GLYFINERY Final Report

GLYFINERY Project 1 March 2008 – 29 February 2012

Summary of project concept, objectives and results

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Executive summary

Due to an increased biodiesel production in recent years, large amounts of the by-product glycerol entered the world market and gradually saturated the demand for glycerol as a chemical. A search for alternative uses for glycerol was therefore relevant. The European Commission funded three research projects investigating "Alternative uses for glycerol", one of these being the GLYFINERY project focussed on "Sustainable and integrated production of liquid biofuels, bioenergy and green chemicals from glycerol in biorefineries". The project has run in the period March 2008 to February 2012. Six partners formed the GLYFINERY consortium: five research institutions from Denmark, Germany and Poland investigated biotechnological conversion of glycerol provided by the sixth partner - Slovakian biodiesel producer Meroco.

Glycerol is currently used as an additive to a wide range of products such as cosmetics, medicines and foods. However, this direct material use is limited and accounts for only a small fraction of the glycerol available on the market. The excess glycerol is considered as an economic burden to biodiesel producers, who have to dispose of the by-product, typically through incineration. The GLYFINERY project has focussed on new biotechnological conversion processes for glycerol in submerged cultivation (fermentation), applying micro-organisms which can grow on glycerol and convert it to value added products which are relevant for modern society. The main interest has been conversion of glycerol to a biofuel, which could be utilised directly as an energy source for vehicles, and on "green chemicals" which could replace existing chemical building blocks based on fossil oil in a wide variety of industries. Production processes for butanol and ethanol as biofuels, and 1,3 – PDO as a green chemical have been developed and optimised during the project. The potential application of glycerol in the production of biogas has also been investigated.

The project included an integrated sustainability assessment covering technological, environmental and economic aspects of the integrated GLYFINERY. Data from pilot scale tests were used as the basis for the integrated assessment, which allowed prediction of viable conversion technologies and where improvements would be needed to improve sustainability of the technologies on a technological, environmental and economic basis.

Overall the GLYFINERY project has demonstrated that biological conversion of glycerol to value added products is a relevant and necessary route to a sustainable society with effective waste management. As with all industrial processes, the economic and environmental benefits can be further increased by continuous improvement of process efficiency.

Summary of project context and objectives

The need for alternative uses for glycerol

The GLYFINERY project is an initiative aimed at the sustainable and integrated production of biofuels, energy and green chemicals from glycerol which can be implemented in a biorefinery setting. The GLYFINERY concept represents a sustainable solution for management of the glycerol by-product from biodiesel refineries improving the economics and environmental impact of existing processes.

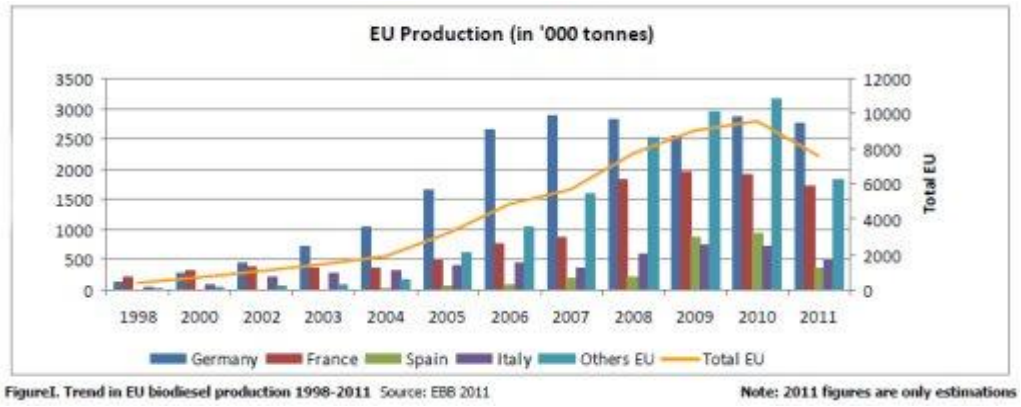

Figure 1.1: European biodiesel production from1998 to 2011.

