



PROJECT FINAL REPORT

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Executive summary

To support the sustainability of the future hydrogen economy, hydrogen has to come from renewable resources. Therefore hydrogen should be produced from water using 'green' electricity from solar or wind energy or from biomass.

In HyTIME, 6 industries, 2 universities and 1 research organisation have worked together to develop a process for production of hydrogen, at low temperature, from biomass resources having a high moisture content. This process is based on fermentation with natural micro-organisms and is an alternative to gasification of biomass. HyTIME leans heavily on the technology of anaerobic digestion but is special in having hydrogen as its product. In anaerobic digestion hydrogen is an intermediate product which is immediately consumed by hydrogen eating bacteria which make methane. In HyTIME the processes in anaerobic digestion are separated to make first a hydrogen fermentation which is then followed by methane production in separate anaerobic digester. Hydrogen is harvested and purified to make a product whereas the methane in the biogas is used to cover the heat demand of the process.

HyTIME has started with the successful mobilization of sugars from verge grass and wheat straw using mechanical and chemical pretreatment, followed by enzymatic hydrolysis. Hydrogen and subsequent methane production from wheat straw hydrolysates were successfully tested in simple stirred tank reactors. Although the hydrogen concentration in the raw gas was high (44% v/v), productivity and yield were fairly low. Hydrogen production from verge grass hydrolysate was tested in a dedicated high cell density reactor. Here hydrogen productivity and yield were high but the hydrogen concentration in the raw gas was lower (19%) due to dilution with nitrogen which is used as stripping gas in the dedicated reactor. Methane production using the effluent from the hydrogen reactor was successful. The volume of the dedicated hydrogen reactor was scaled up to 225L but as a result of contamination causing a decrease in yield, hydrogen productivity halted at 109 g hydrogen/day. For online monitoring and control of the fermentation special devices to measure performance indicators were developed and installed together with sensors for pH, pressure flow rate etc. Automation and visualization was realized to facilitate the management of the fermentation by means of a smart phone.

In order to enable efficient hydrogen upgrading, several membrane contactors were tested for removal of carbon dioxide from the fermenter off gas and the proof of principle was delivered by connecting 2 modules to the hydrogen reactor. At the same time, applicability and cost effects of conventional gas upgrading methods (VSA and PSA) were modelled and simulated for the recovery of hydrogen from the raw gas at low volumetric concentration, pressure and temperature.

For system integration all technological data over the entire process chain were collected to form the backbone of the simulation model for the techno-economic assessment at commercial scale. Optimization options for the process were identified. The foreseen methane production in the anaerobic digester would be sufficient to cover the heat demand of optimized process. Among the assessed process routes the chemical pretreatment of verge grass with lime seems to be the most favourable route concerning economics. In general, major cost contributions to hydrogen production costs resulted from biomass pretreatment. Besides lowering enzyme and chemical costs, an increase of sugar yields and avoidance of sugar losses would improve the techno-economic results.

Project context and objectives

Context

H₂ production by fermentation is a natural process which is part of the well-known anaerobic digestion (AD) which gives biogas as its end-product. In general, biogas contains circa 60 % methane and 40 % CO₂ depending on the type of biomass which has been used as feedstock. Anaerobic digestion has been developed in the past to replace aerobic waste-water treatment where aeration is an undesired cost factor. Currently, AD has become fashionable because of the production of methane which can add to the “green” energy supply whilst at the same time reduce disposal costs. Anaerobic digestion is the result of collaboration of several micro-organisms, which produce organic acids, H₂ and CO₂ in the acetogenic phase and consume organic acids, H₂ and CO₂ to produce methane in the methanogenic phase. The core issue of HyTIME is to separate the 2 phases which together form the AD process. HyTIME starts with a fermentation by specialist, natural bacteria which are selected because their efficient H₂ production from carbohydrates. Hydrogen and CO₂ are the gaseous products from this fermentation whereas the organic acids remain dissolved in the fermentation liquid. Hydrogen is separated from CO₂ by a novel process for gas upgrading. The liquid fraction with the organic acids, mainly acetic acid, is sent to a second vessel for AD. In this second step, biogas with methane and CO₂ is the gaseous end-product. In HyTIME, biomass is converted to pure H₂ and methane and CO₂.

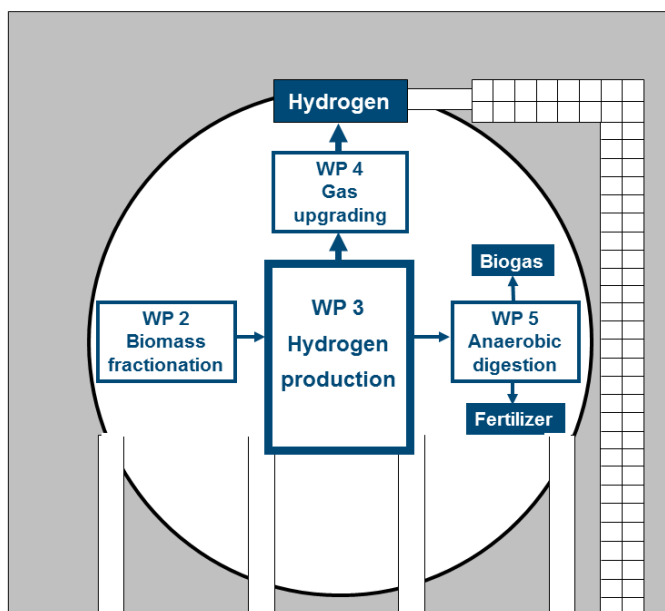


Fig. 1 Production of hydrogen, biogas and fertilizer by integration of hydrogen fermentation and biogas production using biomass as feedstock in HyTIME

The proof of principle of H₂ production from biomass has been delivered in previous RTD projects. These projects have been aimed at achieving a H₂ yield which approaches the theoretical maximum and at preventing the growth of methane producing bacteria. The unique approach of employing thermophilic H₂ producing bacteria rapidly growing at temperatures of above 70 °C has shown its success. HyTIME builds on this knowledge and is geared towards increasing the productivity whilst maintaining the high yield in H₂ fermentation.

The technique of H₂ fermentation is quite similar to the established AD. However, the employment of thermophilic bacteria at larger scale is a new challenge since these bacteria have not been extensively investigated. Since HyTIME is based on the separation of H₂ fermentation from methane production, the optimisation of the H₂ production is extremely important to make up for the additional investments needed to maintain 2 separate vessels plus auxiliary equipment. As a result, all activities to enable an efficient H₂ production system are integrated in the strategy of HyTIME.

The 9 participants, coming from small and large industries, universities and a research institute (Fig. 2) are spread over HyTIME to crosslink 4 technical work packages (WP's) that address the entire value chain from biomass logistics and pretreatment (WP 2) to thermophilic hydrogen production (WP 3) and gas upgrading technologies (WP 4). The integration of all these components of the hydrogen production system is done in WP 5 to bring progress in HyTIME to beyond the state-of-the-art of current fermentative hydrogen production (Fig.3).



Fig. 2 Spread over Europe of 9 partners in HyTIME. Industries are HYGear, Heijmans, ENVIRONMENT PARK, Awite, Wiedemann-Polska Projekt^{*)} and Veolia; RWTH and TUWIEN are universities and Food & Biobased Research is a research institute

^{*)}Wiedemann-Polska Projekt has left HyTIME in month 27

For the production of biomass, road side grass, straw and over-date residues from supermarkets are studied in terms of availability and suitability. Dedicated procedures are developed to decrease energy demand, use of chemicals and enzymes, formation of inhibitors, and to increase fermentability, keepability during fractionation and hydrolysis to mobilise sugars. The hydrolysates are tested for thermophilic fermentation and adjusted with required nutrients for optimum yield. The increase in productivity (kg H₂/ time) is addressed by increasing the concentration of the bacteria through immobilization on carriers or by making bacterial flocs. The approach is to employ designed co-cultures of *Caldicellulosiruptor species* in special bioreactors with a packed bed and gas dis-engager system. For removal and capture of hydrogen, the strategy is twofold: the application of Pressure Swing Adsorption or of a membrane contactor, combining specific absorption and separation, for upgrading hydrogen in the gas phase is investigated. Besides, hydrogen removal from the liquid phase is done by a membrane unit in an internal loop connected to the bioreactor. To guarantee proper process control and operation and, eventually, automation, dedicated measurement devices are developed which allow continuous detection of hydrogen or contaminants like H₂S. The methane production potential of the effluent of the hydrogen bioreactor is prepared for testing in an innovative continuous anaerobic membrane reactor operating at thermophilic conditions. Finally, all mass and energy balances and basic engineering data of the separate process units will be integrated and used for modelling and simulation studies to design a hydrogen production unit with maximum product output, minimum energy demand and low cost.

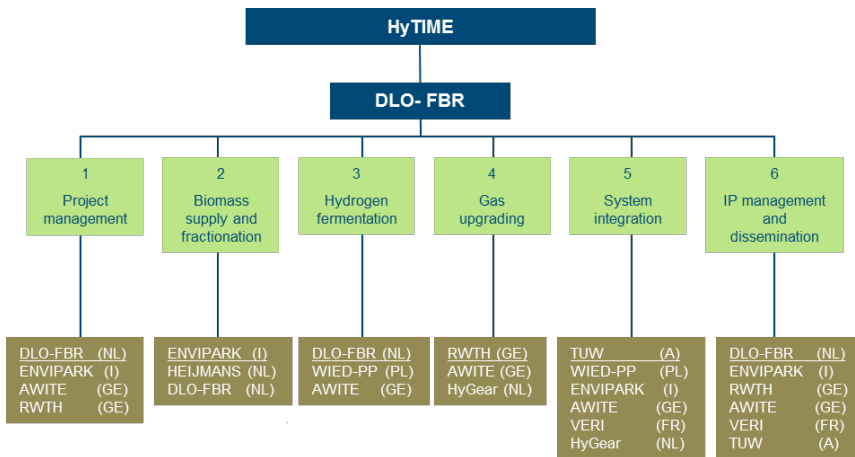


Fig. 3 Participation of 9 partners working together in 6 workpackages of HyTIME

Objectives

HyTIME starts with the supply of suitable feedstock which is followed by the optimisation of the H₂ fermentation in specially designed bioreactors. The rapid recovery of H₂, at ambient pressure, 70 °C and low concentration requires new approaches in gas upgrading techniques and analysers. The integration of all these components, including the last step with using methane from AD for internal heat and power, will bring HyTIME to beyond the state-of-the-art of current fermentative H₂ production.

The main objective of HyTIME is to produce H₂ from second generation biomass with a production rate of 1-10 kg/H₂.day.

Sub-objectives:

- 1) Efficient mobilisation of fermentable feedstock from second generation biomass
- 2) Increased productivity of H₂ fermentation
- 3) Construction of dedicated bioreactors
- 4) Efficient gas purification at low pressure and temperature
- 5) Construction of online measurement and control devices
- 6) Process modelling and simulation studies
- 7) Techno-economic evaluation of a 400 kg H₂/day plant

Main S&T results/foregrounds

WP 2 Biomass supply and fractionation

Two types of biomass have been selected to serve as raw material for HyTIME: straw and grass as primary by-products with a strong seasonal dependence, and unsold fruit and vegetables as secondary by-products available throughout the year. The work with straw was done at ENVIPARK, with grass at DLO-FBR.

Table 1 Availability, cost and moisture content of straw and grass as feedstock for HyTIME

	Availability Kton dry matter/y	Moisture content (%)	Cost € ton dry matter	Special features	Current applications
Straw in NL	700-840	50	40-100	High in ash	Horticulture
Straw in IT			30-40		
Verge grass NL	240-500	10-50	0-80	Heterogeneous	Composting

Straw

The greatest number of pretreatment parameters have been investigated for straw:

- Acid concentration
- Type, dosage and administration of enzymes
- Chemical pretreatment or steam explosion and chemical pretreatment

Figure 4 shows a general outline for acid pretreatment and hydrolysis of wheat straw. Different modes of severity have been applied for the acid impregnation as well as the steam explosion step (Table 2). Generally, steam explosion of acid-soaked straw was executed with the following parameters: 13 bar, 190 °C for 5 min with a severity of 2.233 min. New enzyme cocktails from Novozyme gave less mobilisation but significant decrease in cost: from €300/kg dry straw to €6-18/kg dry straw, and duration of hydrolysis: from 89 to 48 hrs. An alternative approach for cost reduction is the production of dedicated enzymes on site instead of procurement. From a recent study using already pretreated biomass it was estimated that the cost would amount to €55-96 /ton pretreated substrate for *on site* produced enzymes as compared only a little higher cost of €57-125 /ton for off-site produced enzymes, respectively.

The best data have been used for modelling and simulation in WP 5.

Table 2 Mobilisation of sugars from straw after acid pretreatment (CE) or steam explosion after acid impregnation (SEa) and enzymatic hydrolysis

Test	Yield of glucose (g/100g dm)	Yield of glucose (% theoretical)	Yield of xylose (g/100g dm)	Yield of xylose (% theoretical)	Total glu+xyl (g/100g dm)	% theoretical
CE1	28.3	66.5	10.7	33.7	39.1	52.6
CE2	36.1	85.0	16.5	52.0	52.6	70.9
CE3	34.4	80.9	14.8	46.7	49.2	66.3
SE1a	20.9	47.2	2.1	7.37	22.1	29.8
SE2a	24.3	57.1	9.7	34.6	34.0	45.7
SE3a	24.9	58.4	8.7	31.2	33.6	45.2
SE4a	25.6	60.0	3.1	11.2	28.7	38.6
SE5a	22.7	53.4	2.2	7.9	24.9	33.6

Unsold fruit and vegetables

The mobilisation of sugars from vegetable residues was unsuccessful and the use of this feedstock was abandoned in view of the project's duration.

Verge grass

Verge grass was harvested early morning in June in the Netherlands. The effect on solid/liquid separation was compared using a twin screw extruder and a single screw press with the extruder as the most capital

intensive procedure and the single screw press as the option of mobile application. The sugar content in the juice from the extrusion was significantly higher, 35 g sugars/L, as compared to the press juice, 20 g/L. The protein content, regarded as a profitable by-product for the feed industry, was circa 10 g/L in both juices. The solid fraction was chemically pretreated with alkaline at 85-100 °C prior to enzymatic hydrolysis.

