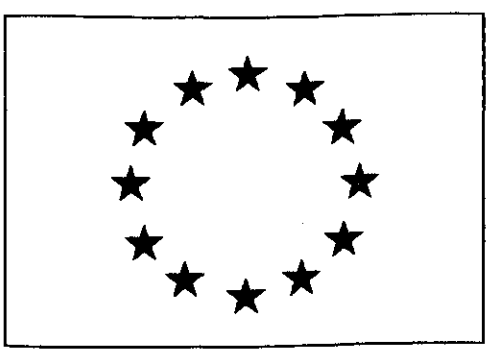
TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

ATO



☐ Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 1 Overview and description of your project and its results

PROGRAMME :	**FP 5**
OJECT TITLE & ACRONYM:	**A NOVEL BIOPROCESS FOR HYDROGEN PRODUCTION FROM BIOMASS FOR FUEL CELLS BIOHYDROGEN**
ONTRACT NUMBER :	**QLK5-1999-01267**
OJECT WEB SITE (if any) :	**www.biohydrogen.nl/biohydrogen**
RTNERS NAMES :	**ATO (CO), NTUA, TUBUD, WU, U-Szeged, Air Liquide**

1.1 Executive summary

Please, synthesise (in 1 or 2 pages) your project original objectives and final outcome.

a) Original research objectives

The main objective of the current project is the **production of hydrogen** from European energy crops and wastes employing anaerobic, thermophilic or hyperthermophilic **micro-organisms** in order to supply the **fuel cell** industry with clean hydrogen gas derived from **renewable resources**. The first objective is the selection, supply, storage and adaptation of **the biomass from energy crops and waste streams** to match the requirements of a suitable feedstock for the 'hydrogen' factory which is composed of two fermentations. The first stage involves the (hyper)thermophilic conversion of the feedstock to hydrogen, carbon dioxide and organic acids. The second fermentation addresses the photoheterotrophic conversion of organic acids to hydrogen and carbon dioxide. There are several objectives in this part of the project. Firstly, existing and newly isolated (hyperthermophilic) micro-organisms will be employed for the **optimisation of heterotrophic hydrogen production**. At the same time, similar research will be done on the **photoheterotrophic production of hydrogen** from organic acids. In both cases, genetic engineering will be used to **improve the selected strains** to finally achieve the theoretically maximal production of 12 moles of hydrogen per mole consumed glucose. The 'hydrogen' factory is ready when the production of hydrogen from both fermentations is coupled to an **efficient hydrogen recovery process** in which hydrogen is collected and carbon dioxide is removed. As stated above, the main objective of this project is to deliver **biohydrogen for application in fuel cells**. Therefore, the final product will be subjected to analysis with respect to specifications of this application.

b) Expected deliverables

- Production of 12 mols hydrogen per mol glucose (=100% of the theoretical maximum) utilised in a two-stage fermentation
- Design of a stable hydrogen producing bioprocess
- Production and conversion of biomass from energy crop and waste up to specifications required for fermentation by (hyper)thermophilic hydrogen producing bacteria
- Delivering biohydrogen which meets specifications required for utilisation in fuel cells

c) Project's actual outcome ***(in terms of technical achievements or if appropriate task per task)***

From several extreme thermophilic bacteria, *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii* were chosen. Both *C. saccharolyticus* and *T. elfii* are able to utilise a vast range of carbohydrates, e.g. cellulose, cellobiose, xylose etc., for growth. Sucrose did not support growth and hydrogen production by *T. elfii*. Further physiological differences between the strains are yeast extract and salt dependency by *T.elfii*, observed during growth on defined media.
The theoretical stoichiometry of the conversion of hexose to hydrogen, acetate en carbondioxide is: 1:4:2:2. In defined media usually circa 83% of the theoretical maximum was obtained, showing that circa 17% is used for biomass production. This high efficiency in hydrogen production makes the hyperthermophiles superior to mesophilic and moderate thermophilic bacteria. An efficiency of 100% appeared impossible since hydrogen production was strongly related to growth.
For the photoheterotrophic fermentation *Rhodopseudomonas* sp. were used. The highest obtained yield on acetate was only 50% of the theoretical maximum. For optimisation of this yield a new photobioreactor has been designed which is aimed at improved growth and hydrogen production by enabling high-density cultures in short ligh-path reactors with pneumatic agitation and gas-recirculation.
Genetic studies were done to unravel the maturation machinery of the hydrogenase enzyme which is responsible for the transfer of electrons to protons to make hydrogen. This has led to the increase of gene dosage and subsequent increase of hydrogenase activity by >50 % of the initial activity.
Two different feedstocks have been used for the thermophilic fermentation.
The first feedstock is sweet sorghum juice, obtained from sweet sorghum which is an energy crop in the Mediterranean part of the EU. Hydrogen production using from numerous samples of sweet sorghum juice showed that it is an excellent substrate for *C. saccharolyticus*. The production efficiency was 63% of the theoretical maximum.
The second feedstock is paper sludge which is an example of an agro-industrial waste stream, prominently available in all EU countries. The main carbohydrate in paper sludge is cellulose and even though these bacteria are able to grow on cellulose, the rate is too low to make a cost-effective bioprocess. Thus paper sludge was hydrolysed prior to fermentation by commercially available enzymes. Hydrogen was produced at $\leq$ 50% efficiency by *C. saccharolyticus*. This fairly low efficiency was due to i) the production of lactate which competes for electrons with hydrogen and ii) the presence of an inhibitor, potentially salt.
From several gas treatment processes, the cryogenic process for hydrogen separation was selected. However, new developments in this area are expected due to the ongoing development of fuel cells.
A preliminary economic evaluation showed that sweet sorghum, produced in Greece, will have a share of 6.9 Euro/GJ hydrogen produced in the bioprocess. This is circa one third of the estimated price of circa 20 Euro/GJ which seems now realistic from the presently available results. The theoretical production in the bioprocess under consideration could amount to 1452 kg hydrogen/ha sweet sorghum.

Conclusion
The biological production of hydrogen in a 2-stage bioprocess shows great promise to convert wet biomass such as sweet sorghum and paper sludge to a pure hydrogen stream. The efficieny of the fermentations is already at 63 and 50% of the maximum theoretical efficieny for thermophilic and photoheterotrophic fermentation, respectively. Genetic engineering has shown that the increase of hydrogenase activity may be regarded as one of the options to increase the production rate.
Future optimisations should address, besides increased performance of the micro-organisms and system efficiency, research and development of processparameters such as bioreactors design. Furthermore, full integration of all process units from well-to-wheel, including e.g. logistics and location of the feedstock production, and the final implementation in society will need to be addressed.

d) **Broad dissemination and use intentions for the expected outputs** *(such as industrial development, standards, regulations and norms, improvement of environment, health, working conditions, employment, net economic benefits, etc)*

A large number of scientific publications, posters and oral presentations at international conferences has been the result of this project.
Furthermore, right from the start in early 2000, an Internet site www.biohydrogen.nl/biohydrogen has been created which has, on the average, a daily number of 15-20 visitors. Also, 2-4 visitors, on a weekly basis, have become member of the Biohydrogen community enabling them to share information related to biological hydrogen production from biomass.

1.2 Overview of all your main project results

No.	Self-descriptive title of the result	Category A, B or C*	Partner(s) owning the result(s) (referring in particular to specific patents, copyrights, etc.) & involved in their further use
1	Selection of the best hydrogen producing extreme thermophilic bacteria	A	WU
2	Determination of factors affecting hydrogen production by from sugars by the extreme thermophilic *C. saccharolyticus*	A	WU
3	Insight in physiological parameters of the extreme thermophilic *C. saccharolyticus* in view of hydrogen production from sugars	A	WU
4	Conversion of acetate to hydrogen and carbon dioxide by photofermentation	A	WU
5	A mathematical growth model for photofermentation	A	WU
6	A photobioreactor with cultivation conditions and a full-scale process design	A	WU
7	Production of hydrogen from sweet sorghum by (hyper) thermophilic bacteria	A	ATO
8	Production of hydrogen from paper sludge hydrolysate by *Caldicellulosiruptor saccharolyticus*	A	ATO
9	Comparative analysis of thermostable hydrogenases	A	U-Szeged
10	Molecular characterization and heterologous expression of hypCD of *Thermococcus litoralis*	A	U-Szeged
11	Accessory genes and the regulation of [NiFe] hydrogenase biosynthesis	A	U-Szeged

12	Production of sweet sorghum	A	NTUA
13	Detailed characterisation protocols for sweet sorghum	A	NTUA
14	Characterisation of sweet sorghum	A	NTUA
15	Pretreatment procedures and optimisation of sugar fractionation from sweet sorghum	A	NTUA
16	Utilisation of the by-product, bagasse, from sweet sorghum for pulp production. Additive to composite materials or biofuel	A	NTUA
17	Composition analysis and evaluation of enzymatic hydrolysis of paper sludge samples for the production of sugars supporting hydrogen fermentation	A	TUBUD
18	Pre-treatment and enzymatic hydrolysis of sweet sorghum bagasse	A	TUBUD
19	Characterisation of thermostable cellulases	A	TUBUD
20	Reactor design for separation of CO2 from H2	B	Air Liquide
21	Analysis of H2 separation processes	B	Air Liquide
22	Safety by risk ranking of the purification process	B	Air Liquide

* A: results usable outside the consortium / B: results usable within the consortium / C: non usable results

1.3 Quantified Data on the dissemination and use of the project results

Items about the dissemination and use of the project results (consolidated numbers)	Currently achieved quantity	Estimated future* quantity
# of product innovations (commercial)		
# of process innovations (commercial)		
# of new services (commercial)		
# of new services (public)		
# of new methods (academic)	4	1
# of scientific breakthrough	2	
# of technical standards to which this project has contributed		
# of EU regulations/directives to which this project has contributed		
# of international regulations to which this project has contributed		
# of PhDs generated by the project		2
# of grantees/trainees including transnational exchange of personnel	1	1

*# = number of... / * "Future" means expectations within the next 3 years following the end of the project*

1.4. Comment on European Interest

l projects are expected to meet European interests. This section should provide an appraisal of your project in terms of European added value and support to the implementation of European Union policies.