The EU target to increase the use of renewable energy in the transportation sector in the near future has already started to boost the production of biodiesel from rapeseed and other vegetable oils. Over 9.5 million tonnes of biodiesel were produced in the European Union in 2010 (Figure 1.1), a considerable increase over the 4 million tonnes produced in 2005. This has led to an immense increase in the production of glycerol (an unavoidable by-product from the transesterification process) in volumes which already exceed the current market demand for direct material use.

In a typical biodiesel process (as shown in Figure 1.2), approximately 10% of the reaction volume ends up as crude glycerol. Glycerol production levels are increasing in line with biodiesel production (in the order of 570,000 tonnes in 2007). Although over 2000 pharmaceutical, food and other uses are known for glycerol, a large (and increasing) fraction is incinerated or stored as excess in an already saturated market. There is an urgent need, therefore, for research and technological development of processes for conversion of glycerol to valuable products, not only to solve waste disposal problems but also to improve the economy of biodiesel production.

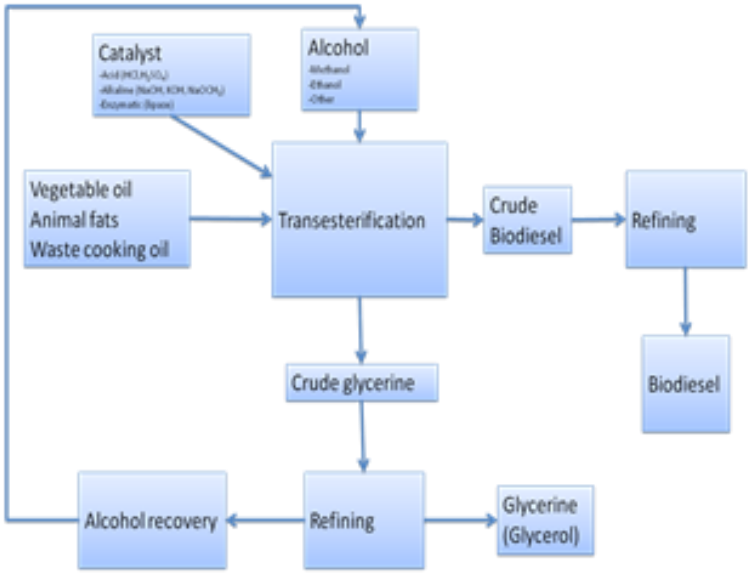

Figure 1.2: Flow chart of a typical biodiesel process showing raw materials and products.

Glycerol is an attractive substrate for current and future bioconversion due to the increasing volumes available on the market concomitant with rising biodiesel production, particularly in Europe. Crude glycerol obtained from biodiesel producers varies in composition dependent on the oil feedstock used. Pure plant oils (typically rapeseed and palm) have primarily been utilized, but there is an increasing trend to blend used cooking oils and other waste oils which results in impurities being present in the glycerol. High concentrations of other inhibitors, such as salts formed during the transesterification, may also be present, which can have a negative effect on the resulting bioprocesses.

Objectives of the GLYFINERY Project

The GLYFINERY project has targeted development of novel technologies based on biological conversion of glycerol by micro-organisms, into known and new advanced liquid biofuels, bioenergy and biochemicals. The aim has been to develop robust bioprocesses based on crude glycerol obtained directly from biodiesel production plants.

The first objective of the project was to isolate a variety of strains suitable for growth on glycerol and production of the desired products. These strains should be subject to characterisation in submerged cultivation to investigate their natural properties and assess their suitability and applicability for industrial scale processes. Three target products were worked on simultaneously: biofuels (ethanol and butanol), the green chemical 1,3-propanediol and biogas.

The second objective of the project was the development and optimisation of the submerged cultivation processes for the production of each of the 3 main product streams. The processes were developed based on the crude glycerol substrate provided by the biodiesel producer. The processes developed should be economically viable in terms of their running costs which should be balanced based on the product titres. In addition, processes robust to impurities and changes in the composition of crude glycerol were desirable to ensure applicability of the developed technologies to the variety of (oil) feedstocks and chemicals used in biodiesel production.