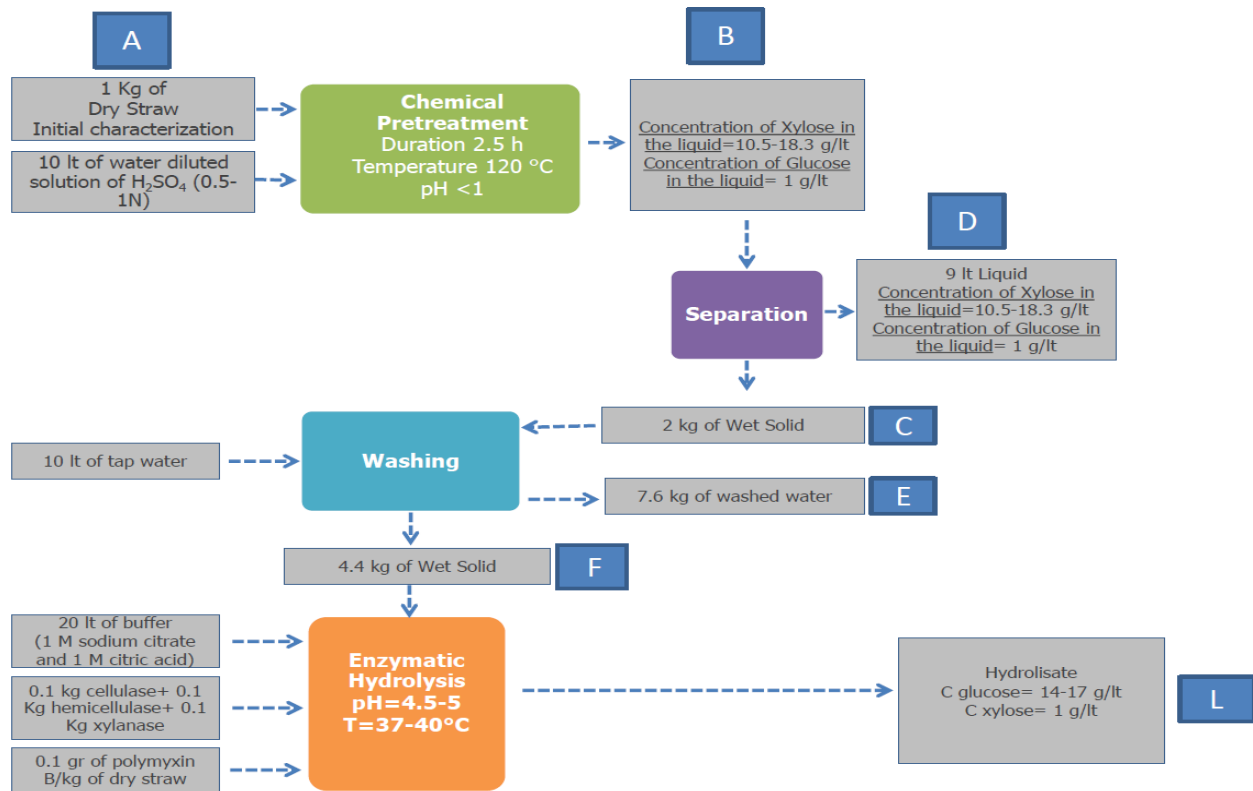


Fig. 4 Acid pretreatment and hydrolysis of wheat straw. In process V1 effluent D goes to the anaerobic digestion and in process V2 effluent D is added to the hydrolysate L for the hydrogen fermentation.

The thermal and electrical energy requirement for the pretreatment processes was extensively analyzed. The data presented in table 3 are applicable to the equipment used in the laboratories of ENVIPARK and DLO-FRB, respectively. It is foreseen that energy demand will decrease with an increase of scale. Table 3 shows little difference between the different processes for pretreatment at ENVIPARK. In contrast, the numbers for energy consumption obtained from DLO-FBR were much lower. This is due to the different biomass types used and the differences in fractionation protocol. Energy consumption at DLO-FBR is lower due to the lower temperature and the much shorter time needed for the enzymatic hydrolysis, i.e. 24 h as compared to 48 h at ENVIPARK. The highest sugar yield from straw is obtained with the chemical pretreatment at ENVIPARK.

The amount of inhibitors as e.g. furfural and hydroxymethylfurfural (HMF), is highest in the hydrolysate prepared by acid pretreatment but the concentrations are lower than that considered toxic for the dark fermentation. It has been reported that at concentrations exceeding 1 g furfural and HMF/L bacterial growth and hydrogen production may be hampered. The effect was tested in the subsequent fermentation (Table 4). The thermal and electrical energy requirements for the pretreatment processes were extensively analysed. It is foreseen that energy demand will decrease with an increase of scale. Table 3 shows little difference between the different processes for pretreatment of straw. In contrast, the numbers for energy consumption obtained for grass pretreatment at lower temperature and half the hydrolysis time were much lower. In all cases the sugar concentration in the hydrolysates was significantly lower as achieved in the first period with similar biomass types. Most probably this reflects the variation in the biomass itself, caused e.g. by time of harvest. The amount of inhibitors as e.g. furfural and hydroxymethylfurfural (HMF), is highest after acid pretreatment. It has been reported that at concentrations exceeding 1 g furfural and HMF/L bacterial growth and hydrogen production may be hampered. The effect was tested in the subsequent fermentation.



Fig. 5 Solid fraction of verge grass used for enzymatic hydrolysis. A: after extrusion and alkali treatment, B: the same after decantation. The decantation device uses centrifugal forces to separate water from solids.

Table 3. Energy demand for fractionation processes of straw and verge grass at pilot scale and concentration of sugars and inhibitors in the hydrolysate.

	Specific thermal energy (kWh/kg)	Specific electrical energy (kWh/kg)	Glucose (g/L)	Xylose (g)	Furfural (mg/kg)	HMF ³⁾ (mg/kg)
ENVI Straw, steam explosion (S+E)	2.1	1.5	10.8	1.8	15	4
ENVI Straw, acid impregnation (C+E)	1.6	1.5	16.7	11.2	399	21
DLO-FBR Verge grass, alkaline	0.03 ¹⁾	0.40	8.9	2.1	n.d. ²⁾	n.d.

1) Estimation since no experimental data were available

2) n.d. is not determined

3) HMF is hydroxymethylfurfural

The fermentability of the hydrolysates produced from straw was validated in a Continuous Stirred Tank Reactor (CSTR) with a working volume of circa 24 L. The first test with acid treated anaerobic sludge from an industrial biogas plant, done at 37°C, showed little hydrogen production. When the temperature of the reactor was increased to 70 °C and inoculation was done with the extreme thermophile *Caldicellulosiruptor saccharolyticus*, hydrogen production was almost 4 times higher. Even though the percentage H₂ in the biogas was a little higher in the hydrolysate with the high furfural and HMF content, the productivity seemed to be retarded. Apparently, the concentration of the inhibitors was below the level of toxicity.

Table 4 Validation of hydrogen production from wheat straw hydrolysates generated by fractionation using steam explosion and enzymatic hydrolysis (S+E) or acid pretreatment and enzymatic hydrolysis (C+E) in a Continuous Stirred Tank reactor (CSTR) at 37 and 70 °C.

Hydrolysate/ inoculum	Reactor (°C)	Hydraulic retention time (h)	Biogas productivity (L/h)	% H ₂ in biogas	H ₂ productivity mmol/L.h	H ₂ productivity in g/day
S+E/ <i>C.saccharolyticus</i>	70	37	3.1	44.3	2.6	3.0
C+E/ <i>C. saccharolyticus</i>	70	37	2.0	47.1	1.8	2.0
C+E/Anaerobic sludge	37	178	4.5	9.6	0.7	0.8

WP 3 Thermophilic hydrogen fermentation

A short survey of extreme thermophilic bacteria confirmed the choice for three *Caldicellulosiruptor* species; *C. saccharolyticus*, *C. owensensis* and *C. kristjanssonii* showing the widest substrate range, lowest demand for additional nutrients and lowest proficiency to produce competing products as e.g. lactic acid, ethanol or alanine.

For creating high cell density the use of flocculants for the congregation of bacteria was investigated. Flocs were formed in cultures of *C. saccharolyticus* with cationic polymers based on polyacrylamide at circa 50 mg/L.d. However, this approach has been abandoned due to the negative effect on hydrogen productivity decreasing from 0.4 to circa 0.1 g H₂/L.d in the control culture and the flocculated culture, respectively, and the high cost for flocculants.

An alternative option for establishing high cell density is through immobilisation of bacteria on a carrier. Carriers made from activated carbon, zeolites or polyethylene or -propylene or composites have been tested with the *Caldicellulosiruptor* species. Figure 6 shows attachment of *C. owensensis* with formation of nanowires on zeolite. Polyethylene carriers gave the best options in terms of attachment, application and cost.

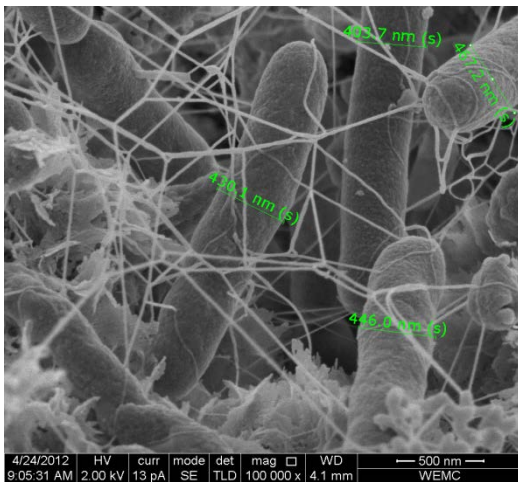


Fig.6 Scanning electron microscopic photograph of *C. owensensis* attached on zeolite

A dedicated lab-scale glass reactor for increase of productivity has been built with one compartment for hydrogen production in a packed bed and one compartment for easy removal of gas (gas-disengager, Fig. 7). The packed bed was designed to enable bacterial cell retention, i.e. increase of biocatalyst, by biofilm formation. Other measures for the increase of productivity are through an increase of organic loading by increasing substrate concentration or the dilution rate (decrease of hydraulic retention time (HRT)). Another feature is the recirculation pump installed between the gas-disengager and the packed bed reactor. By rapid recirculation of hydrogen-lean medium, bacteria are less exposed to hydrogen and expected to increase productivity. Besides, the recirculation was aimed at reducing the rate of gas stripping needed for hydrogen removal. In general, the dedicated reactors were inoculated with at least 2 different bacterial strains. This is because mono-cultures often lack the ability to form biofilms.



Fig. 7 Combined packed bed (left) with gas-disengager (right) (CFBG). The packed bed was filled with Bioflow 9 polyethylene carriers. The diffuser is at the bottom part with the floating balls. The working volume for the fermentation is 5.7 L

The increase of hydrogen in the fermentation inhibits of the bacteria in further production. As a result, the cells shift their metabolism to the formation of more reduced end-products like lactic acid and ethanol, with a detrimental effect on the hydrogen yield. Gas-stripping is one of the most practical but costly methods for hydrogen removal. Figure 8 shows the effect of a variation in nitrogen flow rate in the 5.7 L reactor, with 10 L N₂/h giving the best results in terms of yield (2.5 mol H₂/mol glucose at a dilution rate of 0.042 h⁻¹) but the lowest H₂ concentration in the off gas, 6.1 %. At a flow rate of 0.003 L N₂/h, the yield of mol H₂/mol glucose decreased to 2.0 but the hydrogen partial pressure increased to circa 66 %. In a comparable culture with a slightly higher dilution rate of 0.051 h⁻¹ and 10 L N₂/h, hydrogen yield was circa 3.0 and the partial pressure was 9 %. The hydrogen productivity ranged from 1.3 to 1.5 g H₂/ day for the flow rate of 2.0 and 10 L N₂/h, respectively.

To increase the productivity to 1000 g H₂/day larger reactors, P50 and P1000, were constructed or procured, designed in a similar way as the glass reactor but made from stainless steel. Since for high rate hydrogen production in P50 and P1000 molasses would be used as substrate for hydrogen production, several tests were carried out in small CSTR's to determine the need for addition of nutrients to the medium. Hydrogen yield of *C. saccharolyticus* was a little lower on molasses as compared to sucrose medium due to the production of some lactic acid and ethanol. Omission of yeast extract, cysteine and trace elements was

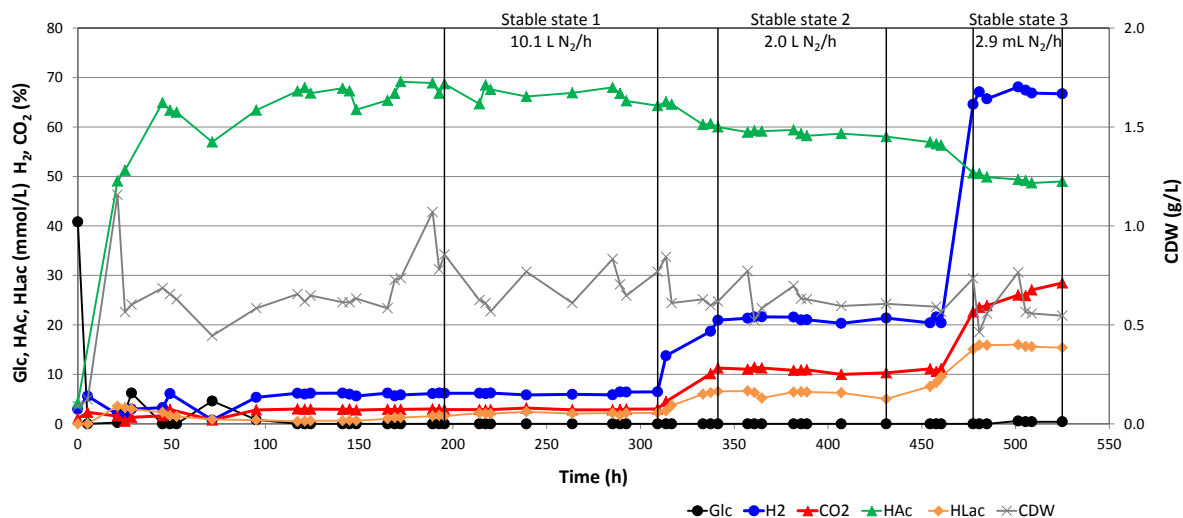


Fig. 8 Production of H_2 , CO_2 , acetic and lactic acid and biomass by a co-culture of *C. saccharolyticus* and *C. owensensis* and the residual glucose concentration in an CFBG. Periods of stable states and N_2 gas flow are indicated.

without effect on the performance of *C. saccharolyticus*. In contrast, *C. owensensis* needed a small amount of cysteine as a source of organic sulphur. Analysis of the elemental composition (P, S, Co, Fe, Mo) of molasses, complete molasses medium and effluents of the *C. saccharolyticus* and *C. owensensis* cultivations showed that none of the elements were limiting but without yeast extract, cysteine and trace elements, limitation of Co and Fe can be expected. The effect of the nutrients on the H_2 productivity was negligible. For studies on gas upgrading in WP 4, the effect of stripping with N_2 or CO_2 to remove hydrogen from the medium was tested. Both cultures were affected but in an opposite way: *C. saccharolyticus* was inhibited by CO_2 sparging and not by pH change whereas *C. owensensis* showed low substrate consumption and H_2 production at the lower pH, and was not inhibited by CO_2 . The effects were, however, not very great. The aim remains to reduce stripping as much as possible in order to prevent dilution of the off gas with another inert gas.

Process control devices for pH, redox, temperature, pressure, liquid level, safety measures etc. for the P50 and P1000 were delivered by AWITE and connected to the reactor. The measurement devices and the power electronic components were integrated in one control box (Fig. 9). Software for the Process Control System, based on standard IEC 61499 was adapted and installed.

The P50 was run with a co-culture of *C. saccharolyticus* and *C. owensensis*, first on sucrose at a low dilution rate of 0.05 h^{-1} to establish biofilm formation. After 7 days, sucrose was replaced by non-sterile molasses medium, without yeast extract and Mg, but fed at the same rate. Figure 9 and Table 5 show an excellent performance of the bacteria when growing on sucrose with a productivity of 23.4 g hydrogen/day. The yield is very high with 3.5 mol hydrogen per mol of C6 sugar, being 88 % of the maximum theoretical yield.

When the sucrose medium was replaced by molasses, a massive change in products was observed. The production of hydrogen and acetate decreased with a concomitant increase in ethanol and CO_2 production.

The results were strongly indicative of the contamination of the hydrogen producing cultures by an extreme thermophilic consortium, producing ethanol and endogenous to molasses.

Therefore, the reactor was steam-sterilized at $105\text{ }^\circ\text{C}$ and molasses was sterilized in the autoclave. The reactor was filled with approximately 10 L *C. saccharolyticus* and *C. owensensis* co-culture and 60 L medium. During the batch phase the production of hydrogen, acetate and CO_2 was representative for growth of *Caldicellulosiruptor sp.* However, after the start of pumping fresh medium, the product profile of *Caldicellulosiruptor* was almost immediately replaced by the profile of the contaminating consortium with high ethanol production as observed in Fig. 10. Several attempts have been made to salvage the *Caldicellulosiruptor* by changing the dilution rate and/or pH. None were successful. This experiment was terminated after 16 days. The effluent was sent to BIOTHANE for testing the biomethane production in the anaerobic membrane reactor in WP 5.