1.4.1. Community added value and contribution to EU policies

a. European dimension of the problem
(The extent to which the project has contributed to solve problems at European level)

Throughout Europe the environment suffers form the use of fossil fuels. This suffering originates from the dependency on import from foreign countries and on the detrimental effect of emissions on the environment.
The biological production of hydrogen from biomass for utilisation in fuel cells offers an escape for the import of foreign fossil fuels by enabling the use of feedstock grown in Europe for energy production. Furthermore, together with the development of fuel cells, the utilisation of hydrogen as an energy carrier will provide for the highly-efficient production of clean-energy and eradicate emissions.

b. Contribution to developing S&T co-operation at international level. European added value
(Development of critical mass in human and financial terms; combination of complementary expertise and resources available Europe-wide)

The involvement of partners throughout Europe has enabled testing the suitability of an energy crop grown at its optimal location and also a waste stream which is available throughout Europe.
Furthermore, the expertise on the genomic level has been successfully combined with the complementary expertise on the physiological level, even though the research centres were separated by great distances.
Through exchange of human resources, sometimes with additional grants, expertise from laboratories involved in hydrogen production, has been effectively transferred to centres for feedstock preparation to enable adjustment of process parameters right from the start.
The spread throughout Europe of this research project has seeded further interest in the area of sustainable energy production.
Because of the work done in this project, the EU is now leader in the field of biological hydrogen production from biomass. The present lead in biological hydrogen production is due to the approach of exploring the very heart of the process, i.e. the fermentations, and relating it to knowledge of biomass, gas and fuel cell technology. This approach, which is based on fundamental insight and combination of various disciplines, differs distinctly from the usual pragmatic approach (black box) carried out in e.g. Asia. The growing significance of our position in biohydrogen is demonstrated by the recent Biohydrogen 2002 conference in Ede, The Netherlands, which drew together 150 participants from around the world.

c. Contribution to policy design or implementation
(Contribution to one or more EU policies; RTD connected with standardisation and regulation at Community and/or national levels)

Biological hydrogen production from biomass for fuel cells is aimed at the supply of clean energy from locally produced feedstock. As such it supports the EU policies aimed at sustainable energy systems for decentral energy production.
The involvement of an international industry with enormous expertise in the area of gas handling, conversion and safety, in the consortium will warrant a timely standardisation and registration once the process is ready for implementation.

1.4.2. Contribution to Community social objectives

a. Improving the quality of life in the Community :

The present supply of has a detrimental impact on the environment, because of emissions but sometimes also because of unfortunate accidents. The energy industry is often associated with the petrochemical industry and localised in heavily industrialised areas in the EU which are fortunate in having the ample supply of petrochemical feedstock at their disposal.

The substitution of fossil fuels by renewable feedstock to supply the energy and chemical industry is a basic solution to this pressing problem. The biological production of hydrogen fulfills here the opportunity to convert wet biomass to a clean fuel which bears no negative impact on the environment. Furthermore, the envisaged bioprocess offers the additional benefit of sound economics on a small scale. This means that instead of the present central mega-installations, small decentral installations will arise at sites where feedstock is being produced. These sites can be dispersed throughout the EU, e.g. in the case of energy crops with preference for the Southern and Eastern EU countries where the highest conversion rates of solar energy to biomass are obtained. In the case of agro-industrial and even domestic waste streams, more densely populated areas in the EU can be selected where small installations can convert the problem of waste streams to the benefit of clean energy.

The concept and now proven biological production of hydrogen from biomass enables the introduction of an elegant, low tech process to convert biomass to hydrogen to power fuel cells which are known for high efficiency and low maintenance. As such this bioprocess fulfills all demands of sustainability which are inherent to the foreseen 'green' Europe.

b. Provision of appropriate incentives for monitoring and creating jobs in the Community (including use and development of skills) :

This project has shown that biomass can be used for hydrogen production and that the non-fermentable part of biomass can be applied for other purposes, having an economic value of their own. This agrees with the whole crop utilisation concept which is one of the prime prerequisites of the future bio-based economy.

The successful utilisation of the agricultural produce from Mediterranean countries has shown that rural economies in the EU can find new market outlets. This will maintain and even enhance rural industries by the increasing demand for crop cultivation but also for pretreatment and processing of the raw material and the local energy supply.

Besides production of the primary feedstock also pretreatment and/or processing industries will need to be developed for upstream activities, and processing or bio-based products industries for downstream activities.

The same development is needed for the industries delivering materials for the bioprocess and also for the related conversion of hydrogen to energy directly or after storage.

All these developments will be the result of the introduction of small scale biohydrogen power plants and will have a strong supportive effect to the Community social objectives related to the provision of sustainable energy in the EU.

1.5. Expected project impact (to be filled in by the project coordinator)

Remark: by replying to the following questions, the coordinator is asked to express his best estimation regarding the impact of the project.

Overall Policy Impact[1]

EU Policy Goals	I SCALE OF EXPECTED IMPACT OVER THE NEXT 10 YEARS[2] -1 0 1 2 3	II other Not applicable to project	II other Project Impact too difficult to estimate
1. Improved sustainable economic development and growth, competitiveness Θ	2		
2. Improved employment Θ	2		
3. Improved quality of life and health and safety Θ	3		
4. Improved education Θ	1		
5. Improved preservation and enhancement of the environment Θ	3		
6. Improved scientific and technological quality Θ	3		
7. Regulatory and legislative environment Θ	3		
8. Other _____ Θ			

[1] Coordinator should respond to section I or, if appropriate, to section II. If the project has had no impact, a "0" should be entered in section I. Scores other than zero in section I will prompt a more detailed subquestion on a separate screen. However, you may access in any case the subquestions by clicking on the symbol" Θ "following each main question.

[2] Indication for scale as follows: -1 represents negative impact, 0 no impact, 1 small positive impact, 2 medium positive impact , 3 is a strong positive impact

Indicate your replies below by putting in each box the number corresponding to the score you chose:

1. Economic development and growth, competitiveness	Scale of Expected Impacts over the next 10 years (2)	
	By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a) Increased Turnover for project participants		2
- national markets		
- international markets		2
b) Increased Productivity for project participants		
c) Reduced costs for project participants		
d) Improved output quality/high technology		
d) the EU community as a whole		2
e)		

2. Employment	Scale of Expected Impacts over the next 10 years (2)	
	By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a) Safeguarding of jobs		2
b) Net employment growth in projects participants staff		
c) Net employment growth in customer and supply chains		2
d) Net employment growth in the European economy at large		2

3. Quality of Life and health and safety	Scale of Expected Impacts over the next 10 years (2)	
	By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a) Improved health care		
b) Improved food, nutrition		
c) Improved safety (incl. consumers and workers safety)		
d) Improved quality of life for the elderly and disabled		3
e) Improved life expectancy		
f) Improved working conditions		3
g) Improved child care		
h) Improved mobility of persons		

4. Improved education	Scale of Expected Impacts over the next 10 years (2)	
	By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a) Improved learning processes including lifelong learning		
b) Development of new university curricula		1

5. Preservation and enhancement of the environment	Scale of Expected Impacts over the next 10 years (2)	
	By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a) Improved prevention of emissions		3
b) Improved treatment of emissions		
c) Improved preservation of natural resources and cultural heritage		3
d) Reduced energy consumption		3

6. S&T quality		Scale of Expected Impacts over the next 10 years (2)	
		By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a)	Production of new knowledge	3	3
b)	Safeguarding or development of expertise in a research area	3	3
c)	Acceleration of RTD, transfer or uptake	3	3
d)	Enhance skills of RTD staff	3	3
e)	Transfer expertise/know-how/technology	3	3
f)	Improved access to knowledge-based networks		
g)	Identifying appropriate partners and expertise		
h)	Develop international S&T co-operation	3	3
i)	Increased gender equality		

7. Regulatory and legislative environment		Scale of Expected Impacts over the next 10 years (2)	
		By Project End -1 0 1 2 3	After Project End -1 0 1 2 3
a)	Contribution to EU policy formulation	3	3
b)	Contribution to EU policy implementation	3	3

8. Other (please specify)	Scale of Expected Impacts over the next 10 years (2)	
	By Project End -1 0 1 2 3	After Project End -1 0 1 2 3

I, **project co-ordinator**, confirm the published information contained in this part 1 of the TIP.

Signature: P Th Claassen **Name: Pieternel Claassen**

Date: December 12, 2003 **Organisation: ATO bv**
(from Oct 1st, 2003: Agrotechnology &Food Innovations)

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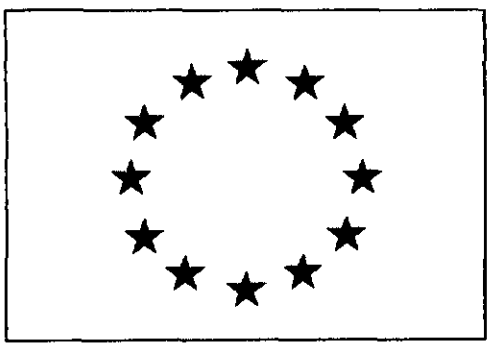


Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

CONTACT PERSON FOR THIS RESULT*)

Name	VAN NIEL, Ed
Position	lecturer
Organisation	Lund University
Address	P.O. Box 124, 22100 Sweden
Telephone	+46 46 2220619
Fax	+46 46 2224203
E-mail	Ed.van_Niel@tmb.lth.se
URL	http://www.tmb.lth.se
Specific Result URL	http://www.biohydrogen.nl

***) This is my new address since 10th March 2003.**

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
1	Selection of the best hydrogen producing extreme thermophilic bacteria

SUMMARY *(200 words maximum)*

From experimental examination of both pure cultures of culture collections and samples taken from hot springs it was concluded that up to now the described bacterium *Caldicellulosiruptor saccharolyticus* is the most promising candidate for hydrogen production at elevated temperatures (70°C). This organism is versatile in its use of substrates, it survives high partial hydrogen pressures (by shifting its metabolism) and showed to be of a robust nature when applied in a pilot plant scale thermoreactor.

Please categorise the result using codes from Annex I

Subject descriptors	61	77	93	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
2	Determination of factors affecting hydrogen production by from sugars by the extreme thermophilic *C. saccharolyticus*

SUMMARY *(200 words maximum)*

The influence and kinetics of the following physicochemical factors on hydrogen production by *C. saccharolyticus* have been studied:

- Hydrogen is the most severe inhibitor; the estimated dissolved hydrogen concentration that inhibits growth: 500 µMol at 70°C;
- Sugars and salts (e.g. the fermentation product sodium acetate) inhibit by increasing the external osmotic potential;
- High concentrations of monovalent ions enhance cell lysis;
- Carbon dioxide can be used as stripping gas to avoid hydrogen accumulation in the cultures. However, through the addition of a significant concentration of bicarbonate it will negatively affect growth yields and enhance the osmotic potential of the system. These effects can be kept to a minimum by keeping a strict pH-regime of 6.5-7.0;
- Hydrogen production activity has a slightly wider temperature range (estimation: 40-90°C) than growth (49.6 – 85.7°C)

Please categorise the result using codes from Annex 1

Subject descriptors	61	77	93	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
3	Insight in physiological parameters of the extreme thermophilic *C. saccharolyticus* in view of hydrogen production from sugars

SUMMARY *(200 words maximum)*

The following major findings were made with respect to the physiology of *C. saccharolyticus*:

- Yeast extract is required for growth, but could be replaced by casamino acids plus proline and vitamins;
- *C. saccharolyticus* prefers xylose, arabinose, sucrose, cellobiose to glucose. This unusual feature makes this organism complementary to other hydrogen producers. This property could be beneficiary in the application of consortia of extremely thermophilic hydrogen producers for optimal performance of hydrogen production from complex sugar mixtures (such as biomass);
- Extreme thermophiles, such as *C. saccharolyticus*, show superior hydrogen yields (83% of the maximal theoretical obtainable) compared to maximal 50% found with mesophiles and moderate thermophiles;
- Sugars are converted to hydrogen and acetate through the EMP-pathway. At a partial hydrogen pressure (pH_2) $\geq$ $1 \cdot 2 \cdot 10^4$ Pa the metabolism shifts partially to lactate formation. There are indications that this pathway to lactate is controlled by the internal redox potential. At high dissolved hydrogen concentrations also the reductive acetate pathway is activated. It is thus of the utmost importance that hydrogen is stripped from the fermentation broth.
- The external redox potential is not an adequate parameter to monitor the internal redox potential to signal any metabolic shifts.

