Final report

1.1 Project details

Project title	BESTF2: CoRyFee - Cost Reduction in Yeast Fermentation for Commercial Production of Cellulosic Ethanol.
Project identification (program abbrev. and file)	64014-0178
Name of the programme which has funded the project	ERA-NET BIOENERGY/Bioenergy Sustaining the Future 2
Project managing company/institution (name and address)	Terranol A/S c/o Aalborg University, Copenhagen A.C. Meyers Vaenge 15 DK-2450
Project partners	SEKAB E-Technology AB SP Technical Research Institute of Sweden (now RISE Research Institutes of Sweden))
CVR (central business register)	30895770
Date for submission	Ultimo January 2019

1.2 Short description of project objective and results

The partners have aimed to reduce production costs of cellulosic ethanol by combining their respective technologies. An efficient pretreatment and enzymatic hydrolysis process, developed by SEKAB, in combination with strategies for (partially) continued fermentation of hydrolyzed biomass, using yeast strains developed by Terranol, have been further refined, upscaled and successfully demonstrated in the Biorefinery Demo Plant (BDP), managed by RISE and operated by SEKAB. The results in 10.000L scale are in accordance with results obtained in 2L laboratory scale. The objectives concerning robust online feed control, improved operating economics and reduced fermentation needs, have been achieved and demonstrated for the overall process; there is a significantly improved yeast economy and the necessary fermentation tank volume for finished production from given biomass quantity per time is also considerably reduced.

1.3 Executive summary

The partners have developed a yeast fermentation process for commercial production of cellulosic ethanol in which production costs are reduced.

A more efficient, and especially cost efficient, 2G ethanol fermentation process based on a flexible production strategy allowing to extend a fedbatch fermentation process into a semicontinuous or even continuous fermentation process by the use of a newly developed process control method allowing control of the feed stream has been developed.

A yeast propagation procedure that differs from standard methods by allowing for far greater carbon source utilization towards yeast biomass was developed in laboratory scale and successfully transferred to the BDP. Balanced yield, e.g. sugar utilization towards biomass was close to theoretical indicating that very little sugar is lost towards undesirable products with this new propagation method.

A base case for CAPEX reduction as a result of implementation of process changes has been made. Large-scale BDP trials have been performed and an investment reduction around 50% lower for the CoRyFee concept compared to conventional yeast propagation based on a batch process can be demonstrated. It is evident that lab scale results are transferable to near industrial scale, and that the cost saving in terms of OPEX lays primarily in the significantly lowered yeast requirement for the process.

After successful lab trials with refractive index (RI) instrument in lab scale it was installed and tested in the BDP and it was found to be very suitable for the type of process control. As in any other production process it was of outmost importance that accurate and stable output data can be obtained in order to accurately control the fermentation process. The "sweet spot" of the fermentation process has been thoroughly tested in laboratory scale and was further verified in PDU scale during the large-scale fermentation trials. Considering that the results from the near industrial scale closely resemble those achieved in laboratory scale shows that the "sweet spot" could be controlled in large scale as well.

Terranol has developed a novel fermentation control program effecting real time feed control based on online measurement of the RI, and the method is the subject of a joint patent application filed by Terranol and SEKAB.

1.4 Project objectives

The overall objective of the project was to reduce production cost of cellulosic ethanol. In a 2G ethanol production the needed amount of enzymes, yeast and energy drives up the operation expenses (OPEX) per produced unit. As more unit operations are needed in order to process cellulosic biomass into ethanol compared with a 1G process, a higher initial capital expenditure (CAPEX) is also needed. To ensure a wide deployment of 2G bioethanol production on market conditions, there is a need for further reduction of the overall production costs.

Development of pentose fermenting yeast was an important accomplishment as this increased obtainable yields by up to 50% due to the additional conversion of pentoses, constituting 20-40 % of the hydrolysed sugars. However, the release of various inhibiting substances during harsh pre-treatment of the cellulosic materials makes the fermentation step more challenging than in a 1G process. Increasing the yeast amount initially added in the fermentation step may partially circumvent this challenge but this increases the OPEX of 2G ethanol production. An additional problem is substantial amounts of weak acids, such as acetic and benzoic acid, released during pre-treatment, which slow down the fermentation, causing a need for higher fermentation tank capacity, higher yeast addition, or an extra process unit for pH shift.