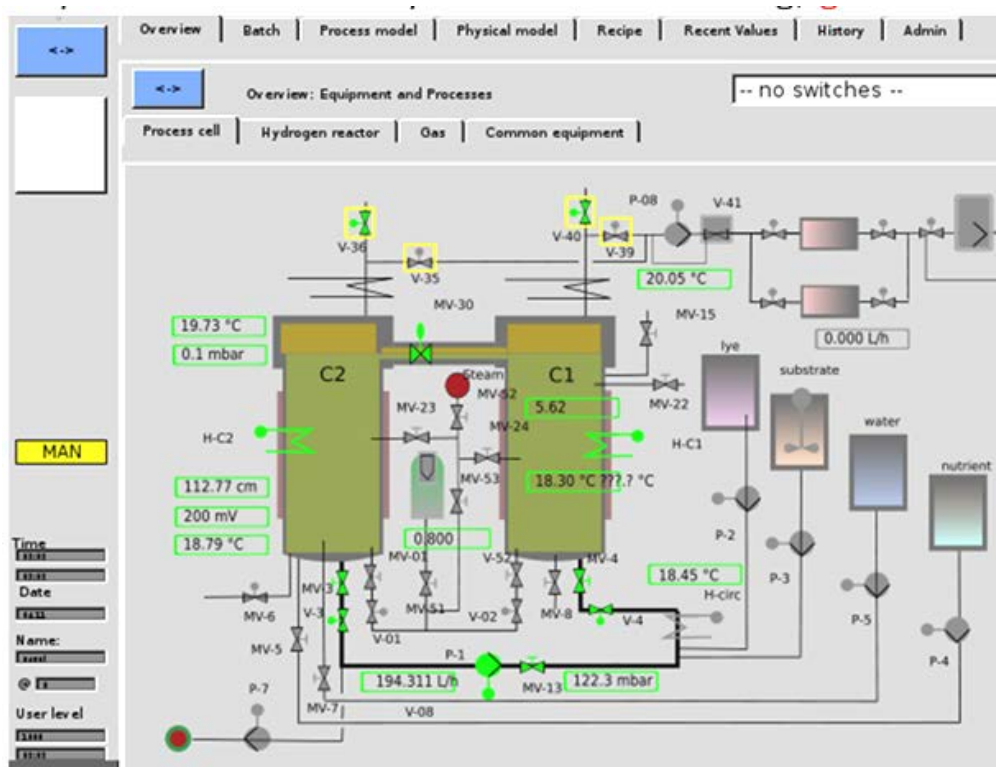


Fig. 9 Screenshot of the AWITE control box for the P50 reactor.

With small scale experiments it was established that the consortium was endogenous to molasses. For annihilation of the bacteria a temperature of 125 °C was needed.

A professional company specialized in disinfecting process equipment was hired to clean the reactor and the auxiliary equipment by chemical disinfection.

Following the disinfection new experiments were carried out. However, in spite of starting with fresh *Caldicellulosiruptor* sp. inocula and sterile medium, the culture turned towards ethanol production of over 90 mmol/L.

Table 5 Data of a co-culture of *C. saccharolyticus* and *C. owensensis* in the P50 bioreactor with a working volume of 58 L. The sucrose solution had been sterilized to prevent growth in the feed. Molasses was used without sterilization. Yeast extract and Mg were omitted in molasses medium. In all cases non-sterile tap water and non-sterile concentrated salt solutions were used where applicable. The initial sucrose concentration was circa 10 g/L. The dilution rate was 0.05 h⁻¹.

Substrate		H ₂ off gas	CO ₂ off gas	H ₂	
				Yield	Productivity
	g/L	%	%	mol/mol C6 sugar	g/day
Sucrose	10.8	17.6	10.8	3.49	23.4
Molasses	9.3	6.6	16.2	1.08	8.1

Increase of sugar concentration and/or decrease of dilution rate appeared of no effect on the contaminant. Table 6 shows some typical data of fermentations in the P50 reactor. The low yield, ranging from 0.7 to 0.9 mol H₂/ mol C6 sugar, is a result from the production of ethanol which is in competition with H₂ production. The highest hydrogen production rate achieved with the contaminated consortium was 18.8 g H₂/day. By increasing the dilution rate to 0.1 h⁻¹ and the sucrose concentration to 15 g/L an improvement of more than 2.6 times in productivity could be achieved. This holds a great promise for the hydrogen production rate by *Caldicellulosiruptor* sp. which showed a productivity of 23.4 g H₂/day at the low dilution rate of 0.05 h⁻¹ and sucrose concentration of 10 g/L (Table 5).

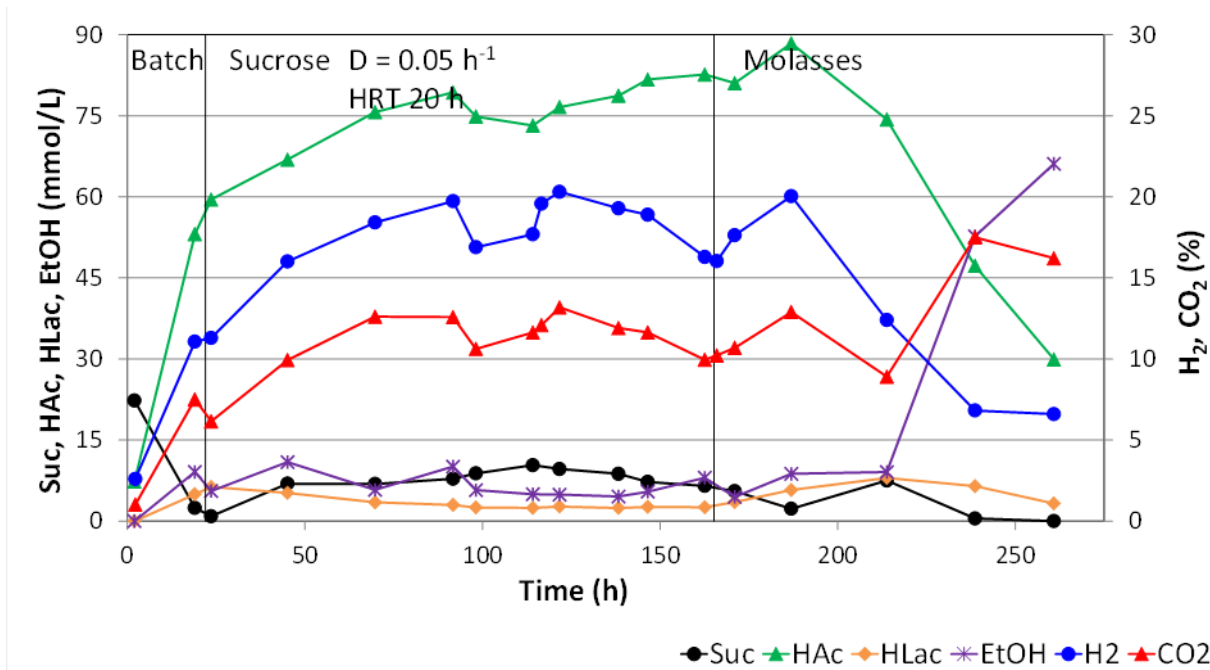


Fig. 10 Production of H₂, CO₂, acetic and lactic acid and ethanol by a co-culture of *C. saccharolyticus* and *C. owensensis* and the residual sucrose concentration in the P50 reactor at continuous cultivation with a dilution rate of 0.05 h⁻¹.

Table 6 Data of a co-culture of *C. saccharolyticus* and *C. owensensis* in the P 50 reactor with a working volume of 58 L. The sucrose solution had been sterilized. Molasses was used without sterilization and supplemented with Mg. Yeast extract, cysteine and SL-10 were omitted. In all cases non-sterile tap water and non-sterile concentrated salt solutions were used were applicable.

Substrate	Dilution rate	H ₂ off gas	CO ₂ off gas	H ₂		
				Yield	Productivity	
	g/L	h ⁻¹	%	%	mol/mol C 6 sugar	g/day
Sucrose	10	0.050	5.7	15.4	0.87	7.1
Sucrose	15	0.109	12.0	29.0	0.70	18.8
Molasses	13.5	0.058	7.1	19.4	0.67	8.6
Molasses	13.5	0.103	11.7	28.5	0.81	18.3

As the severe chemical disinfection had been unsuccessful, the work with the P50 reactor has been abandoned. Apparently the construction of this bioreactor does not allow *in situ* sterilisation due to hold-up areas in the reactor or the heater. An alternative method for sterilisation is the complete deconstruction of the bioreactor and cleaning and sterilisation by autoclaving the separate parts.

A second-hand large scale bioreactor called P1000, without auxiliaries, was purchased from an Austrian company. Before use the bioreactor was cleaned and equipped with auxiliary equipment (mass flow controller, gas flow meter, pumps), a heating system, condenser, valves, electrodes, sensors etc. The process control system developed for the P50 reactor was reused after adaption to meet the requirements for the P1000, a task performed by AWITE.

The P1000 consist of an inner tube filled with carriers for biofilm formation (packed bed) and an outer part surrounding the inner tube (gas-disengager) (Fig. 11). The culture medium is pumped from the bottom to the

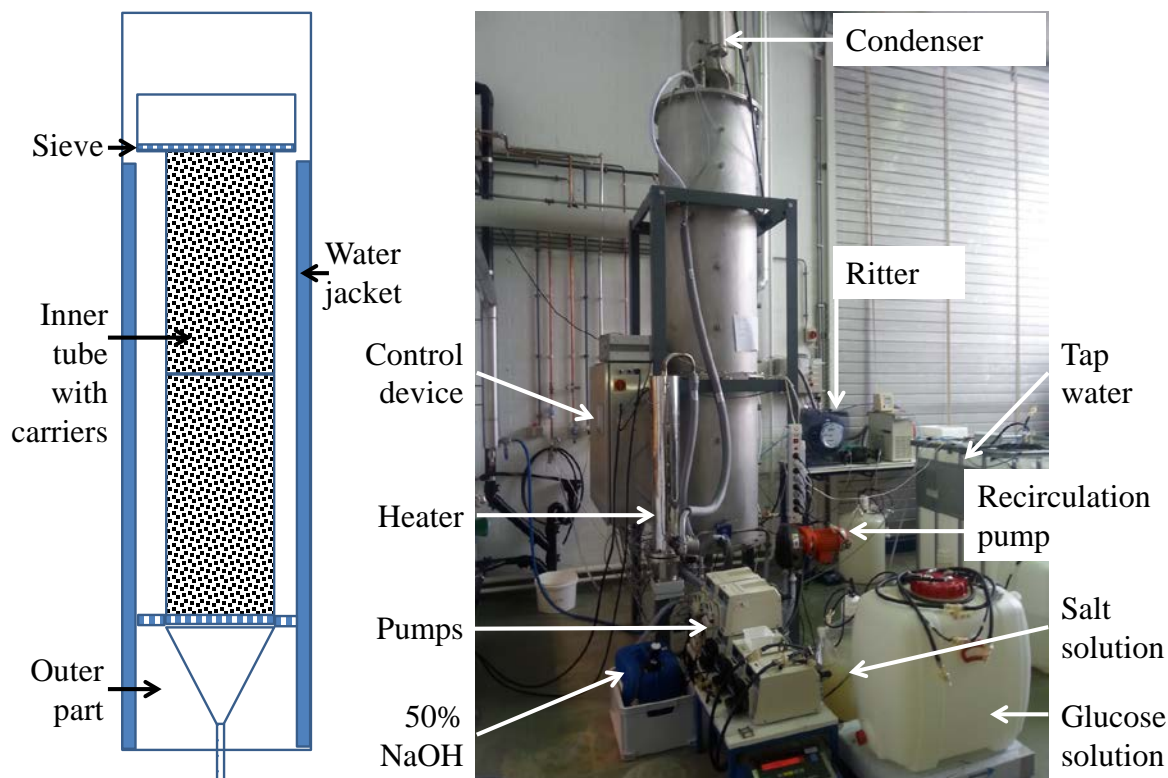


Fig. 11 Schematic drawing (left) and picture of the P1000 bioreactor (right) with equipment and vessels with medium solutions. Later the bioreactor was covered with black insulation.

top of the packed bed and falls down in the outer part from where it is recirculated to the packed bed. Experimental work with the fully equipped P1000 reactor was divided over 3 experiments (I, II and III) under non-sterile conditions with a co-culture of *C. saccharolyticus* and *C. owensensis* and glucose as the substrate. Figures 12 and 13 show the results of experiments I and II and the results of all experiments together are presented in table 7. In the first part of experiment I lactic and acetic acid were the main products (Fig. 12). This changed towards the end of the fermentation with acetic acid as the main product and production of significant amounts of ethanol, butyric and lactic acid. H_2 and CO_2 were produced in equal volumes. A H_2 yield of 2.0 mol per mol of glucose, i.e. 50% of the theoretical yield, was the result of this mixed product fermentation (Table 7). The production of butyric acid is indicative for contamination by other thermophiles since production of this acid by the *Caldicellulosiruptor* species has not been observed nor reported. The H_2 productivity of 62.5 g per day was nevertheless 4 times higher than the productivity with the P50 reactor under similar conditions.

During the 8-days fermentation the recirculation pump (peristaltic pump) regularly stopped and although most of the time the fermentation recovered rapidly it was terminated and another pump (gear pump) was installed.

The second experiment (II) was a continuation of fermentation (I) and its culture after a break of 3 weeks. The fermentation was started in the continuous mode with a dilution rate of 0.027 per h increased to 0.056 and 0.098 per h. The glucose concentration in the feed was step-by-step increased from circa 10 to 20 g/L and the temperature of the medium was increased from 70 to 77 °C (Fig. 13).

Initially the composition of the liquid products was very similar to the composition at the end of experiment (I) but within 40 h after the re-start of the fermentation the product profile changed, and ethanol became the dominant product, whereas lactic and butyric acid were washed out (Fig. 13). Glucose was completely

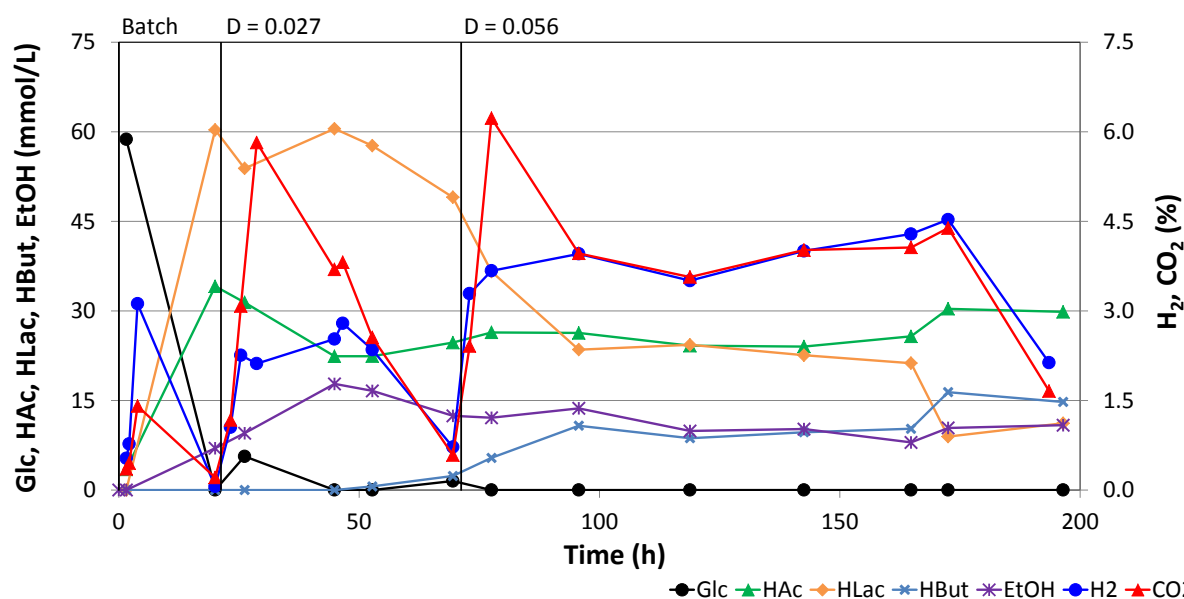


Fig.12 Experiment I. Production of H_2 , CO_2 , acetic, lactic and butyric acid, and ethanol in a continuous cultivation and the residual glucose concentration in a P1000 reactor at 70 °C. The inoculum was a co-culture of *C. saccharolyticus* and *C. owensensis*. The recirculation pump was down from 63 till 69 h and from 182 till 191 h. The dilution rate (D) is shown at top of the figure.