Please categorise the result using codes from Annex 1

Subject descriptors	61	77	93	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
4	Conversion of acetate to hydrogen and carbon dioxide by photofermentation

SUMMARY

The following aspects concerning the photoheterotrophic conversion of acetate to hydrogen by *Rhodopseudomonas* sp. were demonstrated:

- Hydrogen production is catalysed by the nitrogenase enzyme complex. The only hydrogenase which seemed to be active is an uptake hydrogenase.
- Exposure to low concentrations of carbon monoxide (1-2 % v/v in headspace) did not lead to extra hydrogen production and it even led to an increase of the lag phase in batch experiments.
- Hydrogen production can go together with growth, but it is still unclear whether growth itself is a prerequisite for hydrogen production.
- Biomass growth during hydrogen production can be supported with glutamate as a nitrogen source, but this was accompanied with considerable excretion of reduced soluble products. Maximally 60% of the acetate could be directed to hydrogen gas and carbon dioxide at acetate/glutamate ratios of 5 or less.
- Biomass growth during hydrogen production can also be supported with limiting amounts of ammonia. In this situation the major part of the acetate could be accounted for as biomass and hydrogen/carbon dioxide produced. Even more so, preliminary results indicated that severe limiting of ammonia could increase the fraction of acetate directed to hydrogen to more than 80%.
- Using high-density cultures of another purple non-sulfur bacterium, *Rhodobacter*, photosynthetic efficiencies of 4 % were reached at light intensities of 180 W m^{-2} in the wavelength range of 400 to 950 nm (91 mmol H_2 h^{-1} m^{-2} light exposed surface). High-density cultures apparently provide a way to approach the theoretical maximal efficiency, which is approximately 10.6 % considering sunlight between 400 and 950 nm.

Subject descriptors	61	77	201	307	391

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
5	A mathematical growth model for photofermentation

SUMMARY

A preliminary model was developed describing growth and hydrogen production of purple non-sulfur bacteria under ammonia-limited conditions. A number of model parameters were estimated and still have to be determined in future work. Also, the model still needs to be validated. Nevertheless, the model provided valuable insight in the process and the following important aspects of the process of photoheterotrophic hydrogen production were distinguished:

- In order to increase the yield of hydrogen on acetate it is crucial to minimize biomass growth using severe ammonia limitation. As a result of the application of low specific growth rates also (maintenance related) biomass degradation becomes an important factor which should be determined.
- The application of low specific growth rates could lead to the application of voluminous photobioreactors with a long liquid residence time. On the other hand, the residence time and reactor volume could be minimized in case it is possible to immobilize or retain the biomass inside the photobioreactor.
- The specific nitrogenase activity of the biomass is a potential limiting factor and, for this reason, the biomass concentration needs to be adjusted to the volumetric input of light. Model simulations have shown that the biomass densities required are realistic as compared to existing photobiological processes.

Subject descriptors	61	77	201	307	391

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
6	A photobioreactor with cultivation conditions and a full-scale process design

SUMMARY

Different scale-up strategies for full-scale photobioreactors were identified using the results of the mathematical model and previous studies in the area of algal biotechnology. The following guidelines should be taken into account designing a full-scale process:

- Light intensity should be used at maximal efficiency and for this reason the photobioreactor surface should be exposed to low light intensities (roughly, less than 10% of full sunlight). Sunlight dilution is possible using vertical photobioreactor systems with a high area to volume ratio.
- Full sunlight intensities also can be utilized at high efficiency on the condition high-density cultures (> 10 g L^{-1}) are used in short light-path (< 1.5 cm) photobioreactors with adequate mixing (see 3.3).
- The biomass density always needs to fit the volumetric light input given the potentially limiting specific nitrogenase activity of the biomass. Using high-density cultures and low specific growth rates (see 3.4) this would lead to the need of a biomass retention system.

As a first step a lab-scale 2.5 L panel photobioreactor was developed. In this system a short light-path can be applied (3 cm or less). In order to be able to provide adequate mixing a gas recirculation system was developed. This resulted in a pneumatically agitated panel photobioreactor and it was tested successfully. This photobioreactor system is an ideal platform to test and develop the most suitable pilot-scale design considering the guidelines given.
Furthermore, a literature survey was done to make a preliminary cost analysis of a full-scale phototrophic process.

Subject descriptors	61	77	201	307	391

CURRENT STAGE OF DEVELOPMENT

Please tick one category only ✓

Scientific and/or Technical knowledge (Basic research)	X
Guidelines, methodologies, technical drawings	☐
Software code	☐
Experimental development stage (laboratory prototype)	☐
Prototype/demonstrator available for testing	☐
Results of demonstration trials available	☐
Other (please specify.):	☐

DOCUMENTATION AND INFORMATION ON THE RESULT

List main information and documentation, stating whether public or confidential.

Documentation type	**Details (Title, ref. number, general description, language)**	**Status: *PU*=Public *CO*=Confidential**
article	Distinctive properties of high hydrogen producing Extreme thermophiles, *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*. International Journal of Hydrogen Energy 27/2002, pp. 1391-1398	PU
article	Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. Biotechnology and Bioengineering 81/2003, pp. 255-262	PU
Book	Proceedings of *'Biohydrogen 2002'*, Apr 21-24, Ede, The Netherlands. Published as a Special Issue of the International Journal of Hydrogen Energy, Vol. 27, p. 1123-1505.	PU
article	Photobiological hydrogen production: photochemical efficiency and bioreactor design. International Journal of Hydrogen Energy, Vol. 27, p.1195-1208.	PU
article	A pneumatically agitated flat-panel photobioreactor with gas re-circulation: anaerobic photoheterotrophic cultivation cultivation of a purple non-sulfur bacterium. International Journal of Hydrogen Energy, Vol. 27, p.1331-1338.	PU

2.2. Quantified data about the result

Items (about the results)	Actual current quantity [a]	Estimated (or future) quantity [b]
Time to application / market (in months from the end of the research project)		
Number of (public or private) entities potentially involved in the implementation of the result :		
of which : number of SMEs :		
of which : number of entities in third countries (outside EU) :		
Targeted user audience: # of reachable people		
# of S&T publications (referenced publications only)	5	7-8
# of publications addressing general public (e.g. CD-ROMs, WEB sites)	2	
# of publications addressing decision takers / public authorities / etc.	1	
Visibility for the general public	Yes	

[a] *Actual current quantity = the number of items already achieved to date.*
[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve within the next 3 years.*

2.3. Further collaboration, dissemination and use of the result

(Optional; to be completed if partner is willing to set up new collaborations, and seeking dissemination support from the CORDIS services.)

COLLABORATIONS SOUGHT

Please tick appropriate boxes (✓) corresponding to your needs.

R&D	Further research or development	X	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing agreement/Franchising	☐	**INFO**	Information exchange	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

I confirm the information contained in part 2 of this Technological Implementation Plan and I authorise its dissemination to assist this search for collaboration.

Signature: **Name: E.W.J. van Niel**

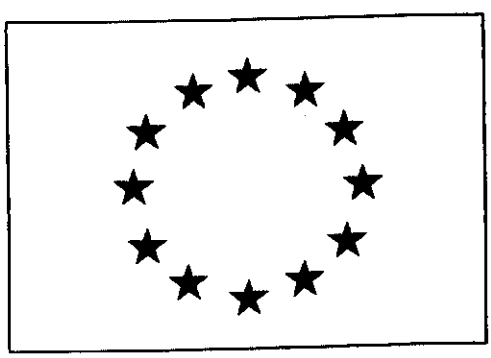
Date: 24 – 03 - 2003 **Organisation: Wageningen University**

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

ATO



Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 2 Description of each result

2.1 : Description of the result(s), one form per result

CONTACT PERSON FOR THIS RESULT

Name	Pieternel Claassen
Position	Senior scientist
Organisation	ATO bv
Address	P.O. Box 17 , 6700 AA Wageningen, The Netherlands
Telephone	31.317.475325
Fax	31.317.475347
E-mail	Pieternel.claassen@wur.nl
URL	www.biohydrogen.nl
Specific Result URL	www.biohydrogen.nl/biohydrogen

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
7	Production of hydrogen from sweet sorghum by (hyper) thermophilic bacteria

SUMMARY *(200 words maximum)*

Twenty-five samples of sweet sorghum juice which had been extracted using a factorial analysis with temperature (24-66 °C), extraction time (0.6-3.4 h), pressure (64-106 bar) and water (-8-48 mL) as variables, were tested for fermentability. Even though some differences in sugar composition, most probably due to remaining invertase activity, was observed, there was no significant difference in hydrogen production by *Caldicellulosiruptor saccharolyticus* using non-filtered and non-sterilized sweet sorghum juice.

Fermentation of non-diluted sweet sorghum juice, supplemented with all required nutrients, showed rapid hydrogen production to a final yield of 63% of the theoretical maximum at a production rate of 21 mmol/L.h. Sucrose and fructose were the preferred substrates. After 16 hours of fermentation growth already stopped, probably due to inhibition by acetate triggering cell lysis. The results showed that sweet sorghum juice is an excellent substrate for hydrogen fermentation. In view of future scaling-up, preliminary experiments were done with *C. saccharolyticus* as well as *Thermotoga elfii*, to reduce the requirements for nutrients in the medium. Here *C. saccharolyticus* came out best although the simultaneous omission yeast extract and salts significantly decreased the hydrogen yield. This may have been due to the limitation of a simple nutrient like Mg or Fe. The other hyperthermophilic organism, *T. elfii* was much more dependent on supplementation with nutrients and thus seems less suited for making hydrogen from sweet sorghum juice.

Please categorise the result using codes from Annex 1

Subject descriptors	61	77	93	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
8	Production of hydrogen from paper sludge hydrolysate by *Caldicellulosiruptor saccharolyticus*

SUMMARY *(200 words maximum)*

The potential of paper sludge hydrolysate for hydrogen production was tested using *C. saccharolyticus*. Firstly, growth and hydrogen production from a mixture of glucose and xylose (the main constituents in paper sludge hydrolysate) was established. Hydrogen production was 65-70% of the theoretical maximum amount and occurred at a maximum rate of 8.5-10.2 mmol H_2/L.h. These yields and production rates are comparable to data from fermentations on glucose or xylose, separately. Xylose seemed to be the preferred substrate for hydrogen production by *C. saccharolyticus*, as usually it was consumed before glucose consumption started. In cultures with paper sludge hydrolysate as the sole carbon and energy source, but supplemented with all nutrients, all carbohydrates were consumed and hydrogen production was circa 50% of the theoretical maximum, occurring at a rate of 3.4-6 mmol H_2/L.h. When only yeast extract was supplied as nutrient, hydrogen yield and rate were similar. These lower yields and rates were due to the production of lactate instead of acetate and hydrogen was produced. Fortunately, lactate is also a prime substrate for the subsequent photofermentation. As such, the energy in lactate will be recovered in more hydrogen being produced in the second fermentation.