The third objective was demonstration of the integrated concept for the treatment of effluent from the processes and product recovery. Novel recovery processes should be developed for the relevant product streams at lab-scale. Treatment of residuals from the bioprocesses (spent biomass and liquid effluent) should be investigated, determining the potential for further energy production in the form of biogas and the possibility for recycling of water and nutrients.

The fourth objective of the project was the scale-up of the optimised processes. Processes should be scaled up to a volume to allow relevant process data to be collected for the technological, environmental and economic assessments. These reports should determine overall viability of the individual processes running in the proposed GLYFINERY, as well as an evaluation of the integrated concept.

The ultimate goal of the project was to demonstrate the suitability and sustainability of the GLYFINERY concept for implementation into large-scale biorefineries. A simplified overview of the process line is shown in Figure 1.3. Fermentation and product recovery are simplified single units where in reality multiple fermentation processes, each with their own recovery step, are envisaged. The possibility for recycling of energy in the form of biogas within the plant, and the reuse of water in the fermentation processes can also be considered. An integrated assessment of the whole production chain of the target products combining technical, economic and environmental aspects was performed and this tool was applied to determine the final optimised process outline.

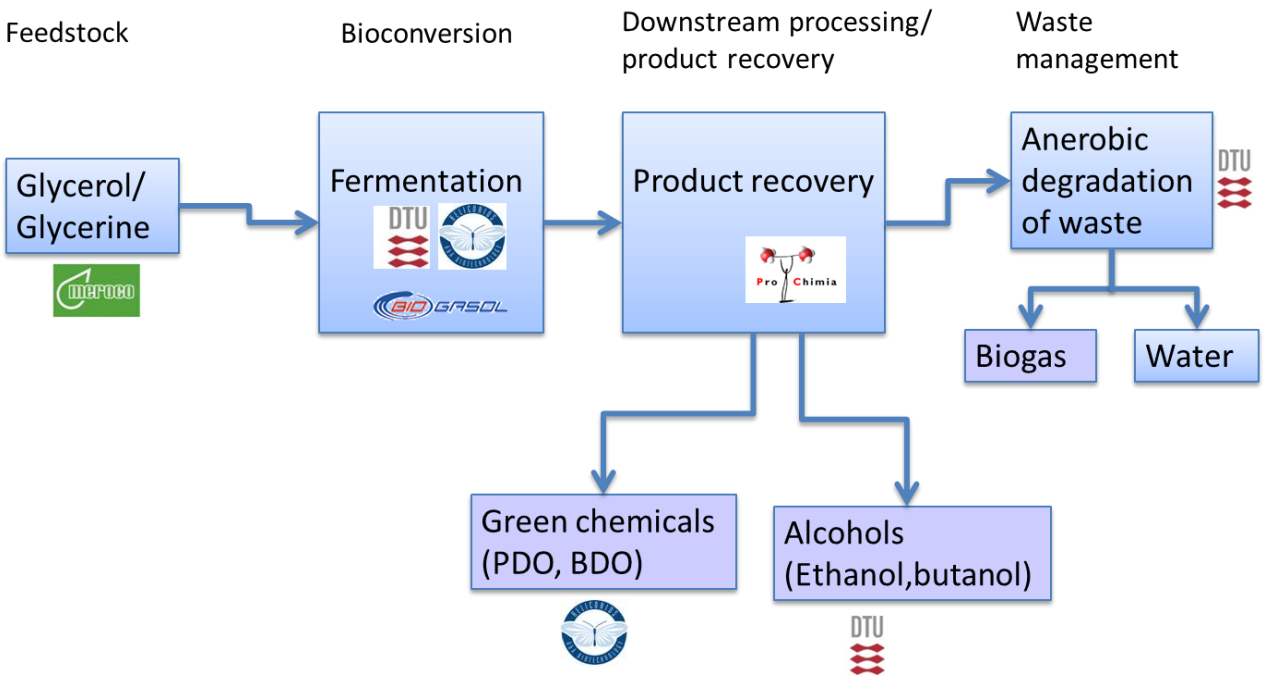

Figure 1.3: Integrated GLYFINERY concept showing simplified process line from glycerol to products, and responsibility of partners for the components.