Table 7 Data of continuous cultivation in the P1000 with a working volume of 225 L. The bioreactor was inoculated with a co-culture of *C. saccharolyticus* and *C. owensensis*. The N_2 flow rate was on average 8.4 NL/min or 4.9 NL/min (III *).

Exp. #	Glucose	Dilution rate	Temp.	H_2 off gas	CO_2 off gas	H_2	
	g/L					h^{-1}	$^{\circ}C$
I	9.1	0.056	70	4.4	4.2	2.0	62.5
II	8.8	0.056	72	2.9	5.3	1.2	35.6
	8.8	0.056	74	3.2	5.2	1.3	39.7
	10.3	0.098	74	5.3	10.5	1.1	66.5
	10.2	0.098	77	5.6	10.8	1.2	71.9
	15.3	0.098	77	8.1	16.6	1.2	108.9
III	10.1	0.098	74	4.8	10.1	1.1	63.6
	15.1	0.098	74	7.1	16.0	1.1	100.1
(*)	15.1	0.098	73	10.3	19.4	1.0	92.3

consumed also at the higher concentrations of 20 g/L in the feed which is normally not achieved by the *Caldicellulosiruptor* species at a dilution rate of 0.1 per h. H_2 production increased with a higher dilution rate and with higher substrate concentrations (Table 10). A slight increase in H_2 production was also observed with increased temperatures (Table 10). At all conditions CO_2 production was higher than H_2 production.

A low H_2 yield of 1.1 – 1.3 mol per mol, i.e. circa 30% of the maximum was obtained. The highest H_2 productivity of 0.11 kg per day was obtained at 15 g/L of glucose in the feed and a dilution rate of 0.1 per h. The composition of the consortium rapidly changed to a culture dominated by ethanol producers.

The second fermentation was stopped because of a disturbed pH control and a high pH of the culture (pH of 10.1).

The conditions of the first part of the third fermentation (III) were the same as in fermentation (II) with similar results (Table 22). In the end the H_2 content of the gas was increased to 10.3 % at an diminished N_2 flow rate of 4.9 NL per min which slightly decreased the H_2 yield and productivity. During this stage gas upgrading units were coupled to the P1000 off gas and effluent stream.

Table 8 shows that at small scale fermentation the productivity was fairly on schedule. However, by upscaling the efficiency of the hydrogen production dropped to 16-25 % instead of 75%. As a result the productivity targets were not achieved.

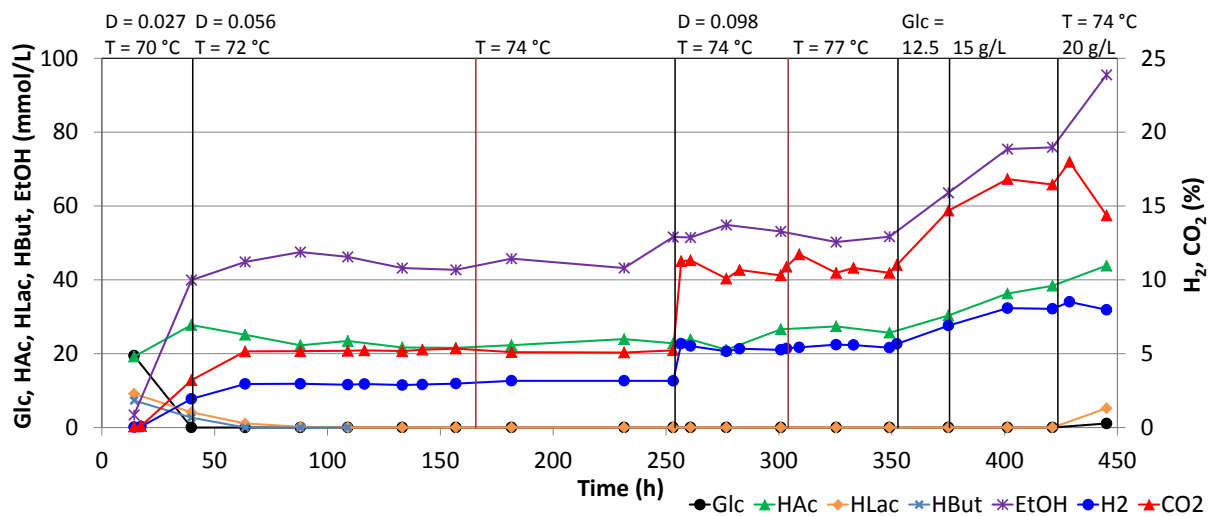


Fig. 13 Experiment II. Production of H₂, CO₂, acetic, lactic and butyric acid, and ethanol in a continuous cultivation and the residual glucose concentration in the P1000 reactor at 70 - 77 °C. The dilution rate (D), temperature and glucose concentration in the feed are shown on top of the figure.

The cause of decrease of efficiency was due to assumed contamination of the culture (Data for identification not yet available). These other bacteria produced a fair amount of ethanol and lactate, competing for hydrogen production. Besides contamination it may also be that the stainless steel had an effect on the bacterial population. Because of the end of HyTIME it was not possible to further investigate and/or validate the assumptions.

Table 8 Upscaling of H₂ production by reactor upscaling and actual H₂ productivities with pure substrates and substrates from 2nd generation biomass.

Foreseen reactor volume (L)	5		50		500	
Actual volume (L)	5.7		58		225	
Substrate	Glucose	Grass hydrolysate	Sucrose	Molasses	Glucose	Molasses
Productivity target (g/day)	10		100		1 000 – 10 000	
Productivity target with actual volume (g/day)	10.9		111		450-4 500**)	
Productivity achieved (g/day) *)	9.1	6.0	23.4	18.3	109	-

*) This is the hydrogen produced in the reactor and measured in the off gas prior to upgrading

***) Initially it was foreseen to have 1-10 kg H₂/day in a 500L reactor. Since only a 225L reactor was available the targeted productivity was decreased proportionally to 0.45 – 4.5 kg H₂/day.

WP 4 Gas upgrading

Recovery of hydrogen from the fermenter off-gas

A first qualitative CG/MS analysis of the gas coming from the hydrogen fermentation showed the presence of H₂S at below 200 ppm. This is assumedly due to the addition of cysteine to the medium needed as sulphur source and for maintenance of the redox potential. Minor contaminants as e.g. ammonia, acetic acid formic acid have not been detected. However, this may be the result of the dilution by the stripping gas.

In view of the shortest time-to-market commercially available adsorbents and modules for use in Pressure Swing Adsorption (PSA) and membrane contactors (MC) have been investigated.

The selected systems have been analysed with respect to energy demand for hydrogen purification from 4 different gases with increasing N₂ content. The standard for the cleaned gas has been set at 98 vol % H₂, 200 bar and 20 °C at a production rate of 1 kg H₂/day.

For the analysis of the MC performance, the PermSelect Poly-dimethylsiloxane-2500 module (membrane area 0.25 m²) from MedArray was selected because of its superior behaviour in CO₂ removal. Using 0.5 M K₂CO₃ and 0.5 M piperazine as absorbent solution, raw gas with 15 vol % CO₂ was purified to an end concentration of 2.3 vol % in one step.

The flow sheet for gas upgrading with a membrane contactor is shown in Fig. 14. The N₂ separator represents another set of membrane modules where separation of N₂ and H₂ takes places on the basis of permeation. At a N₂ concentration exceeding 5 vol%, 3 or more stages are needed to meet the specifications of the standard.

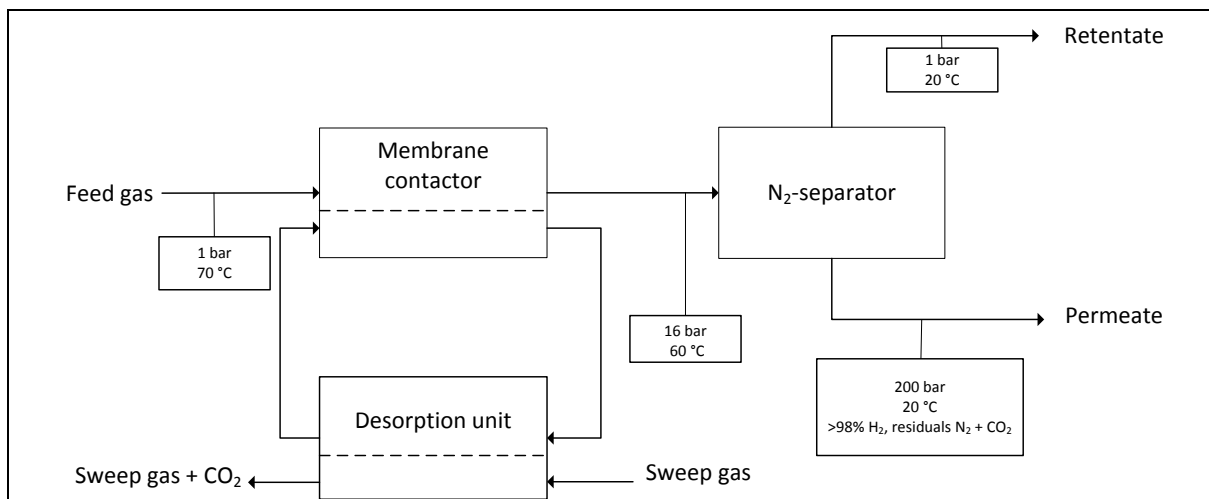


Fig. 14 Flow sheet of the gas upgrading unit with a membrane contactor (MC).

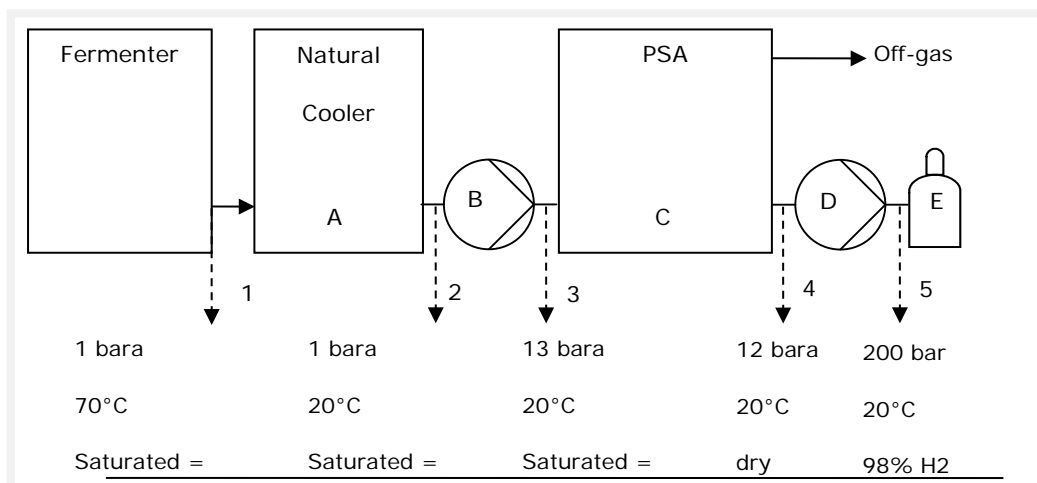


Fig. 15 Process flow scheme for simulation of gas upgrading with Pressure Swing Adsorption (PSA)

The performance of Pressure Swing Adsorption (PSA) in gas upgrading of fermenter gas was simulated after having activated carbon selected as the best adsorbent. The flow sheet for PSA is shown in Fig. 15.

Table 9 shows the simulation of the energy demand of an MC with an experimental poly-vinyltrimethylsilane flat membrane contactor module and a PSA gas upgrading unit. In the simulation with the MC, nitrogen is removed using a 3-end membrane module. Simulation was done using Aspen Plus. The simulation of the PSA is done using HyGEAR's PSA simulation tool. Feed gas 4 could not meet the specifications for the standard. Comparison of the data in Table 9 shows that application of the MC with the experimental membrane is quite promising.

Table 9 Data from simulation of energy demand for purification of different feed gas compositions using a membrane contactor (MC) or pressure swing adsorption (PSA) upgrading unit

Feed gas composition	H ₂ -Purity (%)		H ₂ -Yield (%)		Stages for N ₂ -separation	kWh /kg H ₂	
	MC	PSA	MC	PSA		MC	PSA
H ₂ /CO ₂ /N ₂ (%)							
70/30/0	99.9	98	100	87	0	4.7	5.7
67/28/5	98	98	99.8	87	1	6.3	6
20/10/70	98	98	88.5	40	3	9.5	28.8
6/3/91	98	n.d. ¹⁾	87.4	n.d. ¹⁾	4	11.1	n.d. ¹⁾

1) n.d. is not determined

Further experiments were carried out (using the same absorbent solution of 0.5 M K₂CO₃ and 0.5 M piperazine), with CO₂ concentrations up to 80vol.-%. For a given molar flow and transmembrane partial pressure, the determined permeability, which is defined as the molar flow rate per membrane area and per transmembrane partial pressure for a chosen component, can be used to easily carry out a scale-up. If – as the worst case – a CO₂ concentration in the fermenter off-gas of 80vol.-% is reached and the CO₂-concentration in the cleaned gas shall be below 2vol.-%, about 169 PermSelect Poly-dimethylsiloxane-2.1 modules (membrane area of 2.1 m²) from MedArray will be needed for the gas upgrading (Table 10). If the CO₂-concentration should be decreased even further, more membrane contactor modules are needed.

Table 10 Required amount of membrane contactor modules to have an end -CO₂ -concentration of 2vol.-%

Scale-up for 1kg H ₂ /d	Determined CO ₂ -permeability	H ₂ in raw gas	CO ₂ in raw gas	Required membrane area	Required number of modules with 2.1m ²
	(mol/h.m ² .bar)	(%)	(%)	(m ²)	
Laboratory (Aachen)	1	20	80	354	169
	1	30	70	228	109
P1000 reactor (Wageningen)	1.15	20	80	326	155
	1.15	30	70	210	100

A gas upgrading unit with 4 MC's was constructed (Fig. 16) for gas upgrading including recovery of the absorbent, to be connected and tested with the 225 L-fermenter. The determined permeability of carbon dioxide for the 225 L fermenter was slightly higher than for the laboratory test (Table 10). With the increased permeability a scale-up was carried out. The required membrane area and amount of modules decreases due to the higher permeability. Figure 16 illustrates the set-up in Wageningen.