Please categorise the result using codes from Annex 1

Subject descriptors	61	77	93	201	307

CURRENT STAGE OF DEVELOPMENT

Please tick one category only ✓

Scientific and/or Technical knowledge (Basic research)	X
Guidelines, methodologies, technical drawings	☐
Software code	☐
Experimental development stage (laboratory prototype)	☐
Prototype/demonstrator available for testing	☐
Results of demonstration trials available	☐
Other (please specify.):	☐

DOCUMENTATION AND INFORMATION ON THE RESULT

List main information and documentation, stating whether public or confidential.

Documentation type	**Details (Title, ref. number, general description, language)**	**Status: *PU*=Public *CO*=Confidential**
publication	Feasibility of biological hydrogen production from biomass for utilization in fuel cells. Proc 1st World Conference and Exhibition on Biomass for Energy and Industry, Sevilla, Spain, 2000	PU
publication	Biological hydrogen production from biomass by thermophilic bacteria. Proc 12th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Change Protection, Amsterdam 2002	PU
publication	Dark hydrogen fermentations. In: Bio-methane and Bio-hydrogen: Stautus and perspectives of biological methane and hydrogen production ISBN: 90-9017165-7	PU

MARKET APPLICATION SECTORS

Please describe the possible sectors for application using the NACE classification in Annex 2.

Market application sectors	01	24	40	60	73 1

2.2. Quantified data about the result

Items (about the results)	Actual current quantity [a]	Estimated (o future) quantity [b]
Time to application / market (in months from the end of the research project)		
Number of (public or private) entities potentially involved in the implementation of the result :		
of which : number of SMEs :		
of which : number of entities in third countries (outside EU) :		
Targeted user audience: # of reachable people		
# of S&T publications (referenced publications only)	1	2
# of publications addressing general public (e.g. CD-ROMs, WEB sites)	2	4
# of publications addressing decision takers / public authorities / etc.	2	4
Visibility for the general public	Yes	

[a] *Actual current quantity = the number of items already achieved to date.*
[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve within the next 3 years.*

2.3. Further collaboration, dissemination and use of the result

(Optional; to be completed if partner is willing to set up new collaborations, and seeking dissemination support from the CORDIS services.)

COLLABORATIONS SOUGHT

Please tick appropriate boxes (✓) corresponding to your needs.

R&D	Further research or development	X	**FIN**	Financial support
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership
MKT	Marketing agreement/Franchising	☐	**INFO**	Information exchange
JV	Joint venture	☐	**CONS**	Available for consultancy
			Other	(please specify)

I confirm the information contained in part 2 of this Technological Implementation Plan and I authorise its dissemination to assist this search for collaboration.

Signature: P.A.M. Claassen

Name: Pieternel Claassen

Date: December 12, 2003

Organisation: ATO bv
(from Oct 1st, 2003: Agrotechnology

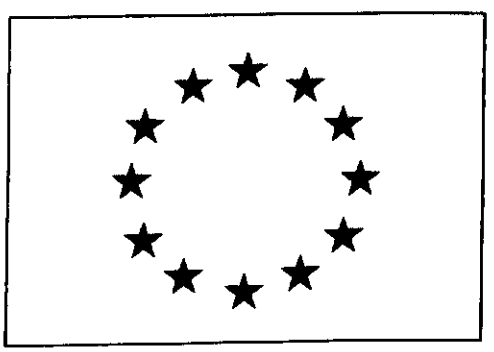
&Food Innovations)

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

U-Szeged



☐ Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 2 Description of each result

2.1 : Description of the result(s), one form per result

CONTACT PERSON FOR ALL RESULTS

Name	Kornel L. Kovacs
Position	professor
Organisation	University of Szeged
Address	6726 Szeged, Temesvari krt. 62, Hungary
Telephone	+36 62 544 351
Fax	+36 62 544 352
E-mail	kornel@nucleus.szbk.u-szeged.hu
URL	http://www.nucleus.szbk.u-szeged.hu
Specific Result URL	http://www.biohydrogen.nl

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
9	Comparative analysis of thermostable hydrogenases

SUMMARY *(200 words maximum)*

Molecular systems have been developed to obtain mutant forms of hydrogenases and to study the gene expression. The recombinant and genetic analysis have been carried out in the phototorphic purple sulfur bacterium *Thiocapsa roseopersicina*. Research focused on the molecular and genetic aspects of thermophilic hydrogen metabolism. Four distinct gene clusters coding for hydrogenases of *T. roseopersicina* have been isolated and sequenced. The hydrogenase gene clusters of Thermococcus litoralis were also identified. Random mutagenesis as well as site directed deletion mutants of various genes showed increased hydrogen evolution activity. One of the mutations was in the gene showing high amino acid sequence similarity to HypF, a pleiotropic protein participating inthe assembly of all [NiFe] hydrogenases. An important consequence of the mutation was the remarkably pronounced hydrogen evolving activity under nitrogen fixing conditions. Hence, this hydrogen evolution activity is most likely due to the nitrogenase complex. The results also indicated that a substantial amount of hydrogen uptake activity has been inactivated in the hypF mutant cells, therefore this mutation offers an important avenue for the development of efficient hydrogen production systems. The structural gene cluster encoding for a soluble hydrogenase has been found and characterized.

Please categorise the result using codes from Annex I

Subject descriptors	61	391	226	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
10	Molecular characterization and heterologous expression of *hypCD* of *Thermococcus litoralis*

SUMMARY *(200 words maximum)*

The first two [NiFe] hydrogenase accessory genes, encoding the counterparts of mesophilic proteins involved in the maturation of hydrogenases, were isolated from the hyperthermophilic archaeon *T. litoralis*. The deduced gene products showed 30-40% identity to the corresponding mesophilic proteins. Heterologous complementation experiments were done in *Escherichia coli* and *Ralstonia eutropha* strains lacking functionally active *hypC* and *hypD* genes. This was the first demonstration of mesophilic hydrogenase processing using a hyperthermophilic archaeal accessory protein to produce and active enzyme. This work also represented the first step in functional characterization of the maturation process of [NiFe] hydrogenases of hyperthermophilic hydrogenases.

Please categorise the result using codes from Annex 1

Subject descriptors	61	391	226	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
11	Accessory genes and the regulation of [NiFe] hydrogense biosynthesis

SUMMARY *(200 words maximum)*

Regulation of hydrogenase biosynthesis was studied in detail for HynSL. In *T. roseopersicina* the activities of both membrane associated [NiFe] hydrogenases (HynSL and HupSL) decreased dramatically in the absence of the HupK protein, whereas the soluble HoxEFUYH enzyme remained apparently unaffected. Remarkably, this protein does not occur in all microbes containing [NiFe] hydrogenase, hence the role of the HupK protein is still uncertain. It resembles the large subunit of the [NiFe] hydrogenases, therefore HupK has been suggested to function as a scaffolding protein during metal cofactor assembly. Although our study did not uncover the precise function of HupK, this was the first demonstration that it made a selection among the various [NiFe] hydrogenases in the cell, and participated in the biosynthesis of the membrane bound ones.

It is worth noting that the Hox hydrogenase produces considerable amount of hydrogen in the HupK mutant under nitrogenase repressed conditions, hence in this case hydrogen is produced by a true [NiFe] hydrogenase.

Please categorise the result using codes from Annex 1

Subject descriptors	61	391	226	201	307

CURRENT STAGE OF DEVELOPMENT

Please tick one category only ✓

Scientific and/or Technical knowledge (Basic research)	X
Guidelines, methodologies, technical drawings	☐
Software code	☐
Experimental development stage (laboratory prototype)	☐
Prototype/demonstrator available for testing	☐
Results of demonstration trials available	☐
Other (please specify.):	☐

DOCUMENTATION AND INFORMATION ON THE RESULT

List main information and documentation, stating whether public or confidential.

Documentation type	**Details(Title, ref. number, general description, language)**	**Status: *PU*=Public *CO*=Confidential**
Article	K. L. Kovács, Cs. Bagyinka, L. Bodrossy, B. Fodor, K. Győrfi, T. Hanczár, J. Ősz, G. Rákhely, M. Takács, A Tóth, J. Tusz, Recent advances in biohydrogen research. Eur. J. Physiol., 439, R81-R83. (2000)	PU
Article	B. Fodor, G. Rákhely, Á. T. Kovács, K. L. Kovács. Transposon mutagenesis in purple sulfur photosynthetic bacteria: Identification of *hypF*, encoding a protein capable to process [NiFe] hydrogenases in α, β and γ subdivision of proteobacteria. Appl. Environ. Microbiol. 67, 2476-2483. (2001)	PU
Article	M. Takács, G. Rákhely, K. L. Kovács. Molecular characterization and heterologous expression of *hypCD*, the first two [NiFe] hydrogenase accessory genes of *Thermococcus litoralis*. Arch. Microbiol., 176, 231-235. (2001)	PU
Article	K. L. Kovács, Utilization of hydrogen metabolism in biotechnological applications. In: Hydrogen as a Fuel: Learning from Nature. Chapter 9.1. (Eds. R. Cammack, R. L. Robson, M. Frey) Taylor & Francis. pp. 181-189. (2001)	PU
Article	P. Pedroni, P. M. Vignais, K. L. Kovács. Hydrogen production by photoheterotrophic and heterotrophic bacteria. Chapter 10. Hydrogen as a Fuel: Learning from Nature. (Eds. R. Cammack, R. L. Robson, M. Frey) Taylor & Francis. pp. 213-220. (2001)	PU

Article	A. Szilágyi, K. L. Kovács, G. Rákhely, P. Závodszky. Homology modelling reveals the structural background of the striking difference in thermal stability between two related [NiFe]hydrogenases. J. Mol. Model., 8, 58-64. (2002)	PU
Article	K. L. Kovács, B. Fodor, Á. T. Kovács, Gy. Csanádi, G. Maróti, J. Balogh, S. Arvani and G. Rákhely. Hydrogenases, accessory genes and the regulation of [NiFe] hydrogenase biosynthesis in *Thiocapsa roseopersicina*. Int. J. of Hydrogen Energy, 27, 1463-1469. (2002)	PU
Article	G. Maróti, B. D. Fodor, G. Rákhely, Á. T. Kovács, S. Arvani, K. L. Kovács. Accessory proteins functioning selectively and pleiotropically in the biosynthesis of [NiFe] hydrogenases in *Thiocapsa roseopersicina*. Eur. J. Biochem. (2003) [in press]	PU
Article	Á. T. Kovács, G. Rákhely, K. L. Kovács. Genes involved in the biosynthesis of photosynthetic pigments in the purple sulfur photosynthetic bacterium *Thiocapsa roseopersicina*. Appl. Environ. Microbiol. (2003) [in press]	PU

2.2. Quantified data about the result

Items (about the results)	**Actual current quantity** [a]	**Estimated (or future) quantity** [b]
Time to application / market (in months from the end of the research project)		
Number of (public or private) entities potentially involved in the implementation of the result :		
of which : number of SMEs :		
of which : number of entities in third countries (outside EU) :		
Targeted user audience: # of reachable people		
# of S&T publications (referenced publications only)		
# of publications addressing general public (e.g. CD-ROMs, WEB sites)		
# of publications addressing decision takers / public authorities / etc.		
Visibility for the general public	Yes	

[a] *Actual current quantity = the number of items already achieved to date.*

[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve within the next 3 years.*

2.3. Further collaboration, dissemination and use of the result

(Optional; to be completed if partner is willing to set up new collaborations, and seeking dissemination support from the CORDIS services.)