The GLYFINERY proposal was worked out in accordance to the Decision No 1982/2006/EC of the European Parliament and of the Council of 18 December 2006, concerning the Seventh Framework Programme of the European Community for research, technological development and demonstration activities (2997-2013). Overall the GLYFINERY project addresses all the major objectives of the program, and the Theme 5 "Energy" by:

1) adapting the current energy system into a more sustainable one
2) reducing the dependence on imported fuels
3) developing energy production based on renewable resources
4) increasing energy efficiency
5) contributing to substantial reductions in greenhouse gas emissions
6) strengthening the competitiveness of the European industries.

In the GLYFINERY project emphasis is given to biofuels for transportation, biochemicals and integration of the bioenergy production into the biorefinery production schemes. The philosophy of the GLYFINERY concept is to achieve full conversion of the glycerol feedstock into biofuels, bioenergy and high-value green chemicals within the frame of the biodiesel production plant.

Potential impact (socio-economic and societal) (10 pages)

Socio-economic impact and impact on society

The strategic goal of the European energy policy defined by the Commission's Green Paper (COM(2006)105 of 8 March 2006) was to secure supply, sustainability and competitiveness of Europe's energy. The progress within the energy technologies applied goes hand in hand with the development of processes and scientific research activities. Particularly, research into energy efficiency and renewable resources and development of new technologies are necessary steps on the way for meeting the overall requirements.

The GLYFINERY project was instigated on the basis of the current EU policy for sustainable, secure and competitive energy production systems and contributes to pursuing the integration of environmental aspects into the common energy policy. At the inception of the project, the proposed method for biological conversion of glycerol was characterized by negligible environmental impact because of using the raw materials that are derived from CO_2-neutral plant biomass. Moreover, generating energy carriers in form of liquid biofuels and bioenergy should aim to significantly contribute to replacement of fuels and energy derived from fossil substrates and the more intensive use of CO_2 will help Europe in meeting the requirement outlined in the Kyoto protocol.

The research and development activities of the GLYFINERY project had the goal of creating a new technological solution for glycerol-management at the biodiesel refinery plants. Biodiesel production is increasing at such a rate that the levels of the byproduct glycerol produced are considered as a burden for biodiesel manufacturers. Glycerol is typically incinerated as a waste product. This represents a waste resource which could otherwise be applied in biotechnology processes converting glycerol to value added products. Inception of such bioconversion processes at biodiesel plants or centrally at a glycerol refinery would represent both an environmentally responsible and economically desirable means for treatment of the abundant glycerol waste.

The proposal for the GLYFINERY (glycerol refinery) was a production line that could be incorporated into biodiesel plants, or at a central facility for glycerol processing. The tight correlation of the proposed biotechnology to the existing, biodiesel production is realized via interconnection that channels the glycerol by-product and other organic residues from transesterification of the oil and plant biomass processing, respectively, to the line of proposed,

biological processing. The GLYFINERY concept also opens the possibility of running the glycerol bioconversion together with the methylester production and in stand-alone biorefineries. The outcome of the GLYFINERY includes four main bioproducts. The known biofuel – ethanol, the advanced type of biofuel – butanol, the green chemical 1,3-propanediol and the bioenergy in form of methane. All these bioproducts will extend the existing range and volume of bioproducts on the market, and form part of the necessary shift in technologies from being fossil based to bio-based.

The target products are made from low-value and biomass-derived material by means of biological conversion process, aiming at maximal conversion of feedstocks to target products. This will strengthen the cost-competitiveness of the biofuels production offering bio-based fuel alternatives in greater quantities.

The basic goals of the GLYFINERY project were:

- Development of new, robust and reliable biocatalysts for glycerol bioconversion
- Development of new bioprocesses for efficient production of alcohols, 1,3-PDO and methane
- Process scale up from the laboratory to the pilot plant
- Development of an optimal process outline for target products based on a balanced analysis of technological, economical, environmental point of view.