Terranol and SEKAB E-Technology have collaborated for a number of years and this project was therefore a natural next step to address these issues. SEKAB E-Technology has a complete pre-treatment and hydrolysis process, CelluAPP^{TM}, that releases only limited amounts of inhibiting substances in general and of weak acids in particular. Terranol has a xylose fermenting yeast, which is capable of simultaneous fermentation of hexoses and pentoses. These Swedish and Danish technologies are of highest international class and coupled with the demonstration facility "Biorefinery Demo Plant" (BDP) in Sweden run by RISE and managed by SEKAB they have complemented each other in a perfect match for this project.

The project was initiated January 1, 2015 and has since been granted extensions and budget change. Both SEKAB and Terranol have been affected by loss of resources in the form of sick leave and termination of employment. This, combined with an unexpected high occupancy in the BDP, has influenced the planning and implementation of activities and major tasks to be carried out would have been difficult to perform and report before the planned end of the project. These unforeseen challenges were, however, met and the project has reached its targets.

A total of six technical milestones were identified:

1. A ready proposal for a basic design of a continuous fermentation system, including flow sheet, and basic unit process flow description for rebuild of demo facility was required.
A proposal for basic flow sheet of the concept to test in the demo plant was made, a review of the functionality needed and the required updates were made and upgrades of fermentor functionality and piping/pumping were installed.

2. Results from the parallel and serial connected secondary tanks of a continuous fermentation should be used as decision point for choice of final fermentation regime in demo facility.
The obtained results resulted in the choice of parallel-connected tanks to be worked into the recommended production scheme.

3. System design for online glucose measurements at the demo facility ready, based on lab scale experiments in WP2.
It was decided that a refractive index (RI) instrument was the best option and should be used for online glucose measurement and this was then installed in the BDP.

4. Demonstration of yeast propagation in PDU scale using hydrolyzed material as carbon source.
A yeast propagation procedure in which very little sugar is lost towards undesirable products and with a very high obtained yeast biomass concentration was developed in laboratory scale and successfully transferred to the BDP.

5. Lab scale verification of total CoRyFee concept including sweet spot for OPEX, CAPEX and yield.
The CoRyFee concept was thoroughly tested in laboratory scale and subsequent verification trials in the BDP show comparable results in terms of yields, productivities and sugar utilization for both fermentation scales.

6. Demonstration of CoRyFee concept in demo scale
Trials in the BDP have been run on wheat straw hydrolysates and the economical benefits of the CoRyFee concept was demonstrated.

1.5 Project results and dissemination of results

WP1: Development and validation of fermentation strategy with continuous feed of hydrolysate, yeast and nutrients.

Task 1:	Determination of growth and fermentation characteristics in batch and fedbatch fermentations of Terranol yeast in SEKAB hydrolysate
Task 2:	Testing and optimisation of continuous fermentation in a one tank system
Task 3:	Setup of fermentation equipment with possibilities for multiple serial and parallel connected tanks, with the chosen online monitors
Task 4:	Testing of various fermentor configurations of two tanks and multiple tanks, choice of suitable configuration
Task 5:	Further testing and finetuning of the chosen fermenter configuration in conjunction with transfer and upscaling into demonstration scale
Task 6:	Specification of investments needed in the BDP to improve control and registration of flows, dosages and requirements on control of enzymatic hydrolysis in a new liquefaction reactor

SEKAB have produced and delivered enzymatically hydrolyzed biomass material allowing Terranol to perform fermentation experiments and determination of fermentation characteristics of Terranol strain cV-110.

Batch fermentations were performed with hydrolyzed wheat slurry with and without solids. The hydrolysate was quite mild and had low sugar content. Strain cV-110 was used with a total yeast pitch of 0.5 g/L, the temperature was kept at 32°C and pH set to 5.0. No nutrients were added, except that ammonia was used to adjust the initial pH and for pH control during the fermentation. Despite the low pH set point, the fermentation was finished after roughly 20 hours with a relatively high cell growth and glycerol formation. A final ethanol concentration of 33 g/L and an ethanol yield of 86% of theoretical were obtained.