After the selection of activated carbon as the best adsorbent, the performance of different PSA strategies, including Vacuum Swing Adsorption (VSA) for the gas upgrading of fermenter off-gas was simulated (Table

11). For all 4 investigated PSA options the highest hydrogen yield, 96% with VPSA and 91% with VSA, respectively, is achieved for a feed gas composition with 30 % H₂ and 70 % CO₂. However, the electrical consumption is highest for VPSA and lowest for VSA. Based on a cost evaluation, clear price benefit is observed for the VSA technology with approximately 3.7 € kg H₂ for upgrading at a productivity of 10 kg H₂/d.

Table 11 (Vacuum) pressure swing adsorption (V)PSA technologies for upgrading fermenter off-gas

Technology	Description
VPSA	Gas adsorption at 7 bar(a), desorption at 200mbar(a)
PSA-7	Gas adsorption at 7 bar(a), desorption at atmospheric pressure
PSA-13	Gas adsorption at 13 bar(a), desorption at atmospheric pressure
VSA	Gas adsorption at 1.5 bar(a), desorption at 200mbar(a)

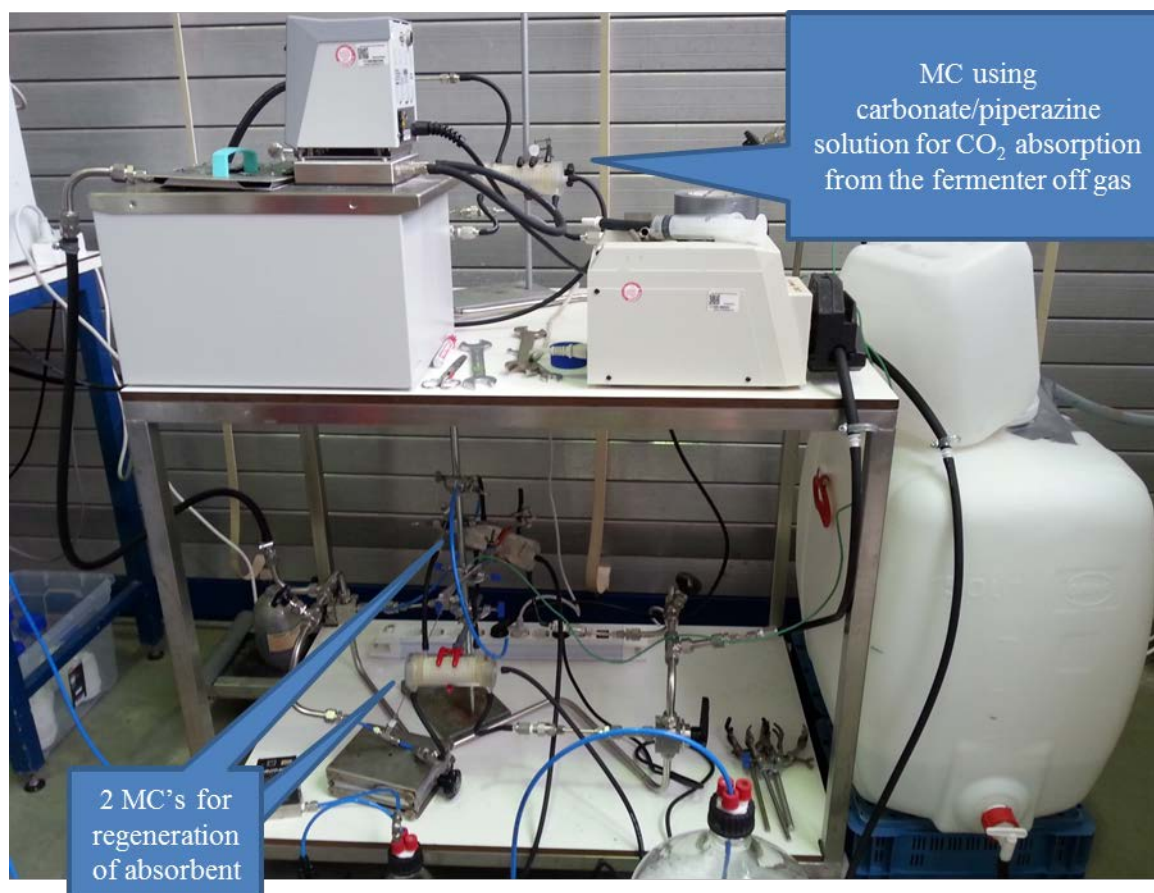


Fig. 16. RWTH Gas upgrading unit with membrane contactors (MC) connected to the off-gas of the P 1000 hydrogen fermenter in Wageningen. Gas upgrading is done by absorption of CO₂ in 0.5M potassium carbonate and 0.5M piperazine. The off-gas flowrate was approx. 6.9 L/min. Absorption (1 MC) is at below 30°C and desorption for regeneration (2 MC's) at circa 55°C.

Recovery of hydrogen from the liquid phase in the hydrogen fermenter

For H₂ removal from the fermentation broth, 2 methods have been selected. In the first H₂ is counter-currently stripped in an external loop by CO₂. CO₂ is removed by stripping with N₂ and the H₂ lean fermentation broth can be recycled to the reactor. Using a 2-step stripping outside of the fermenter, two different stripping gases are needed. But, even if stripping gases with a low solubility within the fermentation broth are chosen, the stripping gases will dissolve partially in the fermentation broth and add at least one more component to the gas that needs to be removed later on.

The other method is based on a membrane based stripping process with a H₂ concentration gradient in the liquid and gas phase, respectively as the driving force. Moderate vacuum might replace the gas phase to prevent dilution of the hydrogen. The advantage of lesser components is in favour of a hydrogen removal system outside of the fermenter using membrane contactors and sweep gas.

The laboratory scale tests, carried out at 25°C, showed that the membrane contactors have a great potential to remove the dissolved hydrogen by applying nitrogen as a sweep gas. With the gained data, even though very limited, a rough scale-up was carried out (Table 12) for successful removal of hydrogen.

Table 12. Results for scale-up estimation for the hydrogen removal by membrane contactor module by MedArray

Process				
Circulation flow rate	60 L/h	120 L/h	500 L/h	1500 L/h
Required membrane area	0.75 m ²	1.5 m ²	3.75 m ²	11.25 m ²
Required modules	1	2 (parallel)	5 (parallel)	15 (parallel)
Module space demand	0.0004 m ³	0.008 m ³	0.0044 m ³	0.0442 m ³
Gas flow rate	6 L/h	12 L/h	60 L/h	180 L/h

Innovative analysers for process gas

For the measurement of H₂S a new system has been developed. In general, H₂S sensors suffer from a high cross sensitivity to H₂. Sensors with low relative sensitivity have only a limited measurement range e.g. 20 ppm which seems too low for the raw gas in HyTIME. Therefore, a new dilution method has been developed with both an electrochemical sensor plus a dilution system using oscillating valves and a thermal conductivity sensor with an iterative mathematical model. With using an oscillating (clocking) valve which provides faster measurement and requires less hardware. The device measures the concentration of hydrogen, carbon dioxide, oxygen, methane, and hydrogen sulphide. For the later, its concentration is measured until its concentration is 1000 times less than the hydrogen concentration.

WP 5 System integration

Mass and Energy Balances

Experimental and simulation data are supplemented with information on technical scale taken from partner's work, comparable projects/processes and literature, forming the basis for up-scaling and techno-economic evaluation with simulation models.

From the experimental work in WP 2 on the steam explosion process, a heat demand of 180-4800 kWh/t feedstock is obtained for the steam explosion step, mainly due to variability in moisture content of the biomass. Besides feeding as dry biomass as possible, implementation of a flashing step as well as continuous operation is suggested, since about 40 % of heat demand of the steam explosion step is needed for reactor pre-heating (Sui et al., 2014).

The different pretreatment procedures of the different biomasses are shown in Table 13. The results have been used as input for the techno-economic evaluation. Validation of Chemical Oxygen Demand (COD) calculation was enabled by COD measurements/calculations using methane potential (BMP) tests of process effluents and a detailed analysis of process streams (Table 14). To meet the COD values reported from DLO-FBR, the conversion factors in thermophilic hydrogen (THF) reactor were adjusted.

As shown in Table 15, performance of hydrogen fermentation for wheat straw routes is considerably lower compared to verge grass, possibly due to lack of nitrogen stripping in the wheat straw fermentations.

To remove hydrogen from fermentation broth to avoid inhibitory effects, stripping with nitrogen is applied at DLO-FBR. For simulation a specific stripping gas flow rate of 0.35 L/L.h is used, corresponding to the 2 NL/h for the 5.7 L reactor. Productivity is kept with the data from verge grass fermentation at 5.7 L reactor scale which is slightly higher (7.2 mmol/L.h) than in that specific experiment.

Raw gas composition for a specific strip gas flow rate of 0.35 L/L.h fits best with a gas composition of 28 % H₂, 17 % CO₂ and 55 % N₂ representing raw gas with highest nitrogen content under consideration in gas-upgrading. Thus, the specific strip gas flow rate of 0.35 L/L.h was applied for all simulation runs involving nitrogen stripping.

Table 13: Comparison of data for pretreatment and enzymatic hydrolysis of verge grass and wheat straw per kg monomeric sugar obtained.

Biomass	Verge grass		Wheat straw	Wheat straw
Pretreatment	Alkali impregnation		Acid impregnation	Steam explosion
Usage/dosage	kg/kg sugar		kg/kg sugar	kg/kg sugar
Chemicals total	0.45	Chemicals total	2.64	0.11
Acetic acid	0.16	NaOH	1.19	0.11
Lime	0.29	H ₂ SO ₄ total	1.46	-
Water	116.8	Water	30.0	95.33
Enzyme solution	0.6	Enzyme solution	1.42	4.63
Buffer	-	Buffer	2.97	6.26
Biomass	4.4	Biomass	1.5	4.8
Heat demand	kWh	Heat demand	kWh	kWh
Pretreatment-heat (95°C)	1.00	Pretreatment-heat (120/190°C)	3.21	36.57
Hydrolysis (40°C)	1.70	Hydrolysis (50°C)	0.42	1.79
Total	2.70	Total	3.62	38.37

Hydrogen recovery in gas-upgrading for this gas composition varies between 51% and 75% depending on adsorption process and desired purity (Table 16). Best upgrading option represents arrangement PSA-7 (see table 11 for description). Table 16 shows the need to decrease N₂ and increase H₂ to obtain higher yields. Calculations without stripping give a raw gas composition (dry gas) of 65 % H₂ and 35 % CO₂. Higher

hydrogen content considerably increases yields. It has to be further considered, that the absence of N₂ boosts adsorption performance. A gas composition of 30 % H₂ and 70 % CO₂ (Figure 17, violet line) shows much higher yields than the raw gas from THF with N₂ stripping (Figure 17, red line). During simulation black box models are applied for gas-upgrading based on process parameters for adsorption (PSA-7).

Table 14: Comparison of Chemical Oxygen Demand (COD) data for process effluents from fermentation of verge grass in the 5.7 L thermophilic reactor (THF) at DLO-FBR.

THF effluent	COD from DLO-FBR	COD simulation (conversion factors from HYVOLUTION)	COD simulation (adjusted conversion factors)
Main components	mg O ₂ /L	mg O ₂ /L	mg O ₂ /L
Acetic acid	7445.8	8371.9	7085.8
Lactic acid	1506.5	1380.6	1507.5
Cysteine	660.4	1338.5	1065.7
Yeast extract	1051.8	1359.8	1351.6
Cell mass	983	1159.1	991.4
Glucose	1586.7	500.3	1587.5
Xylose	259.1	108.7	255.8
Ethanol	777.8	0	773.8
TOTAL defined	14271.1	14210.7	14619.1
Unknown components	6485.8		
TOTAL	20756.9		

Table 15: Fractional conversion THF model based on experimental data

Biomass	Verge grass	Wheat straw	Wheat straw
Pretreatment	Alkaline impregnation	Acid impregnation	Steam explosion
Reaction			
glucose → acetate/H ₂	0.698	0.062	0.01
glucose → cell mass	0.130		
glucose → lactate	0.011		
glucose → ethanol	0.064	0.330*)	
glucose → butyrate/H ₂		0.072	0.15
xylose → acetate/H ₂	0.730		0.6
xylose → cell mass	0.130		
xylose → lactate	0.011		
xylose → ethanol	0.064	0.890*)	
oligomer → glucose			0.4

*) for closing sugar balance

Table 16: H₂ yield for different adsorption processes and purity 3.0 and 1.8 (Source: HyGear)

	H ₂ recovery/yield			
	VPSA	PSA-7	PSA-13	VSA
Purity 3.0	66%	51%	55%	66%
Purity 1.8	74%	67%	69%	75%

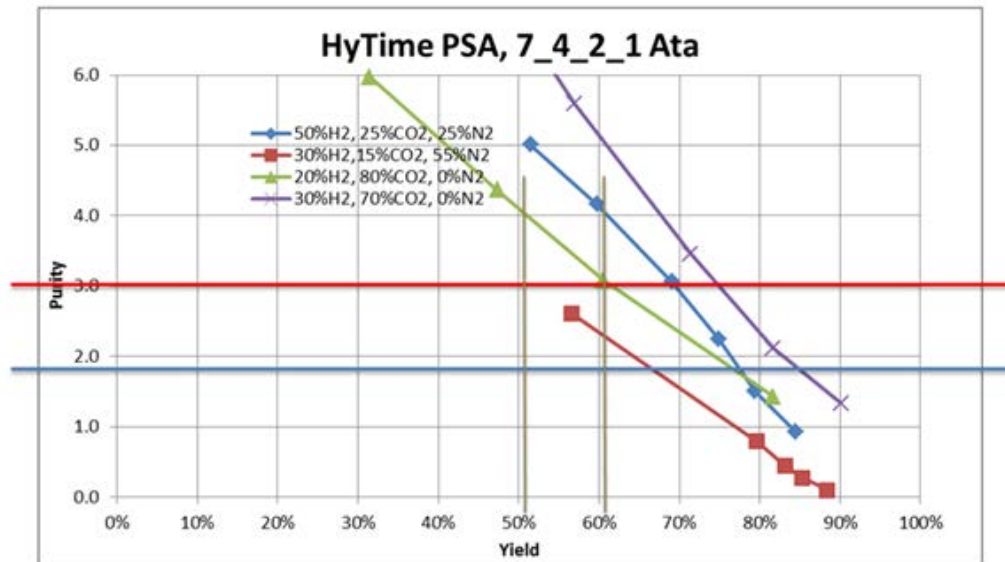


Figure 17: Yield/purity plot for PSA-7 (Source: HyGear)

Conversion factors for methane production in stoichiometric anaerobic digestion (AD) model were changed to 0.85 for carbohydrates and 0.70 for proteins and fat according to VDI guideline 4630. The model showed good accordance with biogas yields for the digestion of sweet sorghum (Theuretzbacher et al., 2013).

Table 17: Comparison of anaerobic digestion (AD) model results with experimental biogas production from influent and effluent of a thermophilic hydrogen fermentation (THF) on grass hydrolysate

	BIOTHANE methane production, m ³ CH ₄ /m ³ input	Simulation methane production from adjusted composition, m ³ CH ₄ /m ³ input	
		COD model	Stoichiometric model
THF feed medium	9.7	9.9	8.5
THF effluent	6.8	6.3	5.8

For validation of the COD based model for experimental BMP tests and detailed analysis of sample composition of THF effluent from grass hydrolysate are used. The reason for lower biogas production in the stoichiometric model (Table 17) is the consumption of substrate for cell mass production. It is assumed that in a real continuous AD process, biomass formation would be higher than during BMP tests and thus correspond more to the results obtained from the stoichiometric model.