COLLABORATIONS SOUGHT

Please tick appropriate boxes (✓) corresponding to your needs.

R&D	Further research or development	X	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing	☐	**INFO**	Information exchange	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

I confirm the information contained in part 2 of this Technological Implementation Plan and I authorise its dissemination to assist this search for collaboration.

Signature: **Name: Kornel L. Kovacs**

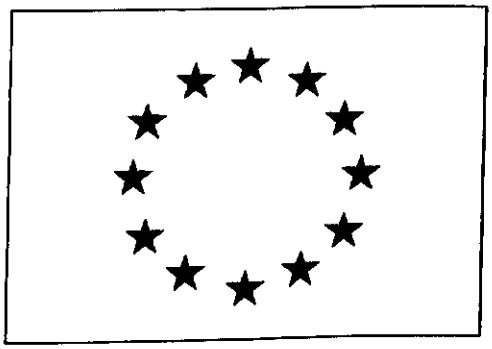
Date:23. 04. 2003. **Organisation: U-Szeged**

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

NTUA



Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 2 Description of each result

2.1 : Description of the result(s), one form per result

CONTACT PERSON FOR ALL RESULTS

Name	Emmanouel Koukios
Position	Professor
Organisation	National Technical University of Athens
Address	NTUA Zografou Campus 15700 Athens GREECE
Telephone	+30 210 7723191
Fax	+30 210 7723163
E-mail	Koukios@chemeng.ntua.gr
URL	http://btu.chemeng.ntua.gr/
Specific Result URL	http://www.biohydrogen.nl

No. & TITLE OF RESULT

No.	Self-descriptive title of the result
12	Production of sweet sorghum

SUMMARY *(200 words maximum)*

The experiment was established at Pirgos, west Greece. The area of the experimental field was 150 m^2 (10 m x 15 m). The soil of the field had a homogeneous profile with a clay loam texture. We cultivated two different sweet sorghum varieties 'Keller' and 'M81E'. The seeds were bought from Kentucky, US. Both varieties were sown on 5th of May 2002. The distance between rows was 70cm and within rows 20 cm. This gives us a plant density of 71000 plants/ha. The nitrogen fertilization rate was 120 kg/ha while the phosphorus rate was 100 kg/ha. The water requirements were less than normal due to the too wet weather. Seven harvests were made during the growing period and the following parameters were measured: fresh and dry matter yields, number of leaves, fresh and dry matter of leaves and crop height. The final harvest was made on 16th of September. The total cost of sweet sorghum production was estimated at about 900€/ha. The production yield for 'Keller' variety was 125 t/ha while for 'M81E' was 89 t/ha.

Please categorise the result using codes from Annex 1

Subject descriptors	13	142	201	496	503

No. & TITLE OF RESULT

No.	Self-descriptive title of the result
13	Detailed characterisation protocols for sweet sorghum

SUMMARY *(200 words maximum)*

Detailed characterisation protocols were designed for sweet sorghum, sweet sorghum juice and for the sweet sorghum bagasse. Stalks were cut to pieces of 5-10 cm and were then ground in a laboratory grind. Moisture and ash content were determined according ASTM D-2016 and ASTM D-1102 respectively. Soluble sugars, i.e. glucose sucrose and other reducing sugars were determined after two successive water extraction in water (2,5 % w/v consistency) at 80^{0}C for 1 hour each. Glucose concentration was determined with the enzymatic method GOD/PAP and reducing sugars by the DNS method (Miller 1959). Sucrose was determined after acid hydrolysis with concentrated HCl to glucose and fructose at 60^{0}C for 15 min. After neutralization, the glucose content was determined enzymically and sucrose was calculated by the difference between glucose content before and after hydrolysis. The residue after water extraction was air dried and then was quantitatively saccharified (Saeman et al., 1945). Cellulose and hemicellulose content was determined from glucose and reducing sugars concentration of the filtrate. Acid insoluble lignin content was determined after filtration and drying of acid insoluble solids at 110 ^{0}C for 24h and weight correction for ash content. Acid soluble lignin was determined with TAPPI UM 250 method.

Please categorise the result using codes from Annex 1

Subject descriptors	28	155	201	496	503

No. & TITLE OF RESULT

No.	Self-descriptive title of the result
14	Characterisation of sweet sorghum

SUMMARY *(200 words maximum)*

Keller stalks are very rich in carbohydrates– almost 90% (w/w dry basis). Total sugar content accounts for 45% of stalks dry mass. Cellulose and hemicellulose content taken together is of the order of 35%. Total lignin content is 9%, while ash content is 3%. Moisture content was about 70% .Sweet sorghum bagasse characterisation (after two successive extractions 2,5 % w/v consistency at 80^0C) showed that it is a lignocellulosic material. Cellulose and hemicellulose together account for 77% and total lignin for 20% of the bagasse dry mass. Ash concentration is 3%. Sugars concentration in the sweet sorghum juice depends on the extraction procedure selected. A simple pressing (70 bar) of ground stalks results in a total sugar concentration of 10% The recovery of sugars in the juice was about 75%. Fermentability test of the juice were carried out and showed that is a suitable feedstock for hydrogen production

Please categorise the result using codes from Annex 1

Subject descriptors	28	142	201	496	503

No. & TITLE OF RESULT

No.	Self-descriptive title of the result
15	Pretreatment procedures and optimisation of sugar fractionation from sweet sorghum

SUMMARY *(200 words maximum)*

Three different procedures where tested:

- Pressing of stalks
- Water addition and thermal pretreatment prior pressing
- Water extraction and water addition prior pressing

Additionally a central composite design based in the second procedure was applied with four design factors (temperature, duration of thermal pretreatment, subjected pressure and the amount of water added before pressing).

From all these experiment it was shown that the quantity and the quality of the sweet sorghum juice depend on the sugar extraction procedure that is used. With the first procedure the resulting juice has a sugar concentration of 108g/l but on the other hand 25-30% of sweet sorghum total sugars remain in the bagasse. With the second procedure the quantity of sugars remaining in the bagasse is reduced but sugar concentration of the juice is up to 89g/l. With the third procedure we can extract up to 98% of total sugar present in sweet sorghum stalks. Sugar concentration of the resulting juice is 9,83g/l. Fermentability tests carried out by ATO showed that the juice produced in all these procedures is suitable for hydrogen production

Please categorise the result using codes from Annex 1

Subject descriptors	142	155	201	496	503

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
16	Utilisation of the by-product, bagasse, from sweet sorghum for pulp production. Additive to composite materials or biofuel

SUMMARY *(200 words maximum)*

In order to achieve 'whole crop' utilisation the following procedures were tested:

- Enzymatic hydrolysis of the bagasse

Experiments carried out by BUTE showed that the bagasse can be used for fermentable sugars production.

- Paper pulp production

Bagasse can be used for pulp production. With alkali delignification, lignin content was reduced by 72% and 80% during the 1and 2 hours process respectively, while the sum of cellulose and hemicellulose was slightly affected

- Composite materials production

Addition of sweet sorghum bagasse to concentrations up to 20% doesn't decrease the maximum load that can be subjected to the samples, but addition of bagasse even at very low concentrations decrease maximum elongation of the material

- Use of bagasse as solid fuel

Gross calorific value of the bagasse is 4150 Kcal/Kg

Please categorise the result using codes from Annex 1

Subject descriptors	93	124	201	462	496

CURRENT STAGE OF DEVELOPMENT

Please tick one category only ✓

Scientific and/or Technical knowledge (Basic research)	X
Guidelines, methodologies, technical drawings	☐
Software code	☐
Experimental development stage (laboratory prototype)	☐
Prototype/demonstrator available for testing	☐
Results of demonstration trials available	☐
Other (please specify.):	☐

DOCUMENTATION AND INFORMATION ON THE RESULT

List main information and documentation, stating whether public or confidential.

Documentation type	**Details(Title, ref. number, general description, language)**	**Status: *PU*=Public *CO*=Confidential**
Article	Glynos A, Koullas D, Koukios E, 2001, 'Hydrogen production from biomass for use in fuel cells' 3rd National Chemical engineering Conference, Vol. 2, p. 1153-1156	PU
Article	Glynos A, Koullas D, Koukios E, 2001, 'Hydrogen production from sweet sorghum for use in fuel cells' Renewable Energy Sources Conference p.498-501	PU
Article	Panopoulos K, Euaggelou D, Glynos A, Koullas D, Koukios E, 2003, 'Biochemical and thermochemical utilisation of sweet sorghum for energy production' 4th National Chemical engineering Conference, p. 585-588	PU

MARKET APPLICATION SECTORS

Please describe the possible sectors for application using the NACE classification in Annex 2.

Market application sectors	01	21	25	40	45

2.2. Quantified data about the result

Items (about the results)	Actual current quantity [a]	Estimated (or future) quantity [b]
Time to application / market (in months from the end of the research project)		
Number of (public or private) entities potentially involved in the implementation of the result :		
of which : number of SMEs :		
of which : number of entities in third countries (outside EU) :		
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# of publications addressing general public (e.g. CD-ROMs, WEB sites)		
# of publications addressing decision takers / public authorities / etc.		
Visibility for the general public	Yes	

[a] *Actual current quantity = the number of items already achieved to date.*
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(Optional; to be completed if partner is willing to set up new collaborations, and seeking dissemination support from the CORDIS services.)

COLLABORATIONS SOUGHT

Please tick appropriate boxes (✓) corresponding to your needs.

R&D	Further research or development	X	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing	☐	**INFO**	Information exchange	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

I confirm the information contained in part 2 of this Technological Implementation Plan and I authorise its dissemination to assist this search for collaboration.