The consortium of participants in the GLYFINERY project believes that the project will put Europe in a leading position when it comes to microorganisms for production of advanced biofuels and green chemicals. Micro-organisms for bioprocess are commonly referred to as cell factories, and as such can perform the conversion of a variety of substrates to an array of products. These cell-factories are central to bioprocesses and gaining knowledge of new and existing micro-organisms which can be utilised as cell-factories is a vital step when developing industrial scale bioprocesses. The work of the GLYFIENRY project paves the way for utilising new strains in the conversion of glycerol as well as gaining knowledge and improving organisms already known to convert glycerol to value added products.

With regard to novelty of the bioprocesses, different types of process lines have been investigated and novel recovery techniques have been developed. The implementation of the integrated concept combining the alcohol or 1,3-PDO fermentation with methane production has been considered. Maximization of the energy output in the target products was the primary goal of the GLYFINERY project. The GLYFINERY project tested several processes in a side-by-side manner and used the integrated assessments to evaluate the benefits of each of the processes.

Dissemination activities

A considerable effort has been spent on disseminating the results of the GLYFINERY project throughout the four years where the project has been running. Generating interest in the topic and discussing the work with other scientist has been facilitated by representation of the consortium at a number of international conferences. Four oral presentations have been given at conferences, as well as the presentation of ten posters. Two articles in popular science magazines directed at the European Community, policy makes, scientists, industry and the general public have also been published.

The high level scientific work has been written and published in the form of scientific research papers, with publication in six international peer reviewed journals. Further publications (3 papers regarding the cell factories developed at the consortium partners) will be submitted for publication in the autumn of 2012.

Towards the end of the project a joint workshop was held together with two other consortia, also funded under the call for projects on “Alternative uses for glycerine”. The successful event was held in Brussels and open to the public, politicians, media, and scientists from academia and industry. The highlights of each of the projects were presented and the general perspectives for implementing the new technologies discussed.

A full list of publications, and other dissemination activities has been submitted with this report. A list of publications can also be obtained from the project website.

Exploitation of results

The technologies developed during the GLYFINERY project will be developed further by the partners of the consortium. The results pertaining to the cell factories developed in the project are accessible to the scientific community in peer reviewed publications in international journals (see above), with the potential for further development at the consortium partners or with other collaborators. This information on the biological conversion of glycerol can be applied in other biorefinery settings or in research and development with application of the various microorganisms as cell factories.

The technological, environmental and economic assessments provide detailed information on the main aspects of the technology compared to reference processes and currently existing technologies. The Integrates Assessment provide a detailed account and evaluation of biological processing of glycerol and alternative uses for this substrate based on the current state-of –the –art and predictions for future scenarios.

Main results

The GLYFINERY project was carried out by a consortium of 6 partners based in 4 countries and ran over a four year period. Multiple staff members contributed to the work of GLYFINERY in each of the partner organisations. The scientific work of the project was divided into 6 main areas which can be described by the figure below. The consortium collaborated in each of the work areas with the main partners being involved being shown below in the relevant task boxes.