Fed-batch fermentations (0.25 l batch, feeding up to a final 2 L volume) were run with another wheat straw hydrolysate from SEKAB, containing a little more sugars and acetic acid. The fermentation conditions were kept at 32°C and pH 5.2. Again, cV-110 was used with a total pitch of 0.5 g/L (4 g/L initially in the batch). In this case a final ethanol concentration of 42 g/L and an ethanol yield of 91 % of theoretical were obtained.

The continuous fermentation scheme developed by Terranol is an extension of the fed-batch fermentations, where the most promising of the tested feed control techniques is used (cf. WP2), bearing in mind that it has to be simple, robust and easily implementable in large scale. The most promising candidate technique is online measurement of refraction, and the feasibility has been tested by proceeding the refraction controlled feed phase while maintaining a fixed tank content by draining an amount equivalent to the feeding. In laboratory scale maintaining the weight of the fermenter within a narrow limit, by using an automatic pump to remove surplus broth, has accomplished this. The same may be obtained in any scale by the use of either tank weight or level sensors in the tank for controlling the broth removal.

The fermentation was thus extended up to five times the volume present in the fermenter, while following CO_2 generation rate (and thereby fermentation rate) and the yeast biomass content in the fermenter. The fermentation rate declines over time, and the same does the biomass concentration, as the wash out of yeast initially is faster than the growth rate. This will at least be the case until the originally inoculated yeast amount (which has been grown externally on sugar in excess of what is present in the tank for yeast growth and thus is a surplus of yeast compared to what can be grown on the sugar content of the biomass) has been washed out. By the determined growth and wash out rates the fresh yeast feed rate that is necessary to maintain a certain yeast concentration can be calculated and thus a certain fermentation rate in the tanks.

Robustness of the refraction based feed control system has been tested by varying the refraction set point that is maintained by the automated feed system.

A laboratory scale setup with tanks in series and in parallel was designed by the use of thermostated flasks of various sizes, equipped with stirring and sampling, but without pH control. The necessary size of the connected tanks were tested, and by the use of SEKAB wheat straw hydrolysate, it appeared that two parallel secondary tanks of half the size of the main fermenter was sufficient to support a continuous fermentation with a realistic start inoculation of 0.5 g/l main fermenter volume. A serial approach with the same hydrolysate appeared to require three tanks in series. The necessary size of the secondary tanks was found to vary with the quality and inhibitory effect of the substrate. An economical way to adapt to this is to install an additional secondary tank, also half the size of the primary tank as a buffer capacity. According to the experiences in lab scale, such an additional tank could be shared between two production lines.

Two new stirrers were installed in the BDP and they improve homogenization and mixing of pre-treated materials, particularly at high solid loads and high dry matter content. In quantitative figures, the new stirrers have improved the possible solids content with more than 50% compared to previous mixing equipment. Current solids loads are around 25% dry matter for raw materials based on wood, e.g. wood chips or saw dust and around 20% for agricultural residues such as wheat and rice straw. This upgrade does in turn improve hydrolysis, pH regulation, fluid dynamics and turbulence as well as overall performance during liquefaction of cellulose to glucose. A refractive index (RI) instrument (see also next WP) from K-Patents was installed for monitoring of sugar concentrations during processing of biomass. The new RI instrument improves overall process control as a complement to the HPLC equipment, which was formerly installed, as it enables sugar formation or consumption to be monitored on-line during hydrolysis and fermentation. It has been tested and evaluated for monitoring of hydrolysis under which glucose formation is measured continuously online but of primary interest for this project it has been used for fermentation control of various feedstocks, most notably wheat straw that this project is based around.

WP2: Test, choice and installation of lab scale online process monitoring and control.

- Task 1: Searching and sampling available technologies for online physical and chemical broth characterization
- Task 2: Small scale, offline evaluation of chosen techniques, using a standardized batch fermentation as model
- Task 3: Installation of suitable online monitoring in lab scale, testing for reliability and stability
- Task 4: Planning and installation and evaluation of online monitoring of process and feed stream in demonstration scale facility

Keeping the glucose concentration low during fed-batch fermentation is a known and efficient method to improve the efficiency of C5/C6 fermentation in lignocellulosic hydrolysates using recombinant yeast. However, reliable online measurement of glucose, suitable for use in industrial scale and environment, has not yet been established.

Different technologies available for physical and chemical online characterization of the fermentation broth were assessed including online measurement of CO2 development during the process in terms of the amount of generated gas effluent. Technologies to monitor speed of sound, refraction and density were also investigated.