Signature: **Name: Emmanuel Koukios**

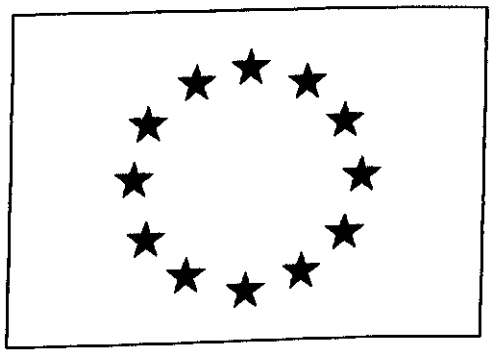
Date: 28. 05. 2003. **Organisation: NTUA**

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

TUBUD



☐ Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 2 Description of each result

2.1 : Description of the result(s), one form per result

CONTACT PERSON FOR ALL RESULTS

Name	Kati Réczey
Position	associate professor
Organisation	Budapest University of Technology and Economics
Address	1521 Budapest Szt Gellért tér 4, Hungary
Telephone	+36 1 463 2843
Fax	+361 463 2598
E-mail	kati_reczey@mkt.bme.hu
URL	http://www.nonfood.bme.hu
Specific Result URL	http://www.biohydrogen.nl

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
17	Composition analysis and evaluation of enzymatic hydrolysis of paper sludge samples for the production of sugars supporting hydrogen fermentation

SUMMARY *(200 words maximum)*

Paper sludge samples were collected from three different paper mills using both secondary and primary fibres for papermaking. Samples were analysed for carbohydrate (cellulose, xylan, arabinan) lignin and ash contents over a certain time periods to see how chances in operation conditions affect the composition. Also the enzymatic digestibility using commercial cellulases (Celluclast 1.5L and Novozyme 188) has been determined first in small scale and then in a 31 litre lab pH and temperature controlled stirred reactor. Over the time periods evaluated no significant changes in paper sludge composition have been observed. The average cellulose content of samples resulting from the technology utilizing secondary fibre was about 40% DM, while the paper sludge resulting from primary fibre processing contained only 14% DM cellulose. The enzymatic digestibility of residues obtained from primary fibre processing were about 60% higher than that of obtained from secondary fibre processing. However, due to the high ash and low cellulose content of primary fibre processing residues (about 70% DM) it is less economically feasible than secondary fibre processing residue utilization for fuel hydrogen production. Based on the results of small scale enzymatic hydrolysis tests some of the paper sludge samples obtained from secondary fibre processing were hydrolysed in lab scale controlled reactor. With these samples about 85% of the potentially available cellulose was converted to glucose.

Please categorise the result using codes from Annex 1

Subject descriptors	59	81	93	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
18	Pre-treatment and enzymatic hydrolysis of sweet sorghum bagasse

SUMMARY *(200 words maximum)*

The primary source of fermentable sugars in this project was the juice of sweet sorghum. After extracting the water-soluble sugars from the crop, substantial part of the produced biomass stays in the bagasse containing significant amounts of carbohydrates in polymeric form such as cellulose and xylan. The enzymatic hydrolysis of the cellulose present in the bagasse has been examined in two sets of experiments. In the first set no pre-treatment, while in the second set chemical pre-treatment (acid and alkaline) of the lignocellulosic biomass has been applied prior to enzymatic hydrolysis. Both alkaline and acid pre-treatment increased the accessibility of the bagasse compared to the untreated material. Alkaline treatment proved to be superior to acid treatment.

Please categorise the result using codes from Annex 1

Subject descriptors	59	81	93	201	307

No. & TITLE OF RESULT (same as in table 1.2)

No.	Self-descriptive title of the result
19	Characterisation of thermostable cellulases

SUMMARY *(200 words maximum)*

In order to support high temperature SSF of lignocellulosics materials for the production of fuel hydrogen, thermostable cellulases are needed. Commercially available thermostable cellulase enzymes has been asked from numerous leading enzyme producing companies, however only one preparation was received. The pH and temperature optima of the sample cellulase have been determined, however the temperature optimum was far lower than needed to support high temperature SSF. From literature survey *Thermoascus aurantiacus* has been selected as it was reported to produce the whole set of cellulases with high temperature optimum. The cellulases produced by this organism have been characterised regarding pH and temperature optima. Also the thermal stability has been evaluated. The optimal pH has been found to be 4.8. In spite of the expected, both the thermal optimum (65°C) and stability (half-life time 3.2 hours) were less than required for high temperature processing of lignocellulosic biomass.

Please categorise the result using codes from Annex 1

Subject descriptors	59	81	93	201	307

CURRENT STAGE OF DEVELOPMENT

Please tick one category only ✓

Scientific and/or Technical knowledge (Basic research)	X
Guidelines, methodologies, technical drawings	☐
Software code	☐
Experimental development stage (laboratory prototype)	☐
Prototype/demonstrator available for testing	☐
Results of demonstration trials available	☐
Other (please specify.):	☐

DOCUMENTATION AND INFORMATION ON THE RESULT

List main information and documentation, stating whether public or confidential.

Documentation type	**Details (Title, ref. number, general description, language)**	**Status: *PU*=Public *CO*=Confidential**
Article	Zs. Kádár, T. de Vrije, G. G. de Haas, M. A. W. Budde, Zs. Szengyel, K. Réczey, P. A. M. Claassen, Hydrogen Production from Paper Sludge Hydrolysate (2003) *Appl. Biochem. Biotech.* (in press)	PU
Article	Zs. Kádár, T de Vrije, G E. van Noorden, M A. W. Budde, Zs. Szengyel, K. Réczey, P. A. M. Claassen, Hydrogen Yields from Paper Sludge Hydrolysate by the Extreme Thermophile *Caldicellulosiruptor saccharolyticus* (2003) manuscript	PU

2.2. Quantified data about the result

Items (about the results)	**Actual current quantity [a]**	**Estimated (or future) quantity [b]**
Time to application / market (in months from the end of the research project)		
Number of (public or private) entities potentially involved in the implementation of the result :		
of which : number of SMEs :		
of which : number of entities in third countries (outside EU) :		
Targeted user audience: # of reachable people		
# of S&T publications (referenced publications only)		
# of publications addressing general public (e.g. CD-ROMs, WEB sites)		
# of publications addressing decision takers / public authorities / etc.		
Visibility for the general public	Yes	

[a] *Actual current quantity = the number of items already achieved to date.*

[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve within the next 3 years.*

2.3. Further collaboration, dissemination and use of the result

(Optional; to be completed if partner is willing to set up new collaborations, and seeking dissemination support from the CORDIS services.)

COLLABORATIONS SOUGHT

Please tick appropriate boxes (✓) corresponding to your needs.

R&D	Further research or development	X	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing agreement/Franchising	☐	**INFO**	Information exchange	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

I confirm the information contained in part 2 of this Technological Implementation Plan and I authorise its dissemination to assist this search for collaboration.

Signature: **Name: Kati Réczey**

Date:15. 04. 2003. **Organisation: TUBUD**

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

Air Liquide

DATA SHEETS

Preliminary version at mid-term (optional, program per program)

[X] Final version before final term (contractual obligation)

Part 1: Overview and description of all your project and its results

Part 2: Description of the intentions for dissemination and use by each partner

Confidential

This section enables each partner - individually or as a consortium – to describe its use and dissemination intentions (including a timetable of its future activities).

2.1 : Description of the use and the dissemination of result(s)
2.2 : Quantified data by partners

Part 3: Search for Collaboration through Commission services (optional)

Publishable

Part 4: Assessment of the European interests

Publishable

Part 2 Description of the intentions by each partner

This part 2 must be completed by each partner who is essential for the dissemination and use (i.e. result owners and/or major project contributors and/or major dissemination and use contributors). Each will detail its own use and dissemination intentions concerning the result(s) they are involved with. This description must be made result by result.

These different parts may be transmitted to the Commission either assembled at the consortium level, or individually by each partner to safeguard confidential matters if necessary (through any appropriate media). Obviously, when all partners are implementing a single dissemination and use scheme all together, a single part 2 is needed.

PARTS 2 WILL ALWAYS BE KEPT CONFIDENTIAL BY THE COMMISSION

2.1 : Description of the use and the dissemination of result(s), partner per partner

MANDATORY INFORMATION :

CONTRACT NUMBER :	**QLK5-CT-1999-01267**
PARTNER's NAME :	**AIR LIQUIDE**
PARTNER's WEB SITE (if any) :	www.airliquide.com

CONTACT PERSON(S):

Name	ALLIDIERES, Laurent.
Position/Title	H2 Fuel Product Manager
Organisation	Air Liquide, Advanced Technology Division
Address	Rue de Clémencière BP 15 38360 Sassenage, France
Telephone	+33 (0) 4 76 43 64 94
Fax	+33 (0) 4 76 43 60 95
E-mail	Laurent.Allidieres@airliquide.com

No, TITLE ***(as in section 1.2)*** **AND BRIEF DESCRIPTION OF MAIN RESULT(S)**

1	**WP5 : Reactor design**
2	Description of main results The use of nitrogen as a stripping gas is difficult as the separation of N2 from Hydrogen can only be done economically with limited hydrogen recovery. The stripping gas was therefore change to CO2 which can be fed to the fuel cell, and which could be easily separated from the hydrogen in case the fuel cell cannot accept CO2. The gas composition at the outlet of the reactor has been found at H2 0.366837 10^5 Pa CO2 0.180118 10^5 Pa CH3COOH 0.000055 10^5 Pa H2O 0.452945 10^5 Pa
3	**WP6 : H2 recovery**
4	Description of main results Different separation processes have been studied for the separation of N2 from a H2+N2 mixture. The use of nitrogen as a stripping gas is difficult as the separation of nitrogen from Hydrogen can only be done economically by molecular sieve reactor with pressure swing adsorption units (PSA) with limited hydrogen recovery. Membrane separation of Hydrogen and Nitrogen was shown not to be economically interesting. Cryogenic separation would be more complex to integrate (use of cold source necessary) Switching from N2 to CO2 as a stripping gas has shown a great interest in the downstream separation reactor design. Separating CO2 from H2 +CO2 is easier than separating N2 from H2 +N2. Moreover, the CO2+H2 mixture could be directly fed to the fuel cell with limited adaptation of the fuel cell. However, the influence of acetic acid (CH2COOH) on the fuel cell membrane has to be checked, as well as all the components which were not listed in the gas analysis coming from the reactor.

5	**WP7 : Safety**
6	Description of main results The H2+CO2 mixture will be fed directly to the fuel cell.

FOR EACH MAIN RESULT, TIMETABLE OF THE USE AND DISSEMINATION ACTIVITIES WITHIN THE NEXT 3 YEARS AFTER THE END OF THE PROJECT

Mention the use and dissemination related activities, the main associated partners, the related milestones and give an indicative timescale		
Activity	**Brief description of the activity, including main milestones and deliverables (and how it relates to data in sections 1.5 and 2.2).**	**Timescale (months)**
WP5 : Reactor design	Gas composition out of the bio reactor. Change of the stripping gas.	M36
WP6 : H2 recovery	Trade off study between different gas purification routes : - Membrane - PSA - Cryogenic purification Optimisation of the biogas composition Reconsideration of the use of a gas separator in case of CO2 stripping.	M36
WP7 : Safety	Rapid risk ranking of the purification processes.	M36

FORESEEN COLLABORATIONS WITH OTHER ENTITIES

Please tick appropriate boxes (✓) corresponding to your most probable follow-up.