Adjusted simulation models described are used to calculate mass and heat balances for the techno-economic analysis. All routes are scaled to a production of 400 kg/d hydrogen in the raw gas leaving the thermophilic fermentation reactor.

The following routes are considered for calculations:

- Alkaline pretreatment of verge grass
 - **Route Alkaline**
- Diluted acid pretreatment of wheat straw
 - **Route Acid**
 - **Route Acid_SetGrass**
 - **Route Acid_SetGrass_Opt**
 - **Route Acid_SetGrass_Enz**
- Steam explosion pretreatment of wheat straw
 - **Route StEx**
 - **Route StEx_Red**
 - **Route StEx_SetGrass_Flash**

Routes **Alkaline**, **Acid** and **StEx** are implemented following the reported experimental protocols.

Route **Acid_SetGrass** uses hydrogen fermentation parameters from verge grass to overcome the high feedstock and chemical demand in pretreatment observed for wheat hydrolysates during thermophilic fermentation, mimicking introduction of nitrogen stripping to these process routes. To enable direct comparison with **Alkaline**, the sugar concentration in thermophilic fermentation is reduced to 10 g/L. Route **Acid_SetGrass_Opt** is further modified with the use of verge grass parameters, omission of citrate and the reduction of enzyme demand (0.14 kg/kg dm). Route **Acid_SetGrass_Enz** uses the same adjustments like but assumes on-site enzyme production by using 1/10 of hydrolysate. Route **StEx_Red** is calculated with a reduced steam demand of 1 kg/kg dm, keeping the experimentally obtained sugar consumption and hydrogen yields in THF. Route **StEx_SetGrass_Flash** is based on fermentation of verge grass, a reduced steam demand of 1 kg/kg dm and flashing after steam explosion and recirculation of steam to preheat feedstock.

It is interesting that the amount of biogas obtained from **Alkaline** is rather comparable with **Acid** and **StEx** (Table 18), although only a little sugar is converted to hydrogen and no by-products are reported. The main reason that **Acid_SetGrass** and **StEx_SetGrass_Flash**, operating at comparable conditions as **Alkaline**, show lower CH₄ amounts seems to be due to the missing COD adjustment for these steps. As seen with COD adjustment of **Alkaline**, considerable amounts of biomass/hydrolysed biomass are not covered by the performed analytical procedures.

Table 18: Produced biogas and obtained heat and power for selected process routes.

Route		Alkaline	Acid	Acid SetGrass	Acid SetGrass Enz	StEx	StEx SetGrass Flash
Gas Total	kg/h	1614	2307	391	424	3823	370
CO ₂	kg/h	1184	1687	292	325	2857	271
CH ₄	kg/h	429	591	98	96	966	99
NH ₃	kg/h	0.54	27	0.64	2.46	0.00	0.44
Work	kW	2407	3376	554	547	5419	555
Heat	kW	2616	3669	602	594	5888	602

Heat integration is performed on basis of minimum utility demand obtained via Pinch analysis and on the generation of heat exchanger networks (HEN). Heat demand of the integrated case for **Alkaline** is 67%, for optimized utility 77% and assuming the minimum utility 80% lower as compared to the non-integrated case. Reduction of heat demand for **Acid** is comparable to the **Alkaline** with 66%, 74% and 80% for options with low heat exchanger area, low utility demand and minimum utility demand compared to the non-integrated case, respectively. Only a few heat exchangers are necessary and heat exchange areas are low for **StEx** as part of heating is done by direct steam injection and already included. Thus, heat integration only slightly lowers the heat demand of the integrated options by 21%, 29% and 44% for low heat exchanger area, low utility demand and minimum utility demand, respectively.

Technological Data

Relevant technical process data are collected over the entire process chain from feedstock supply through tgas upgrading to enable the up-scale to 400 kg/d raw biohydrogen. Topics are heat integration, comparison and evaluation of equipment for continuous operation (heat exchangers, solid/liquid separation, steam explosion), detailed design of gas up-grading and the use of off-gas from the process. Furthermore, reported results are scaled to 400 kg H₂/d after thermophilic fermentation. Technological data include experimental investigation of AD of effluents as well as the developed process visualization system, enabling a virtual connection to HyTIME plant via the internet. For equipment specific heat exchanger design with easy cleaning options have been selected as e.g. the HRS Unicus heat exchanger `series or spiral heat exchangers (Sondex) to handle fouling fluids, fluids containing fibers, particles and other viscous or abrasive media. The reactor sizes in pretreatment and fermentation for the different process routes are estimated for the production of 400 kg H₂/d in the raw gas (Table 19). Highest difference in reactor size for different process routes is observed in enzymatic hydrolysis due to retention times and substrate concentration. Lowest difference is observed in AD, when neglecting cases with poor performance in THF. In chemical hydrolysis a factor of 10 is observed. The volume for flash operation is not included in the steam explosion installation.

Table 19: Reactor sizes in m³ for selected process routes

	Pretreatment (chemical, steam explosion)	Enzymatic hydrolysis	Thermophilic fermentation	Anaerobic digestion
	m ³	m ³	m ³	m ³
Route Alkaline	77	103	1169	7053
Route Acid	401	11504	5555	24111
Route Acid_SetGrass	34	1001	1159	4443
Route Acid_SetGrass_Opt	34	893	1159	4156
Route Acid_SetGrass_Enz	37	1062	1131	4156
Route StEx	12	9357	3030	26455
Route StEx_Red	8.5	9364	3030	26455
Route StEx_SetGrass_Flash	1.7	2052	1101	5651

Process residues from pretreatment and thermophilic fermentation are collected and fed into AD to produce methane, to cover the heat and energy demand of HyTIME. The potential of the effluent for anaerobic digestion is evaluated and validated by performing BMP (biomethane potential) tests and larger scale tests in a continuously stirred tank reactor (CSTR) and an anaerobic membrane bioreactor (AnMBR).

Usually samples show fast conversion, with more than 90% of its final conversion reached after 1 day of incubation. Digestibility (TCOD converted into methane) varied between the samples depending on their origin and the applied pretreatment procedure. ENVIPARK successfully tested effluents from thermophilic biohydrogen fermentation in a CSTR (working volume 150 L) for biogas production at 50°C. Two different effluents were used for AD (Fig. 18). The results are shown in Table 20.

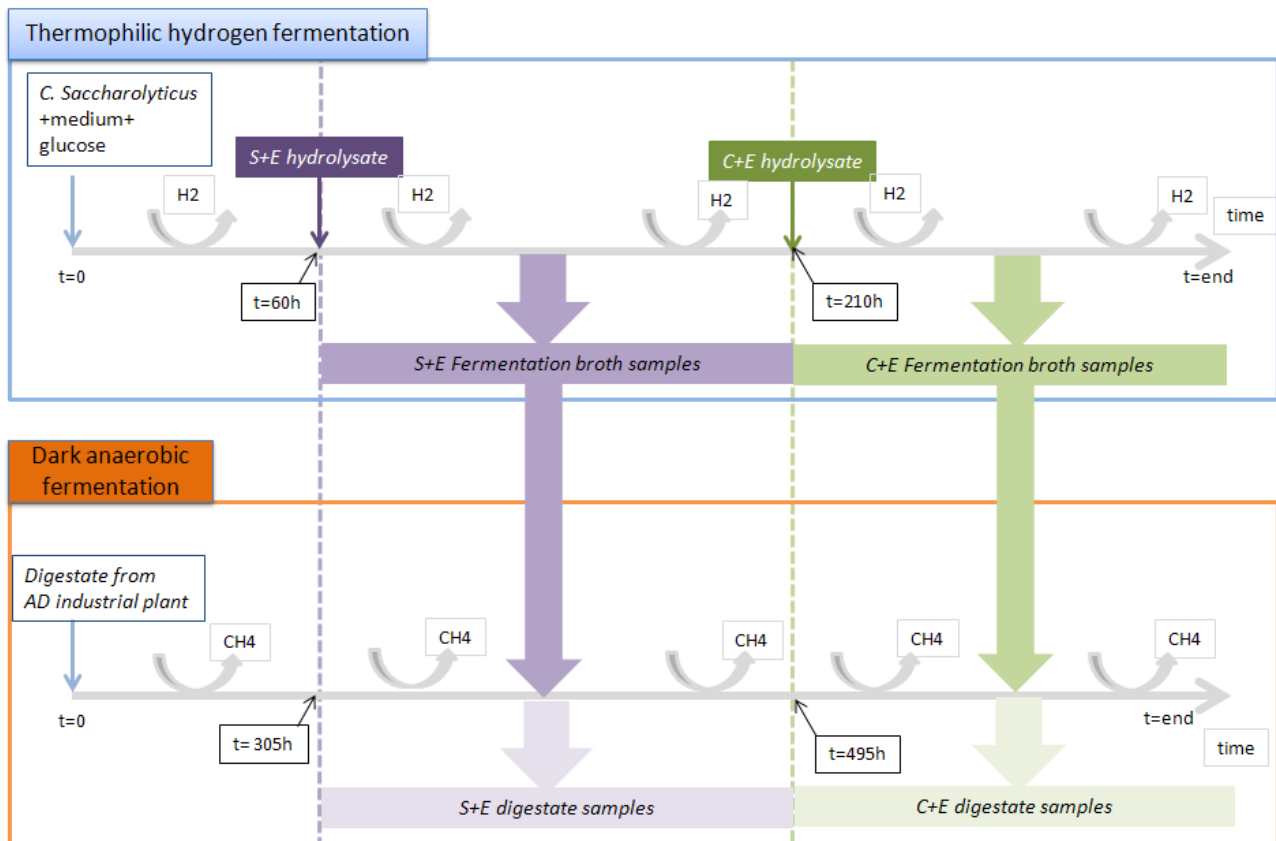


Fig. 18 Use of effluents from thermophilic hydrogen fermentation with wheat straw as biomass. Phase 0: start-up with industrial digestate, phase 1 (purple): effluent from fermentation of hydrolysate after steam explosion and enzymatic hydrolysis (S+E) and phase 2 (green): effluent from fermentation of hydrolysate after acid pretreatment and enzymatic hydrolysis (C+E),

Table 20 Results of anaerobic digestion of effluents from thermophilic fermentation using hydrolysate after steam explosion (S+E) or acid pretreatment (C+E), including enzymatic hydrolysis, of wheat straw.

Anaerobic digestion	Start-up phase	S+E fermentation (phase 1)	C+E fermentation (phase 2)
Produced biogas (L/L. d)	0.7	0.9	1.9
Maximum CH ₄ (%)	59.4	53.9	53.9
Average CH ₄ (%)	48.4	46.8	44.3

BIOTHANE Systems Int. has explored the AD of effluent from the thermophilic biohydrogen production on diluted sugar beet molasses with their special anaerobic membrane bioreactor (Memthane® AnMBR) designed to increase the biogas production rate by sludge recycling. The membrane was made of PVDF with a mean pore size of 0.03 μm. Operation took place at 37 °C. Two different effluents from the thermophilic biohydrogen production on molasses prepared in WP 3 were investigated showing severe inhibition with increasing volatile fatty acids (VFA) levels even at a low volumetric loading rate. Toxicity was also evident from the lack of biomass growth, as reflected by the constant sludge VSS concentrations. Activity tests carried out with AnMBR sludge samples at the end phase of the trial showed that the methanogenic activity had dropped significantly (Figure 19). From these results it was clear that the AnMBR was suffering from irreversible toxicity by components in the molasses effluent.

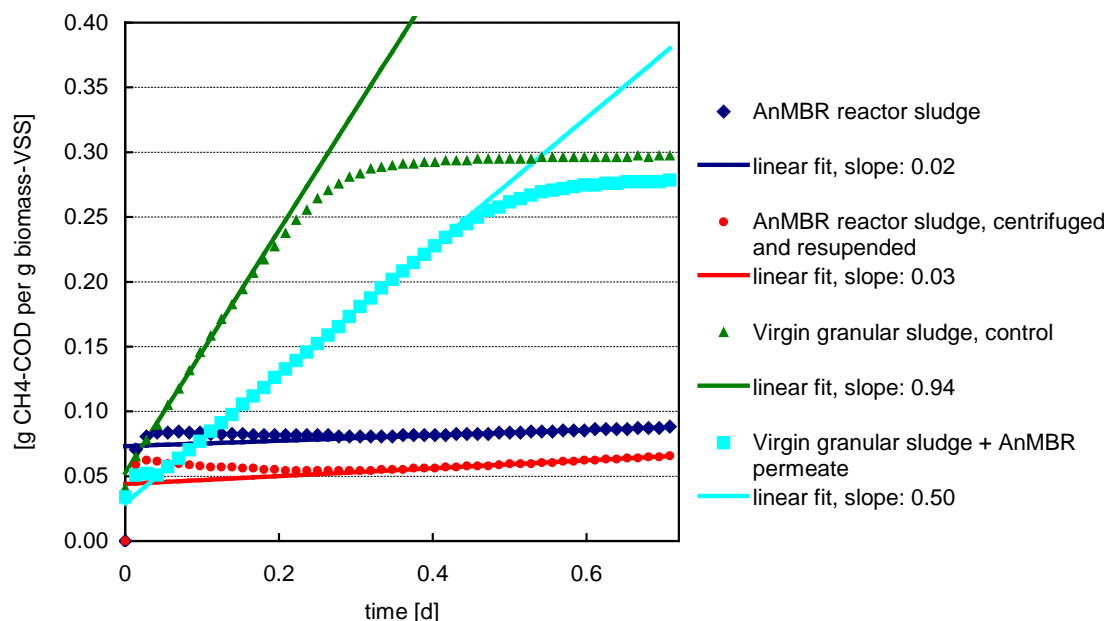


Fig. 19 Activity tests with sodium acetate added to the anaerobic membrane bioreactor (AnMBR) sludge and virgin granular sludge in the presence and in the absence of AnMBR permeate. Methane production is expressed as chemical oxygen demand (COD) relative to the biomass-volatile suspended solids (VSS) concentration. The slope of each curves represents the specific acetoclastic methanogenic activity.

The processing of fermenter gas downstream of the gas upgrading unit was evaluated by HyGear to provide input for TUW for the techno-economic study. Cost and sizing information for a demister and bacterial filters for the 400 kg H₂/d. fermenter case was collected. As the most cost effective technology for H₂S removal non-regenerable solids were selected with a combination of a guard bed (H₂S removal down to 5 ppm) and a polish bed (from 5 ppm H₂S to 0.05 ppm). A process flow diagram of the gas processing and upgrading (based on VSA) unit, including a cooling, was prepared (Fig. 20).

The utilisation of highly diluted hydrogen in the off-gas has been evaluated. Options are mixing with biogas and thermal utilisation of the mixture or a stand-alone use in a dedicated unit operation, able to cope with the low calorific value. Critical parameter for the utilisation of low calorific value gas is the flammable limit of the mixture. Data for the systems H₂/N₂, CH₄/CO₂ and H₂/CH₄ in air have been collected and the theoretical flammable limits for expected off-gas from gas-upgrading has been estimated (Table 21).

To control all steps of HyTIME process (biomass supply and fractionation, fermentation, gas upgrading and anaerobic digestion) a dedicated system for control and visualization was developed. Due to the low acceptance of the international standard for distributed control systems IEC 61499 "Function Blocks" (since 2005) and the lack of suitable tools available on the market, a process control system fully based on open source is proposed. A distributed control system was suggested, whose structure is based on IEC 61512 (batch control) and the MQTT protocol is used for connection of all the modules (Figures 21 and 22).

Table 21: Flammable limits (FL) for off-gas mixtures with H₂, CO₂ and N₂ from gas upgrading.