Initially, a new control program for substrate supply optimized for the purpose of co-fermentation of glucose and xylose based on CO2 development was further refined by Terranol. This method was first described during the previously supported EUDP project. Instead of the laboratory control program that continuously calculates the concentration of sugars and adapts substrate supply during the process, then control of substrate supply is instead based on changes in CO2 production. When the CO2 production begins to decline due to low sugar concentration, i.e. a CO2 development peak is detected; a predetermined amount of substrate is further fed to the reactor with maximum pump speed. This results in an increase of CO2 production. When the fermentation rate again decreases, a new portion of substrate is fed. This control strategy has been demonstrated to be very robust in a number of fed-batch fermentations, using different hydrolysates and sugar concentrations, and the advantage of this strategy is that it is not necessary to know in advance the concentration of sugars in the hydrolysate. Successful fed-batch fermentations were performed with SEKAB hydrolysate without solids employing 0.2 g/L of strain cV-110, pH 5.5 and 32°C using the CO2 concentration in the off-gas as control variable.

A potential online measurement of CO2 in large scale would be to measure the amount of generated exhaust gas from the fermenter. This measurement is a simple and inexpensive method that could be realistic to implement in a full-scale production facility.

In the BDP two methods were investigated in order to evaluate if online measurement of CO_2 is feasible. In the first method controlled airflow was utilized to investigate if process control based on valve opening controlled by maintaining stable pressure within the fermentor at 1.5 kPa is an option and in the second method the use of controlled airflow was utilized to examine if pressure build-up within the fermentor could be employed. In both instances the experiments were conducted with 10 m3 tank contained water or hydrolysate.

Experimental results from the gas trials showed promising results as high correlation between theoretical estimates based on the general gas law and practical results using airflow and pressure buildup were achieved. The trials were positive and showed promising potential as applicable for "peak CO_2 control" but the fact that the rate-of-change as the derivative function of the gas rate or gas buildup does have an inherent risk of causing volatility, even if small fluctuations of gas measurements occur. In practice, this means that even small changes in gas flow measurements may result in strong indications of rate-of-change for gas formation, indicating a slowdown in fermentation rate although such a slowdown has not actually occurred. All together, further development and optimization of the "peak CO_2 control" method was eventually abandoned as an alternative monitoring and control principle proved to be more stable and accurate.

This alternative approach is based on refractometer measurement, which relies on the fact that the refractive index (RI) value is linearly related to the sugar concentration. Terranol has developed a novel fermentation control program effecting real time feed control based on online measurement of the RI, and the method is the subject of a joint patent application filed by Terranol and SEKAB.

Initially batch fermentations were performed in order to evaluate a RI-detector purchased from Krüss, Germany as well as related equipment and software. It turned out to be somewhat of a challenge, but after numerous trials, the online RI-detector was eventually connected to the control program (MFCS/win) via an OPC server and it proved to work well.

Dissolved matter in the hydrolysate, as well as ethanol and glycerol formation during the fermentation contribute to the RI signal, but by taking a "relative" approach, i.e. finding important transitions in the fermentation rather than using quantitative values, the problem with interfering compounds can be avoided to some extent. The method used here was to use the online RI value to find a suitable starting point as well as set point for the fed-batch feed control. The similar principle as used in Terranol's "CO_2 peak control", where CO_2 in the off-gas is monitored, was used. By calculating the rate of change of the RI value in a small time window (i.e. the derivative of the RI value), a negative peak, indicating the maximum fermentation rate could be found. This is the opportune moment to start the feed. The RI value at this time point was subsequently used as set point in the feed phase, since here the sugar consumption rate is at it's maximum. This point in the fermentation corresponds to the CO_2 peak previously used to determine the starting point for the feed.

Fed-batch fermentations were performed to test the new control strategy. When the initial batch phase was over, the control program automatically detected the decrease in sugar consumption rate (the derivative minimum) and the feed pump was started. The RI set point used was the RI value at that time point. This RI value was automatically kept throughout the whole feed phase. When the volume reached the maximum fermenter volume the feed was turned off. After about 8 additional hours all the residual sugars in the bioreactor were consumed.