R&D	Further research or development	X	**FIN**	Financial support	
LIC	Licence agreement		**VC**	Venture capital/spin-off funding	
MAN	Manufacturing agreement		**PPP**	Private-public partnership	
MKT	Marketing		**INFO**	Information exchange, training	X
JV	Joint venture		**CONS**	Available for consultancy	
			Other	(please specify)	

2.2 : Quantified data for each partner's main result

Items	Currently achieved quantity [a]	Estimated future quantity [b]
Economic impacts (in EURO)	0	0
# of licenses issued (within EU)	0	0
# of licenses issued (outside EU)	0	0
Total value of licenses (in EURO)	0	0
# of entrepreneurial actions (start-up company, joint ventures…)	0	0
# of direct jobs created [c]	0	0
# of direct jobs safeguarded [c]	0	0
# of direct jobs lost	0	0

[a] *The added value or the number of items already achieved to date.*

[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve in the future (i.e. expectations within the next 3 years following the end of the project).*

[c] *"Direct jobs" means jobs within the partner involved. Research posts are to be excluded from the jobs calculation*

 # = number of ...

I confirm the information contained in part 2 of this Technological Implementation Plan and I certify that these are our exploitation intentions

Signature: **Name : Laurent ALLIDIERES**

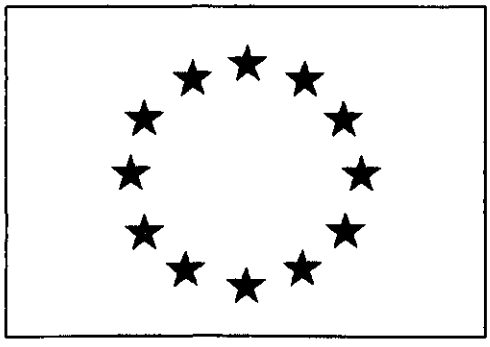
Date: 24/01/03

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

ATO



Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 3 Description of the intentions by each partner

General comment concerning part 3

Part 3 is only partially applicable to the Biohydrogen project since the project requires further Research and Development before coming into the implementation stage. Attempts will be made by all partners to acquire future funding for research related to the production of hydrogen from biomass.
Unfortunately, the first attempt, i.e. the submission of an Integrated Project, Stairway to Hydrogen, in the first call of FP 6 , Sustainable Energy Systems, March 18th 2003, did not succeed. The main comments referred to the enormous size of the consortium (45 participants). As a result, a second project proposal will be prepared by the same coordinator, which will be reduced in size and submitted in the first available call of FP6.
Besides, attempts for obtaining funding from national organisations have been made.
The coordinator Agrotechnology & Food Innovations (formerly ATO) has succeeded in obtaining a national grant for studying biological hydrogen production from Miscanthus and potato steam peels.

3.1 : Description of the use and the dissemination of result(s), partner per partner

MANDATORY INFORMATION :

CONTRACT NUMBER :	**QLK5-1999-01267**
PARTNER's NAME :	**ATO**

CONTACT PERSON(S):

Name	Pieternel Claassen
Position	Senior scientist
Organisation	ATO bv
Address	P.O. Box 17 , 6700 AA Wageningen, The Netherlands
Telephone	31.317.475325
Fax	31.317.475347
E-mail	Pieternel.claassen@wur.nl

No, TITLE AND BRIEF DESCRIPTION OF MAIN RESULT(S)

7	Production of hydrogen from sweet sorghum by (hyper) thermophilic bacteria Twenty-five samples of sweet sorghum juice which had been extracted using a factorial analysis with temperature (24-66 °C), extraction time (0.6-3.4 h), pressure (64-106 bar) and water (-8-48 mL) as variables, were tested for fermentability. Even though some differences in sugar composition, most probably due to remaining invertase activity, was observed, there was no significant difference in hydrogen production by *Caldicellulosiruptor saccharolyticus* using non-filtered and non-sterilized sweet sorghum juice. Fermentation of non-diluted sweet sorghum juice, supplemented with all required nutrients, showed rapid hydrogen production to a final yield of 63% of the theoretical maximum at a production rate of 21 mmol/L.h. Sucrose and fructose were the preferred substrates. After 16 hours of fermentation growth already stopped, probably due to inhibition by acetate triggering cell lysis. The results showed that sweet sorghum juice is an excellent substrate for hydrogen fermentation. In view of future scaling-up, preliminary experiments were done with *C. saccharolyticus* as well as *Thermotoga elfii*, to reduce the requirements for nutrients in the medium. Here *C. saccharolyticus* came out best although the simultaneous omission yeast extract and salts significantly decreased the hydrogen yield. This may have been due to the limitation of a simple nutrient like Mg or Fe. The other hyperthermophilic organism, *T. elfii* was much more dependent on supplementation with nutrients and thus seems less suited for making hydrogen from sweet sorghum juice.

8	Production of hydrogen from paper sludge hydrolysate by *Caldicellulosiruptor saccharolyticus* The potential of paper sludge hydrolysate for hydrogen production was tested using *C. saccharolyticus*. Firstly, growth and hydrogen production from a mixture of glucose and xylose (the main constituents in paper sludge hydrolysate) was established. Hydrogen production was 65-70% of the theoretical maximum amount and occurred at a maximum rate of 8.5-10.2 mmol H_2/L.h. These yields and production rates are comparable to data from fermentations on glucose or xylose, separately. Xylose seemed to be the preferred substrate for hydrogen production by *C. saccharolyticus*, as usually it was consumed before glucose consumption started. In cultures with paper sludge hydrolysate as the sole carbon and energy source, but supplemented with all nutrients, all carbohydrates were consumed and hydrogen production was circa 50% of the theoretical maximum, occurring at a rate of 3.4-6 mmol H_2/L.h. When only yeast extract was supplied as nutrient, hydrogen yield and rate were similar. These lower yields and rates were due to the production of lactate instead of acetate and hydrogen was produced. Fortunately, lactate is also a prime substrate for the subsequent photofermentation. As such, the energy in lactate will be recovered in more hydrogen being produced in the second fermentation

FOR EACH MAIN RESULT:

TIMETABLE OF THE USE AND DISSEMINATION ACTIVITIES WITHIN THE NEXT 3 YEARS AFTER THE END OF THE PROJECT

Mention the use and dissemination related activities, the main associated partners, the related milestones and give an indicative timescale		
Activity	**Brief description of the activity, including main milestones and deliverables (and how it relates to data in sections 2.2 and 3.2).**	**Timescale (months)**
Publication in scientific journals	**Parts of main results 7 and 8 will be published in 1-2 articles**	**24-48**
Public availability	**After the publications are in print, electronic copies become publicly available at our website www.biohydrogen.nl**	**24-48**
Seminars	**The main results 7 and 8 will be used for presentation at conferences**	**0-24**

FORESEEN COLLABORATIONS WITH OTHER ENTITIES

Please tick appropriate boxes (✓) corresponding to your most probable follow-up.

R&D	Further research or development	x	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing agreement/Franchising	☐	**INFO**	Information exchange, training	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

3.2 : Quantified data for each partner's main result

Items	Currently achieved quantity [a]	Estimated future quantity [b]
Economic impacts (in EURO)		
# of licenses issued (within EU)		
# of licenses issued (outside EU)		
Total value of licenses (in EURO)		
# of entrepreneurial actions (start-up company, joint ventures…)		
# of direct jobs created [c]		
# of direct jobs safeguarded [c]		
# of direct jobs lost		

[a] *The added value or the number of items already achieved to date.*

[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve in the future (i.e. expectations within the next 3 years following the end of the project).*

[c] *"Direct jobs" means jobs within the partner involved. Research posts are to be excluded from the jobs calculation*

 # = number of...

I confirm the information contained in part 3 of this Technological Implementation Plan and I certify that these are our exploitation intentions

Signature: PAn Claassen

Date: December 12, 2003

Name: Pieternel Claassen

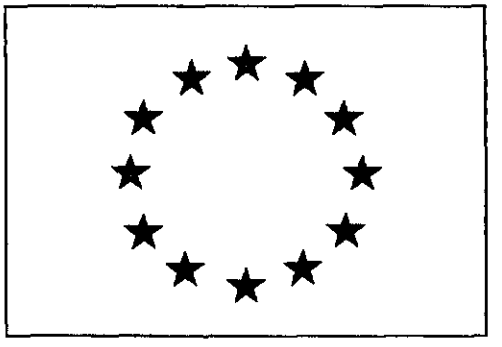
Organisation: ATO bv
(from Oct 1st, 2003: Agrotechnology &Food Innovations)

TECHNOLOGICAL IMPLEMENTATION PLAN

A Framework for the further development, dissemination and use of the results of EC RTD Projects (including also thematic networks and concerted actions)

DATA SHEETS

WU



Preliminary version at mid-term (optional, programme per programme)

X Final version before final term (contractual obligation)

Part 3 Description of the intentions by each partner

3.1 : Description of the use and the dissemination of result(s), partner per partner

MANDATORY INFORMATION :

CONTRACT NUMBER :	**QLRT-1999-01267**
PARTNER's NAME :	**WAU**

CONTACT PERSON(S): Dr A.J.M. Stams & Dr E.W.J. van Niel

Name	Van Niel, Ed
Position/Title	Senior lecturer/Dr
Organisation	Lund University
Address	P.O. Box 124, S-221 00 Sweden
Telephone	+46 46 2220619
Fax	+46 46 2224203
E-mail	Ed.van_Niel@tmb.lth.se

No, TITLE AND BRIEF DESCRIPTION OF MAIN RESULT(S)

1	**Selection of the best hydrogen producing extremely thermophiles.** From experimental examination of both pure cultures of culture collections and samples taken from hot springs it was concluded that up to now the described bacterium *Caldicellulosiruptor saccharolyticus* is the most promising candidate for hydrogen production at elevated temperatures (70°C). This organism is versatile in its use of substrates, it survives high partial hydrogen pressures (by shifting its metabolism) and showed to be of a robust nature when applied in a pilot plant scale thermoreactor.
2	**Factors affecting hydrogen production by *C. saccharolyticus*.** The influence and kinetics of the following physicochemical factors on hydrogen production by *C. saccharolyticus* have been studied: • Hydrogen is the most severe inhibitor; the estimated dissolved hydrogen concentration that inhibits growth: 500 µMol at 70°C; • Sugars and salts (e.g. the fermentation product sodium acetate) inhibit by increasing the external osmotic potential; • High concentrations of monovalent ions enhance cell lysis; • Carbon dioxide can be used as stripping gas to avoid hydrogen accumulation in the cultures. However, significant concentrations of bicarbonate will be formed enhancing the osmolality of the system, which negatively influences the performance of the organism. This effect can be kept to a minimum by keeping a strict pH-regime of 6.5-7.0;