		Mol% H ₂ in air	Mol% mixture in air
9 % H ₂ / 91 % inert, inert = CO ₂ + N ₂ as N ₂	Lower FL	4	44
	Upper FL	7	78
16 % H ₂ / 84 % inert, inert = CO ₂ + N ₂ as N ₂	Lower FL	4	25
	Upper FL	12	75

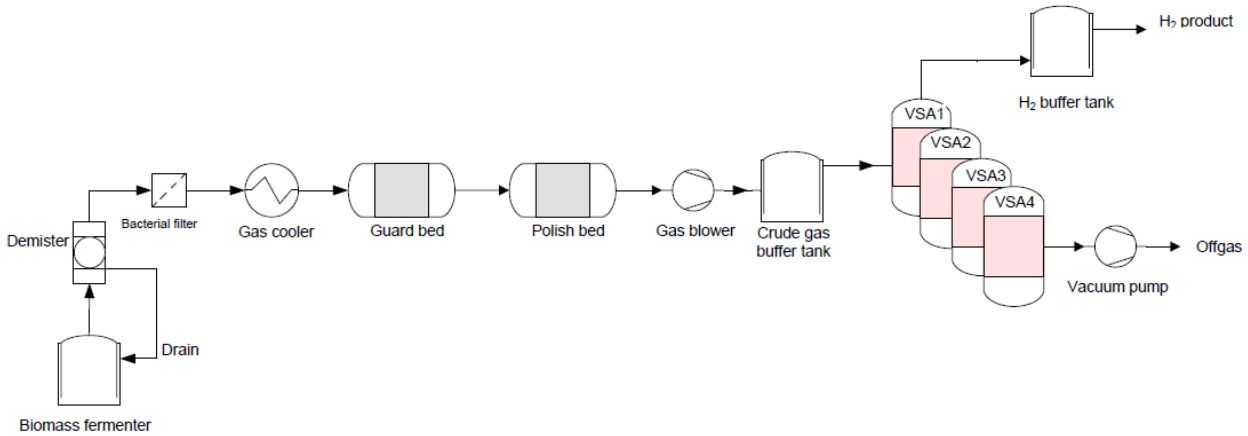


Fig. 20 Process flow diagram (PFD) of vacuum swing adsorption (VSA) gas-upgrading step

The use of an open system enables the exchange of single modules and is vendor independent. The system is developed in connection with the base automation “awicontrol”. However, the base automation could be any system if a MQTT interface or a MQTT interface gateway is connected. The visualisation uses "scenarios" to assemble charts and tables e.g. showing data from the hydrogen fermentation running at DLO-FBR in The Netherlands and displayed and printed at AWITE in Germany. A scenario can be flexibly configured by selecting relevant sensors and events. In this way different scenarios can be defined and configured using the Scenario Manager.

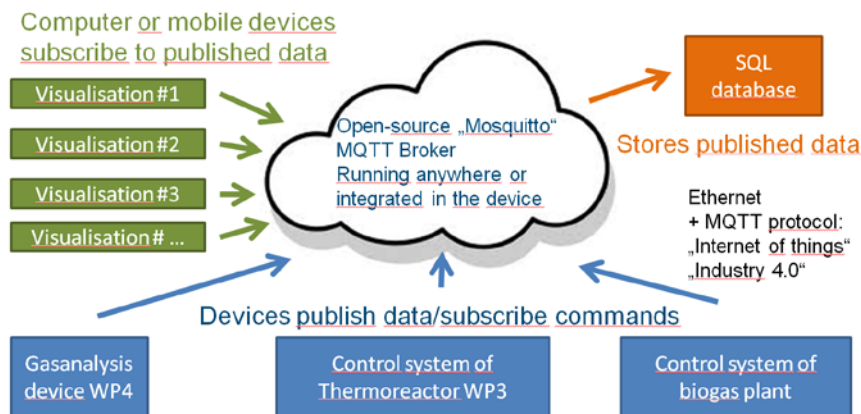


Fig. 20 Communication concept between automation modules.

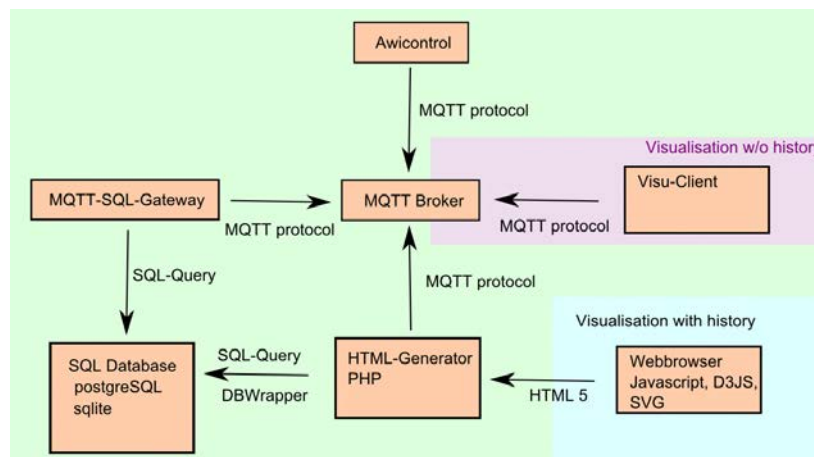


Fig. 21 Interaction scheme of base automation system (awicontrol) with database and visualisation.

Techno-economic Evaluation

The process routes are scaled to obtain 400 kg H₂/day produced by the thermophilic fermentation. The three process routes (alkaline pretreatment of verge grass, acid pretreatment of wheat straw and steam explosion pretreatment of wheat straw) were each economically investigated for different scenarios with highest and lowest prices for different capital and operating costs (Table 22).

The results for verge grass as biomass for H₂ production show 2.4 – 4.8% reduction of the costs after heat integration. At 50 % enzyme costs (case 3) hydrogen production costs are 33% lower than with full costs (cases 1 and 2) and with a decrease to 30% enzyme costs, another 17% cost reduction can be established. Optimisation of gas upgrading costs in case 4 reduces the production costs by 11 – 16%. Without N₂ stripping and with a theoretical increase in the gas upgrading yield to 85% and 95% (case 5), the lowest hydrogen production cost of 34 and 31 €/kg H₂, respectively, is achieved.

Table 22. Hydrogen production costs for process routes using alkaline-pretreated verge grass

Process parameters	N ₂ stripping	Heat integration	50 % enzyme cost	30% enzyme cost	Yield in PSA upgrading (%)		Production cost (€/kg H ₂)	
					3.0	1.8	Lowest	Highest
Case 1	+				51		106	213
						67	82	163
						75	72	144
Case 2	+	+			51		102	208
						67	78	159
						75	68	141
Case 3	+	+	+		51		72	-
						67	55	-
						75	48	-
Case 4:	+	+		+	51		59	-
						67	46	-
						75	40	-
						67 ^{*)}	40	-
						75 ^{*)}	36	-
Case 5		+		+		75	38	-
					85 ^{**)}		34	-
					95 ^{**)}		31	-

^{*)} optimized gas upgrading costs

^{**)} theoretical improvement of gas upgrading yield

Techno-economic analysis has also been done for wheat straw after acid pretreatment and after pretreatment by steam explosion. The unrealistically high costs showed that the current yields from the lab experiments are not appropriate for a commercial production plant: after several assumptions for process improvement including on-site production of the enzymes, production costs for the acid pretreatment route could be reduced to 56 – 85 €/kg H₂.

The operating cost fractions for the verge grass route are shown in Figure 23 with the costs of enzymes as the major share of costs.

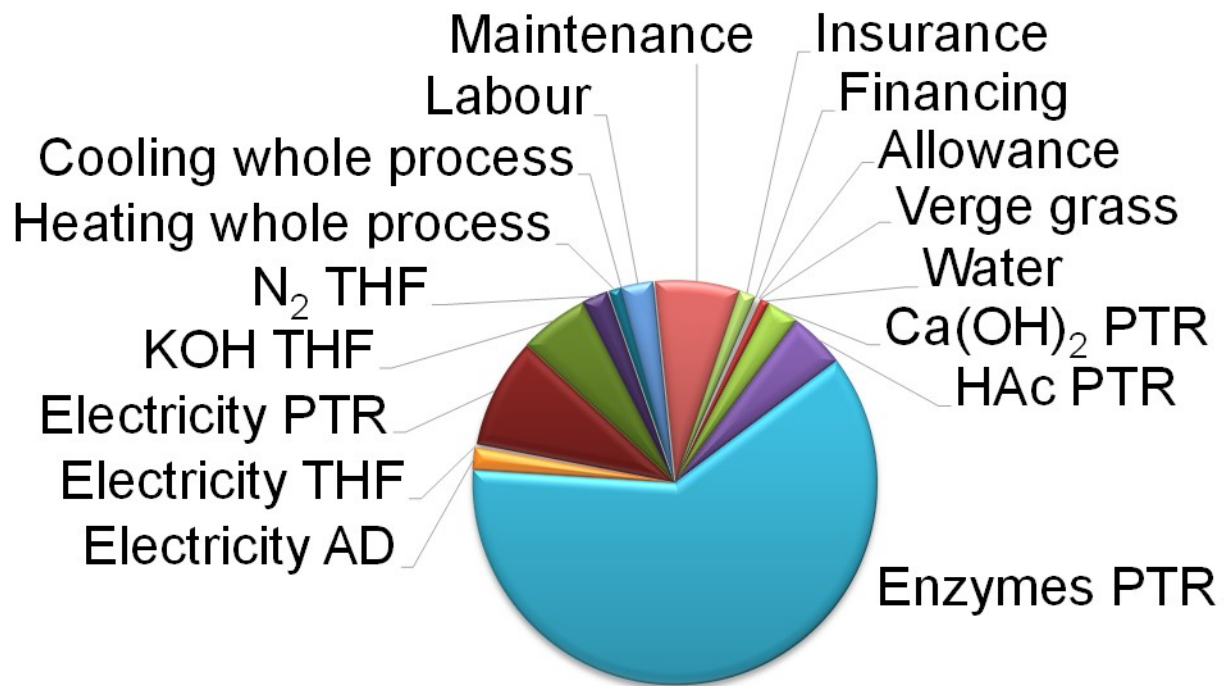


Fig.23 Operating cost fractions for hydrogen production from verge grass after alkaline pretreatment, enzymatic hydrolysis and thermophilic fermentation followed by anaerobic digestion and with heat integration (Case 2). PTR: pretreatment, THF: thermophilic hydrogen fermentation, AD: anaerobic digestion.

Potential impact, main dissemination activities and exploitation of results

Strategic impact

The impact of HyTIME is right in the heart of the expected outcome for research oriented at biological processes, described under topic SP1-JTI-FCH.2010.2.4: Low temperature H₂ production processes, because the following features form the core of this project :

- ▲ Efficient fractionation of 2nd generation biomass and different biomass residues
- ▲ Installment and operation of a continuous fermentation process for the production of 1-10 kg hydrogen/d using biomass
- ▲ Design and construction of devices for gas cleaning and process control specifically designed for bioprocesses operating at low temperature and pressure
- ▲ Development of a biohydrogen production system as a stepping stone to pre-commercial application.
- ▲ Enlarging the technology base of participating SMEs for innovations in the field of design and construction of reactors and process control equipment.

The combination of basic research in microbial physiology with applied research and development is spread over the partners in academia and the industrial partners, including 3 SME's, respectively. The 5 partners initially involved in the previous HYVOLUTION project have already shown their complementarity in hydrogen production by thermophilic fermentation. The expansion with 4 new partners coming from industry supports the movement towards application by increased involvement and emphasis on the activities in HyTIME.

The overall impact will be the acceleration of the implementation of an industrial bioprocess for decentral hydrogen production systems using 2nd generation biomass.

HyTIME builds on the findings in the former FP5 BIOHYDROGEN and FP6 HYVOLUTION projects which have founded the basis for the approach of small scale hydrogen production from locally produced biomass enabling security of supply and independence of fossil fuels. The former projects have also underpinned the combination of co-products in agro-industries with hydrogen production to reduce hydrogen production costs and to provide for a hydrogen production technology which does not compete with food production.

The technology of HyTIME leans on the technology of anaerobic digestion which has been developed to provide a solution to the undesired environmental burden of bio-waste streams. In the past, aerobic waste water purification was replaced by anaerobic digestion because of the costly aeration procedures. With the recognition of the energetic value of methane in biogas coming from anaerobic digestion, addition of co-products from agro-industry to anaerobic digestions installations became fashionable. This is precisely the area where the technology of HyTIME will have its major impact as the technology is expected to replace anaerobic digestion at numerous locations in the future, providing hydrogen instead of methane as its end-product.

Impact on the European level

In the EU funded project HyWays the overall hydrogen production mix for transport and stationary end-use applications in 10 Member States has been analyzed (Fig 24). Each country outlined its own preferences but biomass, besides natural gas and wind energy based pathways, was always selected by all Member States participating in HyWays. After imposing a least-cost solution on the pathways shown in figure 24, the share of the renewables (solar, wind and biomass), increased considerably with the highest final share for hydrogen from biomass. The study in HyWays has taken into account the competition for biomass by other energy uses. However, an in-depth role of biomass was out of the scope of this particular project and therefore the focus in HyWays has been on the use of woody biomass for gasification in large installations.

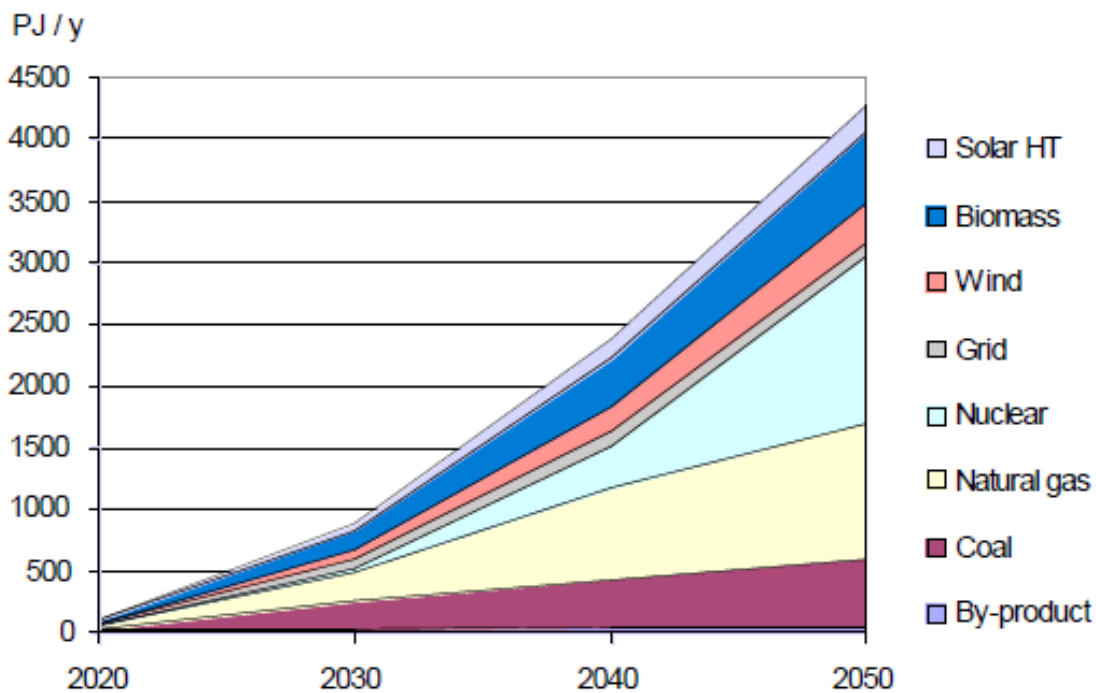


Fig. 24 Overall hydrogen production mix for the 10 HyWays countries based on the visions developed in the Member State workshops. Source: HyWays (2008) - The European Hydrogen Roadmap.