In conclusion, it was demonstrated in laboratory scale that it is feasible to control the fermentation using online RI determination and therefore SEKAB commissioned K-Patents from Finland to deliver and install such an instrument. This instrument and method results in a robust online measurement method for accurately controlling the rate-of-change in sugar formation or consumption and thus allows monitoring of the rate of hydrolysis as well as fermentation. Testing and validation of the equipment showed strongly repeatable hydrolysis experiments as well as fermentation trials that closely resemble those achieved in laboratory scale with RI-monitoring and feed-control. The instrument was used to accurately control the feed of additional sugars into the fermentation process which also showed good stability over time with minimal drift in output values and thus allowed for a very accurate process control. Hence allowing the fermentation to be performed at the highest rate of sugar consumption, in the PDU tests this rate corresponded to approx. 6 percent fermenter volume per hour. The rate expressed in turnover hours is

14-16h operational time to fill a primary fermenter after which additional 8-10h are needed to complete the fermentation. Those numbers can be compared to the required residence time for a batch fermentation, which are usually between 46-48h. Furthermore, the maximum rate of fermentation that was achieved with RI-controlled feed in the BDP closely resembles the results that were achieved in laboratory scale.

WP3: On-site production of yeast with hydrolysed biomass as carbon source.

Task 1: Establishment of suitable propagation conditions in lab scale, such as pH, temperature and aeration rate

Task 2: Investigation of propagation performance of the yeast in hydrolysate in lab scale with subsequent test in PDU scale

Task 3: Process design and testing of (semi-)continuous propagation in both lab scale and PDU scale with the chosen conditions

Task 4: Demo scale yeast propagation trials in BDP using the most feasible conditions obtained from lab scale and PDU scale propagations

SEKAB have produced and delivered molasses allowing Terranol to perform the propagation experiments. Propagation procedure of strain cV-110 on beet molasses using controlled feeding based on ethanol off-gas was established. The conditions were 0.4 to 2L fed-batch fermentation (10 g/L initially and 210 g/L sugars in the feed), pH 5.5, 30°C, 1000-1600 rpm. Only salts (N,P,K,Mg) and vitamins were added to the beet molasses. The aeration was 1 vvm (up to 1.5 L in this case) and ethanol in the off-gas was controlled around 100 ppm. We obtained 70 g dw/L cells and above 80% yield of theoretical in a not yet optimized protocol, which is satisfactory. Less satisfactory was the finding that cell lysis was taking place, and that appeared to reduce the yield a bit. Attempts to increase the biomass amount be feeding in more molasses increased the cell lysis and further decreased the yield. Substituting the molasses with glucose enabled us to increase the yield up to about 100 g DW yeast per liter and obtain a yield of about 95 % on the theoretical glucose yield, starting with an inoculation with 1 g/liter of dry yeast. So the lower price of sugar in the form of molasses was partially offset by the increased yield and amount by using glucose. Adding that to a reduced risk of contamination by using glucose caused us to prefer to use glucose instead of molasses. An additional advantage by the use of glucose is that we then were able to develop a protocol that allowed to save the produced yeast in liquid form for several weeks prior to use, and thus introduce a higher degree of flexibility in the total ethanol production process. The highly concentrated yeast also allows for direct inoculation of the ethanol production tank, eliminating the need for an up concentration step.

Results from further development of the CoRyFee concept in laboratory scale setups have indicated that further yeast additions after the initial inoculum may not be needed in order to sustain the desired fermentation rate throughout the fermentation step. That makes the slightly higher price for propagation substrate by using glucose further insignificant, argumenting that the higher yeast quality and reduced contamination risks are more important determinants of the choice.

Propagation of yeast for the large-scale experiments was done in a 400L bioreactor, but only using one third of the volume. A batch phase of 90 liters was inoculated with 100 grams of rehydrated dry yeast (cV-110), and was grown until the sugar had been utilized and the produced ethanol were metabolized, too. A total feed amount of 30 liters was fed into the fermenter in two tempi, each lasting 24 hours. This protocol was developed for using in fermenters where ethanol controlled feed stream is not available. This protocol resulted in 86 grams of yeast DW per liter or slightly above 10 kg DW yeast in total. Carbon utilization and theoretical yield was close to 95 percent in the propagation in large scale. In these experiments the carbon sources were based on glucose, for the above mentioned reasons. Previously used batch methods in the BDP facility commonly resulted in yeast concentrations of 25-30 g/L after which the yeast would be centrifuged with a decanter centrifuge prior to inoculation. This was not necessary with the new protocol, as the yeast concentration obtained was so much higher. Balanced yield, e.g. sugar utilization towards biomass was close to theoretical indicating that very little sugar is lost towards undesirable products with the new propagation method.