	• Hydrogen production activity has a slightly wider temperature range (estimation: 40-90°C) than growth (49.6 – 85.7°C)
3	**Physiology of *C. saccharolyticus*.** The following major findings were made with respect to the physiology of *C. saccharolyticus*: • Yeast extract is required for growth, but could be replaced by casamino acids plus proline and vitamins; • *C. saccharolyticus* prefers xylose, arabinose, sucrose, cellobiose to glucose. This unusual feature makes this organism complementary to other hydrogen producers. This property could be beneficiary in the application of consortia of extremely thermophilic hydrogen producers for optimal performance of hydrogen production from complex sugar mixtures (such as biomass); • Extreme thermophiles, such as *C. saccharolyticus*, show superior hydrogen yields (83% of the maximal theoretical obtainable) compared to maximal 50% found with mesophiles and moderate thermophiles; • Sugars are converted to hydrogen and acetate through the EMP-pathway. At a partial hydrogen pressure (pH_2) $\geq 1 \cdot 2 \cdot 10^4$ Pa the metabolism shifts partially to lactate formation. There are indications that this pathway to lactate is controlled by the internal redox potential. At high dissolved hydrogen concentrations also the reductive acetate pathway is activated. It is thus of the utmost importance that hydrogen is stripped from the fermentation broth. • The external redox potential is not an adequate parameter to monitor the internal redox potential to signal any metabolic shifts.
4	Conversion of acetate to hydrogen and carbon dioxide by photofermentation The following aspects concerning the photoheterotrophic conversion of acetate to hydrogen by *Rhodopseudomonas* sp. were demonstrated: • Hydrogen production is catalysed by the nitrogenase enzyme complex. The only hydrogenase which seemed to be active is an uptake hydrogenase. • Exposure to low concentrations of carbon monoxide (1-2 % v/v in headspace) did not lead to extra hydrogen production and it even led to an increase of the lag phase in batch experiments. • Hydrogen production can go together with growth, but it is still unclear whether growth itself is a prerequisite for hydrogen production. • Biomass growth during hydrogen production can be supported with glutamate as a nitrogen source, but this was accompanied with considerable excretion of reduced soluble products. Maximally 60% of the acetate could be directed to hydrogen gas and carbon dioxide at acetate/glutamate ratios of 5 or less. • Biomass growth during hydrogen production can also be supported with limiting amounts of ammonia. In this situation the major part of the acetate could be accounted for as biomass and hydrogen/carbon dioxide produced. Even more so, preliminary results indicated that severe limiting of ammonia could increase the fraction of acetate directed to hydrogen to more than 80%. • Using high-density cultures of another purple non-sulfur bacterium, *Rhodobacter*, photosynthetic efficiencies of 4 % were reached at light intensities of 180 W m^{-2} in the wavelength range of 400 to 950 nm (91 mmol H_2 h^{-1} m^{-2} light exposed surface). High-density cultures apparently provide a way to approach the theoretical maximal efficiency, which is approximately 10.6 % considering sunlight between 400 and 950 nm.

5	A mathematical growth model for photofermentation A preliminary model was developed describing growth and hydrogen production of purple non-sulfur bacteria under ammonia-limited conditions. A number of model parameters were estimated and still have to be determined in future work. Also, the model still needs to be validated. Nevertheless, the model provided valuable insight in the process and the following important aspects of the process of photoheterotrophic hydrogen production were distinguished: • In order to increase the yield of hydrogen on acetate it is crucial to minimize biomass growth using severe ammonia limitation. As a result of the application of low specific growth rates also (maintenance related) biomass degradation becomes an important factor which should be determined. • The application of low specific growth rates could lead to the application of voluminous photobioreactors with a long liquid residence time. On the other hand, the residence time and reactor volume could be minimized in case it is possible to immobilize or retain the biomass inside the photobioreactor. • The specific nitrogenase activity of the biomass is a potential limiting factor and, for this reason, the biomass concentration needs to be adjusted to the volumetric input of light. Model simulations have shown that the biomass densities required are realistic as compared to existing photobiological processes.
6	A photobioreactor with cultivation conditions and a full-scale process design Different scale-up strategies for full-scale photobioreactors were identified using the results of the mathematical model and previous studies in the area of algal biotechnology. The following guidelines should be taken into account designing a full-scale process: • Light intensity should be used at maximal efficiency and for this reason the photobioreactor surface should be exposed to low light intensities (roughly, less than 10% of full sunlight). Sunlight dilution is possible using vertical photobioreactor systems with a high area to volume ratio. • Full sunlight intensities also can be utilized at high efficiency on the condition high-density cultures (> 10 g L^{-1}) are used in short light-path (< 1.5 cm) photobioreactors with adequate mixing (see 3.3). • The biomass density always needs to fit the volumetric light input given the potentially limiting specific nitrogenase activity of the biomass. Using high-density cultures and low specific growth rates (see 3.4) this would lead to the need of a biomass retention system. As a first step a lab-scale 2.5 L panel photobioreactor was developed. In this system a short light-path can be applied (3 cm or less). In order to be able to provide adequate mixing a gas recirculation system was developed. This resulted in a pneumatically agitated panel photobioreactor and it was tested successfully. This photobioreactor system is an ideal platform to test and develop the most suitable pilot-scale design considering the guidelines given. Furthermore, a literature survey was done to make a preliminary cost analysis of a full-scale phototrophic process.

FOR EACH MAIN RESULT:

TIMETABLE OF THE USE AND DISSEMINATION ACTIVITIES WITHIN THE NEXT 3 YEARS AFTER THE END OF THE PROJECT

Mention the use and dissemination related activities, the main associated partners, the related milestones and give an indicative timescale		
Activity	**Brief description of the activity, including main milestones and deliverables (and how it relates to data in sections 2.2 and 3.2).**	**Timescale (months)**
Publication in scientific journal	**Parts of main results 2 and 3 will be published in 3-4 articles**	**24-48**
Public availability	**After the publications are in print, electronic copies become publicly available at our website www.hydrogen.nl**	**24-48**
Seminars	**The main results 1 to 3 will be used in seminars to be held at universities and workshops in the near future**	**0-24**
Research	**Research will focus on scale up of the photobioreactors for different phototropic processes**	**12**

FORESEEN COLLABORATIONS WITH OTHER ENTITIES

Please tick appropriate boxes (✓) corresponding to your most probable follow-up.

R&D	Further research or development	x	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing agreement/Franchising	☐	**INFO**	Information exchange, training	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

3.2 : Quantified data for each partner's main result

Items	Currently achieved quantity [a]	Estimated future quantity [b]
Economic impacts (in EURO)		
# of licenses issued (within EU)		
# of licenses issued (outside EU)		
Total value of licenses (in EURO)		
# of entrepreneurial actions (start-up company, joint ventures…)		
# of direct jobs created [c]		
# of direct jobs safeguarded [c]		
# of direct jobs lost		

[a] *The added value or the number of items already achieved to date.*
[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve in the future (i.e. expectations within the next 3 years following the end of the project).*
[c] *"Direct jobs" means jobs within the partner involved. Research posts are to be excluded from the jobs calculation*
 # = number of...

I confirm the information contained in part 3 of this Technological Implementation Plan and I certify that these are our exploitation intentions

Signature: **Name: E.W.J. van Niel**

Date:15-12-03

Part 3 Description of the intentions by each partner

This part 3 must be completed by each partner who is essential for the dissemination and use (i.e. result owners and/or major project contributors and/or major dissemination and use contributors). Each will detail its own use and dissemination intentions concerning the result(s) they are involved with. This description must be made result by result.

These different parts may be transmitted to the Commission either assembled at the consortium level, or individually by each partner to safeguard confidential matters if necessary (through any appropriate media). Obviously, when all partners are implementing a single dissemination and use scheme all together, a single part 3 is needed.

PARTS 3 WILL ALWAYS BE KEPT CONFIDENTIAL BY THE COMMISSION

3.1 : Description of the use and the dissemination of result(s), partner per partner

MANDATORY INFORMATION :

CONTRACT NUMBER :	**EC QLK5-1999-01267**
PARTNER's NAME :	**AIR LIQUIDE**

CONTACT PERSON(S):

Name	ALLIDIERES
Position/Title	Product Manager H2 FUel
Organisation	Division des Technologies Avancées
Address	2, rue de Clémencière, BP15 38360 Sassenage, France
Telephone	+33 (0) 4 76 43 64 94
Fax	+33 (0) 4 76 43 60 95
E-mail	Laurent.allidieres@airliquide.com

No, TITLE AND BRIEF DESCRIPTION OF MAIN RESULT(S)

1	Review of N2 separation in CO2 / H2 mixtures
2	Trade off of separation processes
3	Selection of most appropriate process
4	
5	

FOR EACH MAIN RESULT:

TIMETABLE OF THE USE AND DISSEMINATION ACTIVITIES WITHIN THE NEXT 3 YEARS AFTER THE END OF THE PROJECT

Mention the use and dissemination related activities, the main associated partners, the related milestones and give an indicative timescale		
Activity	**Brief description of the activity, including main milestones and deliverables (and how it relates to data in sections 2.2 and 3.2).**	**Timescale (months)**
FP6 – Stairway to Hydrogen	Not applicable. Program is not accepted by the commission.	

FORESEEN COLLABORATIONS WITH OTHER ENTITIES

Please tick appropriate boxes (%) corresponding to your most probable follow-up.

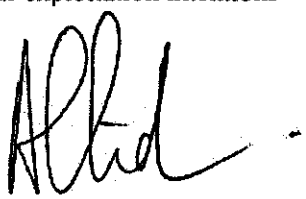
R&D	Further research or development	X	**FIN**	Financial support	☐
LIC	Licence agreement	☐	**VC**	Venture capital/spin-off funding	☐
MAN	Manufacturing agreement	☐	**PPP**	Private-public partnership	☐
MKT	Marketing agreement/Franchising	☐	**INFO**	Information exchange, training	☐
JV	Joint venture	☐	**CONS**	Available for consultancy	☐
			Other	(please specify)	☐

3.2 : Quantified data for each partner's main result

Items	Currently achieved quantity [a]	Estimated future quantity [b]
Economic impacts (in EURO)	0	0
# of licenses issued (within EU)	0	0
# of licenses issued (outside EU)	0	0
Total value of licenses (in EURO)	0	0
# of entrepreneurial actions (start-up company, joint ventures…)	0	0
# of direct jobs created [c]	0	0
# of direct jobs safeguarded [c]	0	0
# of direct jobs lost	0	0

[a] *The added value or the number of items already achieved to date.*
[b] *Estimated quantity = estimation of the quantity of the corresponding item or the number of items that you foresee to achieve in the future (i.e. expectations within the next 3 years following the end of the project).*
[c] *"Direct jobs" means jobs within the partner involved. Research posts are to be excluded from the jobs calculation*
 # = number of...

I confirm the information contained in part 3 of this Technological Implementation Plan and I certify that these are our exploitation intentions

Signature: 

Name: Laurent ALLIDIERES

Date: 12/12/2003