The technology of HyTIME is especially suited for hydrogen production from other biomass streams in small scale installations, thus complementing the thermochemical conversion addressed in HyWays and increasing the security of supply of resources for hydrogen production.

In the EU, between 118 and 138 million tonnes of bio-waste (garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises as well as comparable waste from food processing plants) are currently produced on a yearly basis (COM(2010)235 final). This bio-waste could be used for methane production in biogas installations with a theoretical production of 2 million tonnes methane. Using the same assumptions, the theoretical hydrogen production from the same bio-waste would be 0.34 million tonnes hydrogen with an additional 1.3 million tonnes methane on the side when using the technology of HyTIME. If only half of the theoretical amount of hydrogen would be produced, a production 20 PJ/year from bio-waste would be the result. This amount is envisaged to increase because of the 10% increase in bio-waste production foreseen in the EU and because it is expected that in the global transition to a bio-based economy, new bio-waste streams will arise. Besides, the technology may grow from its infancy to full maturity after 10 years following the start of HyTIME. The impact for the EU is twofold: a significant contribution to the triple 20% by 2020' objective with green hydrogen and a contribution to improved bio-waste management.

Besides the bio-waste streams mentioned above, HyTIME is also designed to convert primary waste streams in agriculture to hydrogen. The availability of such waste streams in the EU-27 has been listed in HYVOLUTION (table 23). The Biomass-to Hydrogen route as an efficient biofuel pathway evidently offers new opportunities for agricultural policy to expand innovative outlets for EU farmers. This whole chain approach is further solidified by the trans-Europe setting of the partners in HyTIME which will be supportive to achieve national impacts on clean energy.

Finally, there is an increased interest in the utilization of aquatic biomass for the production of food and chemicals. Again, this development will come with an additional bio-waste stream which is very suitable for conversion by fermentation as in HyTIME.

Table 23. Inventory of primary residues in agriculture of the 27 EU countries. Data were kindly provided by the National Technical University in Athens and derived from Agriculture in the EU statistical and economic information 2004 (EU DG for Agriculture and Rural Development, Feb 2005).

Crop Category	Crop	Main product	Primary by-products		
			Leafy biomass	Stems and stalks	
Million ton dry matter					
Crops already cultivated for nutritional needs	Sugar Crops	Sugar beet	18.1	7.3	-
	Starch Crops	Potato	12.9	8.8	-
		Wheat	97.5	-	82.6
		Barley	48.2	-	40.8
		Maize	43.8	-	74.5
		Other cereals	25.7	-	18.3
		Rice	2.3	-	1.7
		Grapes	12.7	-	10.9
	Other Food Crops	Apples	1.7	-	2.0
		Other fruits	3.6	-	15.1
		Vegetables	5.4	7.3	-
		Oil seeds	9.4	-	40.3
	Energy crops	Sugar Crops	Sweet sorghum	19.5	4.9
Lignocellulosic crops					
		Miscanthus	21.6	5.6	-
		Total annual production for EU 27	322.4	33.9	294.1

The maximization of biomass exploitation, extensive process integration and continuous operation will reduce the hydrogen production costs, giving economic justification to the HyTIME process. HyTIME may operate at small-scale stand-alone or add-on production level, which will:

- ▲ Minimize the transport and infrastructure costs and subsequently improve energy efficiency
- ▲ Secure implementation in rural regions, thus contributing to strengthening regional economies
- ▲ Enable the introduction of a novel sustainable technology at a faster pace.

The concept of HyTIME is fully aligned with the concept of sustainability since it is independent of fossil imports. All these benefits ensure an impact of HyTIME on the reinforcement of European competitiveness in clean energy technologies.

Extensive activity in the developments of biohydrogen production occurs globally, but is fragmented across the Member States of the EU. In view of the diversity of challenges that will be met in the development of a HyTIME process, a collective approach at the European level is desired. The consortium of HyTIME is especially designed to deal with the following issues:

- ▲ Feedstock and biomass supply logistics to obtain security of supply
- ▲ Tailor-made optimization of biomass fractionation to obtain improved productivity
- ▲ Integration of various process streams to increase production efficiencies and decrease environmental impacts
- ▲ Process development and scale up to increase marketing opportunities.

The scale of investment and risks for developing a bioprocess for hydrogen production from 2nd generation biomass are large and beyond a national level. HyTIME exploits transnational co-operation by the continuation of HYVOLUTION with an interdisciplinary and cross-sectoral consortium that is expanded with 4 new partners to pool together competences in biomass, biotechnology and energy. In addition, international projects like HyTIME reinforce long-lasting European research alliances among the different disciplines that enable necessary breakthroughs to arrive at a cost-effective process. The participation of new industrial partners in HyTIME, together with EU biohydrogen leaders in the relevant scientific fields from HYVOLUTION indicates that industrial support for such an endeavour is increasing. The HyTIME consortium is derived from the world-recognised group of partners in HYVOLUTION (evident from the nomination for the IEA award) with complementary expertises. Furthermore, the consortium is embedded in a network of many international and national activities and know-how concerning production of hydrogen in HYVOLUTION, BIOHYDROGEN, and FISR Hydrogen vector, Sustainable Production of Hydrogen in Austria, of methane in GasHighWay and SEBE, of renewable energy and chemicals biorefinery projects such as SOLREF, BIOSYNERGY, and other relevant projects such as FITUP dealing with fuel cells, Austrian Advanced Automotive Technology, and HyProfessionals developing educational programmes and training initiatives related to hydrogen technologies and fuel cells, to name a few. Besides these links with hydrogen associated projects, the consortium is embedded in a network of international activities in development of lignocellulosic bio-refineries for the production of renewable energy and chemicals such as BIOREF-INTEG and Star-COLIBRI. This will consolidate profound synergies that will support and speed up the research and guarantee breakthroughs in HyTIME, applying previously built up knowledge.

Impact on the market

Hydrogen produced in HyTIME offers substantial efficiency gains over biofuels (and conventional fuels) when used to feed a fuel cell, thus contributing to energy conservation goals. This efficiency gain over biofuels is specifically important since the availability of biomass is limited and strong competition exists (e.g. power sector, feedstock for chemical industry as chemical building blocks and/or fibers). These features will promote the use of hydrogen amongst the general public facilitating the transition of the market to an hydrogen economy.

The expected market size of HyTIME is evident from the world-wide prospects of hydrogen and merely depends on the availability of biomass. Since the 1990's the global market for hydrogen in the (petro)chemical industry has been expanding with an average annual growth percentage of 6.3%, due to enforcement of ever more stringent environmental legislation in the EU and the US. Green hydrogen offers the industry the advantage of a sustainable energy carrier for fuel cells in transport and stationary appliances, but also as a green chemical and the flexibility to meet international agreements for cleaner products, such as desulfurization and upgrading of fossil fuels.

Besides green hydrogen, novel and improved technologies will be developed in HyTIME with the intention to form a stepping stone to commercialization, potentially also in related processes. For example, more specific knowledge based on devices for upgrading of gas streams with components usually not observed in conventional gas streams will add to creating new opportunities in gas industries. The same is true for basic engineering issues, new process operation strategies and materials, also applicable in the foreseen development of bio-refineries. The work and results coming from HyTIME will lead to higher reliability of these appliances smoothening the market introduction of these systems.

Dissemination of project results

The previous biohydrogen projects have learnt that, in general, the public is enchanted by the production of hydrogen from bio-waste streams. Wherever the public considers the transition to a hydrogen economy as 'another' innovation, interest becomes for real when hydrogen is produced from renewable sources. Usually, the public is not aware of the potential of 2nd generation biomass or biomass waste streams for hydrogen production. The partners in HyTIME have considered the link between simple renewable biomass streams and hydrogen as an essential and crucial issue for dissemination.

HyTIME has followed the regular route for dissemination involving an attractive website, for project, hydrogen and biofuels information, a press release, and dissemination of project results in specialist

journals as well as presentations (conferences, fairs, fora etc.) at the national and international level (table 24).

During the course of HyTIME, there have been 10 dissemination activities especially geared towards the general public and policy makers as e.g. an interview in the leading Dutch Financial Daily, an interview in the EU Research Magazine, entitled Turning bio-waste into green hydrogen, papers in local magazines and oral presentations at national workshops and fora on ‘green’ innovations. In more restricted settings, numerous contacts have been made with representatives of Ministries or other governmental organisations. These representatives have come from HyTIME-partner countries but also from countries like Morocco, Russia, Greece, India and Japan.

Table 24. Dissemination activities in HyTIME

Type of dissemination activity	Number
Oral presentations aimed at general public and policy makers	10
Articles for a wider public	3
Interviews for a wider public	5
Exhibitions	2
Organisation of workshop	1
Press release	1
Publications in peer reviewed journals	6
Oral presentation at scientific conferences	20
Poster presentation at scientific conferences	4

The highest number of dissemination activities is found amongst the presentations, 20, at international conferences. Usually the audience of these conferences is a mixture of researchers, industrial delegates and policy makers, all working in the field of increased sustainability or a the greening of society. In table 24 the number of actual presentations at conferences has been listed. Generally these conferences have come with proceedings in which the abstracts or manuscripts of the presentations have been published. These are not listed under publications in peer reviewed journals. The coordinator of HyTIME actively contributed to the IEA-Hydrogen Implementing Agreement (HIA) Task 21 on Biohydrogen and the (International Association of Hydrogen Energy) IAHE with a Biohydrogen Division. These global knowledge platforms provide excellent opportunities to disseminate the results of HyTIME and the result is a global recognition of the successful integrated approach.

All information is available on the HyTIME website, for distribution upon request.

Several students have worked on HyTIME issues throughout the project, especially at the academic partners in order to acquire their degrees. Table 25 is an overview of how students have been trained through courses or dedicated lectures at seminars or have participated with experimental work in HyTIME.

Table 25. Educational activities in HyTIME

Type	Type of audience	Location	Main topic	Partner responsible /involved
Internship	Students University of Turin	Turin	Biomass pretreatment and fermentation	ENVIPARK
Internship	PhD student	Wageningen	Fermentation	DLO-FBR
Seminar	Students of Natl U of Malaysia	Bangi, Malaysia	Biohydrogen, biology and chemistry	DLO-FBR
Master thesis	International engineering society	Vienna	Process design	TUW
Seminar	Students of Pusan Natl University	Busan, Korea	The approach in the EU towards Biohydrogen	DLO-FBR
TCA Symposium Beyond Biogas	Students, researchers and national industry	Wageningen	Production and upgrading of hydrogen	HyGEAR
TCA Symposium Beyond Biogas	Students, researchers and national industry	Wageningen	Integrated approach of HyTIME	DLO-FBR
Lecture	International students and co-workers at DLO-FBR	Wageningen	Hydrogen applications	DLO-FBR
Training course	Students Industrial Chemistry, U of Turin	Turin	Biohydrogen and biogas	ENVIPARK
Training and education	1 PhD, 2 Masters and 1 Bachelor student	Vienna	Biorefinery concepts, process simulation etc.	TUW
Training and education	1 Master, 2 Bachelor and 1 Diplom student and circa 15 undergraduate students	Aachen	Gas separation devices etc.	RWTH

Exploitation of project results

HyTIME is special in being a mixture of TRL's: very low in the area of hydrogen fermentation and higher in the area of some gas upgrading devices.

At the upstream site, i.e. where biomass is pretreated and hydrolyzed, technologies have been validated. This will enable the partners involved to exploit this knowledge in case biomass owners are searching for turning their biomass into higher-value products. Several biomass streams have been utilized for hydrogen production, albeit with unforeseen low yields. This technology will require further R&D before exploitation is feasible. The same is true in the area of novel gas upgrading techniques. However, where measurement and control systems have been involved, the TRL has significantly increased. The developments in HyTIME have already brought some of these devices to the biogas market.

Exploitation plans more specifically:

ENVIPARK

The experiences gained during the HYTIME project will be used to promote and strengthen the connection between ENVIPARK and private companies in technology transfer, e.g. by giving support for future scale up of bioreactors for hydrogen production and for developing a ready-to-market system for a two stage system for production of biohydrogen and biogas.

The exploitation plan includes the biomass pretreatment processes and, in particular, the optimization of enzymatic processes. Besides, the possibility to obtain chemical building-blocks with high added value will be evaluated.

DLO-FBR

Exploitation of the findings in HyTIME by DLO-FBR will be similar as described for ENVIPARK. In the area of biomass pretreatment and hydrolysis and the subsequent fermentation of mobilized sugars to hydrogen, it has been shown that biohydrogen production is a realistic approach. DLO-FBR can offer

contract research to biomass owners e.g. by testing suitability of biomass streams for hydrogen production potential. However, the unforeseen events encountered when upscaling the reactor necessitate further basic research prior to exploitation.

TUW

TUW will use the models developed in HyTIME for simulation, integration and analysis of different biorefinery processes. Since the principles of procedures for pretreatment of biomass are very similar independent from the subsequent downstream processing, the developed models can easily be adapted to other biorefinery processes. The same is true for the exploitation of heat and process integration strategies.

AWITE

This SME has developed exploitable results in 3 areas:

A) New measurement device.

The hydrogen measurement device which was developed in the previous project HYVOLUTION was designed for measurement of high hydrogen sulphide concentrations.

In HyTIME a new device was developed which not only can be used for discontinuous measurement but also for continuous measurement. It is planned to use this device as the basis for a commercial measurement device for measurement of hydrogen and hydrogen sulphide in very high concentrations. Awite expects that there is a market in the power-to-gas business.

B) Remote control

One part of Awite's contribution to the HyTIME project was the system integration of the control and measurement devices. Not only the parts of such a plant can be integrated, it is also possible to secure interconnection of the parts even over the internet to enable all the project partners to access the devices from their homes. After having been able to connect to the HyTIME reactor in the Netherlands from Awite's home in Germany without any problems, it was decided to integrate the remote control part into all the Awite devices already in 2015. This is especially important for customers outside Europe, as Awite can connect to the device by any internet connection and give advanced product support. This makes it possible to sell Awite devices into areas which otherwise would not have been taken into account. This development (a half open reverse ssh tunnel) can also be used by the old proprietary protocols which are used by Awite now.

C) Advanced visualization

Awite has incorporated the "MQ Telemetry transport" protocol (MQTT) within HyTIME in order to use an open standard (MQTT is an OASIS internet standard since 2014) as a base for system integration. Awite plans to use this protocol for integration of all Awite products, as this protocol is one of the transports protocols often mentioned together with "Industry 4.0". In 2015 an internal project will be started in order to use MQTT as base for a new open source visualization and also for an open database connection. The goal is to access automation systems and gas measurement systems from many places with many devices (e.g. by using smartphones).

HyTIME has shown that hydrogen fermentation at the larger scale can suffer from low yield due to the production of end-products which compete with the production of hydrogen. The assumed selection pressure, i.e. anaerobiosis and high temperature, have been insufficient to enable hydrogen fermentation under non-sterile conditions. Further research and development in this specific area is needed. The nature of this research is even more fundamental than was envisaged for the current project. The focus would be on population analysis and dynamics, on identification of major environmental effects and the management of the bacterial consortium. At the same time there is a need for improvement of bioreactor design which should contribute to maintaining the right culture conditions for hydrogen production. The astounding results obtained with the digestion of the effluent from the molasses to hydrogen fermentation are also in need of further research to identify and eradicate the toxic effects.

Besides the need for future more basic biotechnological R&D, progress has been made in novel gas upgrading techniques to a higher TRL as compared to the start of HyTIME. This will need further upscaling and testing at the applied level.