WP4: Creation of a model that calculates the CAPEX reduction as a result of implementation of process changes.

Task 1: Making a calculation model of CAPEX necessary to build a full-scale facility.

A base case for CAPEX reduction has been drafted and builds on SEKAB's CelluAPP® process. The process has further been developed within a pre-feasibility study for a client with the help of French engineering firm Technip into an engineering process with cost estimates on the level of +/- 25% of actual investment cost. The results of the engineering work shows two different results albeit equally important insights in the workings of the developed process. The base case was a batch fermentation employed within a plant capacity of 60,000 tons of ethanol annually. The investment cost was set as the normalized value for the CapEx. Subsequent estimates of the CoRyfee process resulted in two different cases, the first case maintained the same volumetric inflow of material per hour as the batch while the second maintained the same output per hour. The results affecting the CapEx were thus estimated to be 5% lower with the CoRyFee concept as the maintained in-flow of material would require almost the same fermenter volumes to be used. But the difference in fermentation process rate allows the CoRyfee process to produce the 60,000 tons in less than half the time compared to batch. In reality this increases the capacity of the plant from 60,000 to 138,000 tons with the same investment cost. If the same output is used as a parameter the CapEx of the CoRyFee facility decreases with 53% as the fast fermentation time allows for a smaller fermentation setup to be used while maintaining the same annual output. These results meet the target of lowering the CapEx with 50% based on the same annual processing volumes and product output as it is achieves the output of ethanol 2.3x smaller fermenter volume.

WP5: Creation of a model that performs complete parameter calculation OPEX at full-scale production. Combining with CAPEX calculation model constructed in WP4.

Task 1: Modelling of OPEX of a full-scale fermentation process.
Task 2: Combining models of CAPEX and OPEX into a complete calculation instrument.

Following completion of WP4 to adjust the CAPEX model to better reflect the CoRyFee concept that was defined and tested in lab scale and further scaled to near industrial scale it is also possible to more accurately model and estimate OPEX. The two models are combined to form a comprehensive model covering CAPEX, OPEX as well as mass balances of raw used materials and formed products. After the scale-up tests were performed and evaluated in the BDP it was evident that lab scale results are transferable to near industrial scale with almost identical results. It was also clear that the lower OPEX lays primarily in the lowered yeast requirement for the process. The targeted concentration that was set in the project goals was a yeast concentration of 0.5 g/L. This was achieved in both laboratory and PDU scale where concentrations of as low as 0.05 g/L fermented broth were demonstrated. It is calculated that a large-scale process based on the CoRyFee concept would require 14 times less yeast than batch fermentation process. This would in term have a significant impact on OPEX. Absolute numbers are strongly dependent on the size of the biorefinery but considering the more efficient yeast propagation as described in WP3, task 3 in combination with lower yeast inoculation will undoubtedly have a significant impact on CAPEX, far more than the 50% lower OPEX that was set as one of the goals of this project. This assumption is derived from the assumption that the protocol that was used to propagate yeast during this project resulted in more than 95 percent biomass yield with biomass concentration reaching around 100 g/L. If it can be assumed that the OPEX for each process in terms of yeast production would essentially be the same, and with the utilization of around 14x less yeast for the CoRyFee process the goal of 40% OPEX reduction would be met with a significant margin. This is achieved although the yeast propagation protocol in the project was performed without a cost optimized nutrient composition.

WP6: Definition of process parameters and demonstration of sweet-spot concept in lab scale

Task 1: Final model use for determination of the "sweet spot" parameters for the CoRyFee process
Task 2: Testing and verification of the model results of the "sweet spot" concept in lab scale.

At the demo plant the process design and existing infrastructure inventory was reviewed in order to optimally complement, extend and rebuild so that the planned process concept could be demonstrated and verified. A review of analytical equipment, which is essential for the process control, has also been conducted and the primary upgrade involved concerned the purchase of equipment for measuring sugar concentrations. See WP1, WP2 and WP8 for details on completed improvements and adjustments intended to verify the CoRyFee process in PDU scale.

The fermentation process, "sweet spot" and "peak control" was thoroughly tested in laboratory scale and was subsequently verified in PDU scale during the large-scale fermentation trials. Considering that the results from the near industrial scale closely resemble those achieved in laboratory scale shows that the maximum fermentation rate could be controlled in large scale as well. In fact, as previously described it was possible to achieve very similar fermentation rates in lab and PDU scale. System robustness and scalability is vital for further work with commercialization and application of the technology in large-scale production facilities. Lab results indicate higher product yields and fermentation rates, which were the main purposes of the development of the CoRyFee process. Verification trials in the BDP show comparable results in terms of yields, productivities and sugar utilization for both fermentation scales.

WP7: Testing and verification of the resulting sweet spot CoRYFee processes in large scale.

Task 1: Testing and verification of the model results in demo scale.

Trials have been performed regarding "peak-detection control", a control strategy to monitor and control the substrate feed into the fermenters. One such strategy that was tested was to simulate the CO_2 generation during fermentation and evaluate if that would correlate to theoretical pressure build up during fermentation. The results were positive and showed potential, after which a subsequent trial was performed during a fermentation run with yeast. As previously described in section WP2, these trials showed potential but were deemed too sensitive when smaller additions would be made to larger volumes, compromising accuracy and thus decreasing the robustness and weakening processing control.

After successful lab trials with refractive index (RI) instruments in lab scale it was decided that such processing controls would be installed and tested in the BDP instead of as a strategy for "peak detection control", see WP8 below for further details. The installation and subsequent tests and verification of RI-instrumentation in PDU scale showed that the equipment is very suitable for the type of process control. As in any other production process it was of outmost importance that accurate and stable output data can be obtained in order to accurately control the fermentation process. This was evident in both lab and near industrial scale where high ethanol titers (>50 g/L ethanol), yields (>90 % of theoretical) and faster fermentation rate (6% of fermenter volume per hour) was achieved and demonstrated during the development and verification steps.

WP8: Adaption of the Biorefinery Demo Plant to enable verification of new process configuration

Task 1: Evaluation and preparation/ engineering
Task 2: Purchase and modifications of existing equipment and installation of new process equipment and monitoring instruments
Task 3: Commissioning and initial testing of the functionality of the modified and new solutions

Extensive infrastructure improvements of the BDP have been performed and verified. These improvements include installation of piping and pumps that connects the four 10 m^3 fermentation vessels across each other, increasing process flexibility and improving pumping of materials between each vessel. After the improvement it is now possible to transfer materials from any given vessel to each of the other vessels with high pumping capacity resulting in accurate and swift transfers. This was not possible prior to the project and was a limiting bottleneck prior to the modification within this project.

Two large and dynamic stirrers with wider impellers have been installed in one of the 20 m^3 storage vessels where the pre-treated biomass is homogenized and stored. One additional stirrer of the same kind was also installed in the primary hydrolysis/fermentation vessels of 10 m^3. These improvements further improve processing and homogenization of pre-treated biomass as well as hydrolysis and fermentation that can now be performed with higher solids loads. Previous limit of solids loads was around 16-17 weight percent but that capacity is now increased to levels of up to 25 percent.

Although promising laboratory results in lab scale experiments at Terranol and the previously described off-gas measurements in the BDP unit by SEKAB it was found that an online refractive index (RI) instrument is both extremely robust and provides more accurate results concerning the fermentation state in the fermenter, which is necessary to improve online monitoring of sugar concentrations and rate of fermentation. RI measurement was thus the chosen solution and SEKAB commissioned K-Patents from Finland to deliver and install such an instrument. The new instrument did indeed result in a robust online measurement method for accurately controlling the rate-of-change in sugar formation or consumption and thus allowed monitoring of the rate of hydrolysis as well as fermentation. The instrument allowed the fermentation to be performed at the highest rate of sugar consumption, validating the CoRyFee concept in demonstration scale in the BDP. See previous sections for details quantitative regarding the results of verification and testing of the concept in near industrial scale in the BDP.

The project goals as stated in the application concerned improved yield, and reductions in CAPEX and OPEX:
Yield improvements:
Increasing fermentation efficiency from 85% to 93% amounting to an overall increase of yield from 66% to 75% of theoretically maximum. This translates to an increase from 290 L/bone dry ton to 320 L/bone dry ton of biomass.
CAPEX reduction:
Increasing volumetric productivity 2 fold, thereby reducing fermentation related CAPEX need by 50 % (reduction in needed installed fermentation capacity).
OPEX reduction:
Lowering needed yeast pitch and on-site, biomass based yeast production, thus reducing the yeast expenses. Reducing needed yeast pitching to 0.3 g/L total fermentation volume thereby reducing yeast expenditure from 6 ¢/L to 3.6 ¢/L.

All in all, the project objective to reduce production costs of cellulosic ethanol by combining technologies, developed by SEKAB, for efficient pretreatment and enzymatic hydrolysis process and strategies for continued or partially continued fermentation using yeast developed by Terranol was accomplished.

Dissemination of results

EU progress reports were delivered on a yearly basis during the project.

The CoRyFee project was represented at the EUBCE 2016 conference in the session ”Production improvements in cellulosic ethanol” and at the parallel event ”EU support for bioenergy demonstration projects: current state and developments” in which updates were given in the programmes NER300, BEST ERANET, FP7 and H2020. The CoRyFee project was also presented at the side event “Bioenergy - From Research to Market Deployment in a European Context” at the EUBCE 2017 conference in Stockholm.

Project results were presented by invitation at the “40th Symposium on Biotechnology for Fuels and Chemicals” in Clearwater, Florida in May 2018 (see attached presentation).

Three manuscripts are in preparation and are intended to be submitted for the journal “Biotechnology for Biofuels”.

1.6 Utilization of project results

A novel fermentation control program effecting real time feed control based on online measurement of the RI was developed, and the method and the process has been the subject of a joint European patent application filed by Terranol and SEKAB with the title “Process and System for Microbial Fermentation”. The parties share the rights and have entered into a preliminary joint intellectual property and patent application ownership agreement and begun discussions how to agree on commercial terms and conditions of joint worldwide commercialization of the patented technology. First attempt to commercialize the developed concept has been initiated in that a Scandinavian energy company, who is planning a 150 million Euro investment in a commercial 65.000 MT per year 2G bioethanol facility to be operational in 2021, has been presented with the concept to consider for their facility. An Indian company has currently licensed a Terranol yeast strain for testing in their precommercial scale facility, and the description of the CoRyFee concept and the work and results obtained with it has likewise been sent to them. A major engineering company has been engaged to perform estimations of the Capital investments necessary to erect a 60.000 MT/year 2G bioethanol facility according to the CoRyFee concept, and to provide similar and comparable estimate of a traditional facility running batch or fed batch processes. The marketing of the CoRyFee concept has thus already been initiated, and search for further potential customers is ongoing, without limiting the use of this fermentation concept for 2G bioethanol production.

1.7 Project conclusion and perspective

Proprietary technologies for an efficient pretreatment and enzymatic hydrolysis process, developed by SEKAB, in combination with strategies for continued or partially continued fermentation of hydrolyzed biomass, using yeast strains developed by Terranol, have been further refined, upscaled and successfully demonstrated in the Biorefinery Demo Plant (BDP), managed by RISE and operated by SEKAB. The results in 10.000L scale are in accordance with results obtained in 2L laboratory scale. The objectives concerning robust online feed control, improved operating economics and reduced fermentation needs have been achieved and demonstrated for the overall process. There is a significantly improved yeast economy as the needed yeast amount per produced unit of ethanol has been reduced with 80 % or more. The necessary fermentation tank volume for finished production from given biomass quantity per time is also considerably reduced, amounting to a 50 % reduction in size.

Implementation of the process is facilitated by a novel fermentation control program developed by Terranol, effecting real time feed control based on online measurement of the RI, and the method and the process is the subject of a joint European patent application filed by Terranol and SEKAB.

The main conclusion of the project is that the CoRyFee process is technically feasible as proved in 10.000 liter fermentation scale, and that a positive business case for commercial 2G ethanol projects can be established. The partners have formed a strong consortium that has the competencies, personnel and equipment, which provides promise of a low risk transfer to commercial scale. The substantial improved 2G bioethanol production economy is considerably strengthening of the argument for including 2G ethanol production as part of the worldwide efforts to reduce the CO_2 footprint of transportation.

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