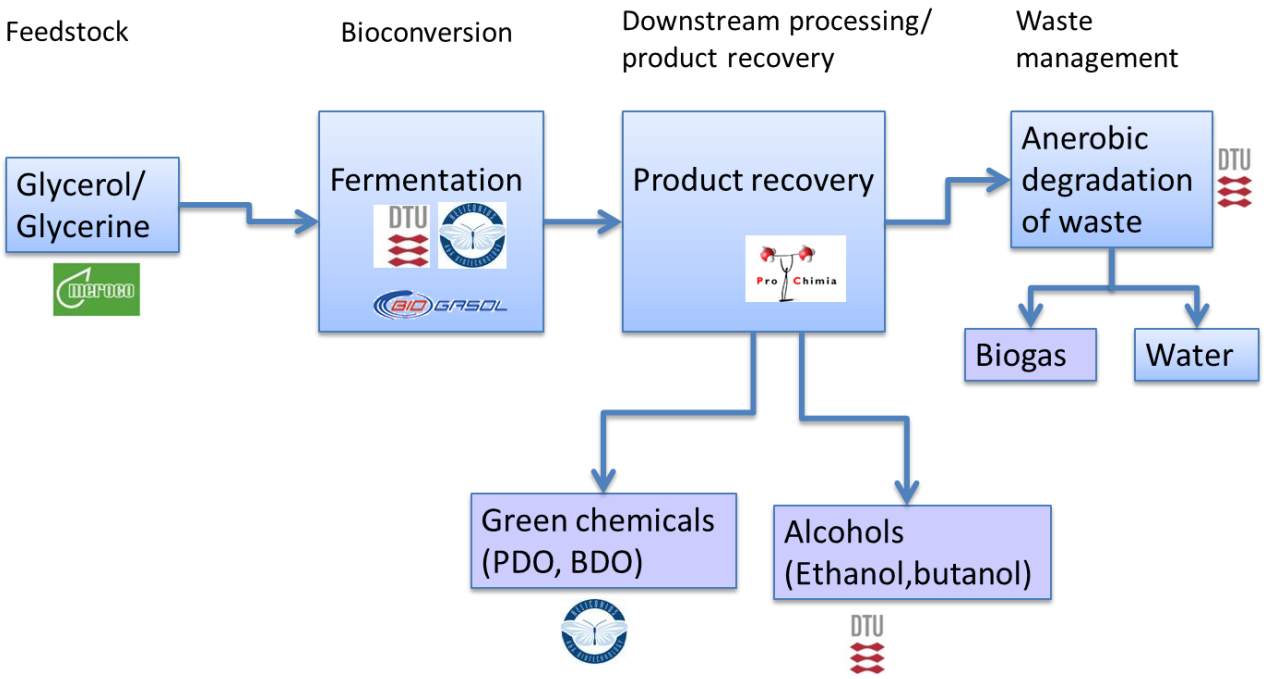

Figure 3.1: Schematic of the work flow of the GLYFINERY project.

The main scientific work areas for the project were:

- Characterisation of the glycerol feedstocks
- Discovery of micro-organisms
- Biological conversion of glycerol
- Product recovery
- Process integration at pilot scale
- Integrated assessment

Characterisation of the glycerol feedstock

The aim of this work task was to perform the necessary chemical analysis of the glycerol feedstocks to be used throughout the project in all experimental work tasks and work packages. A basic and reliable chemical analysis was required to provide a baseline for the planning and implementation of research in the GLYFINERY project. This was not only to determine that all beneficiaries had representative samples of the glycerol feedstock for their research, but also to ensure that results obtained at lab scale could be reproduced at pilot plant scale when larger supply volumes of glycerol will be required. Regular chemical analysis of the glycerol feedstock at Meroco (where the biodiesel by-product glycerol is obtained) indicated a relative constancy in purity and salt content. Independent analysis was also carried out by 2 beneficiaries and revealed similar results to those obtained at Meroco. Additional chemical analysis (total solids, volatile solids and chemical oxygen demand) was carried out at DTU, providing necessary background data for work on the biological conversion of glycerol to biofuels, green chemicals and biogas.

In the beginning of the project, the glycerol provided by one biodiesel producer in Slovakia (Meroco) was analysed and the composition for further bioprocessing was determined. Chemical analysis of the glycerol feedstock was performed routinely at Meroco at regular intervals over the course of the first 6 months of the GLYFINERY project. The glycerol by-product from the biodiesel production process at Meroco was provided to DTU, BioGasol and A&A Biotechnology at the start of the project, where work was performed on biological conversion of the glycerol feedstock. Data on chemical analysis was provided at this time as a reference for designing experiments. As a check on reproducibility of analysis and to determine variability between batches, more regular analysis was performed on the glycerol during the phase of the project where exact information on medium components was critical for medium design.

The primary feedstock for the GLYFINERY processes is crude glycerol derived as a waste stream from biodiesel production. In the previous report the composition of glycerol was reported to vary between producers [ref del.7.1?]. Furthermore the production biodiesel by a single producer can also be subject to variation. In the GLYFINERY project we have in total received three different batches of crude glycerol from Meroco:

1. (B1) Based on 100% rape seed oil feedstock

2. (B2) Based on a mix of 90% rape seed oil with a blend of 10% waste cooking oil

3. (B3) Based on 100% rape seed oil feedstock

The characteristics of each batch vary since they are derived from different production runs. A picture of the three batches can be seen below:

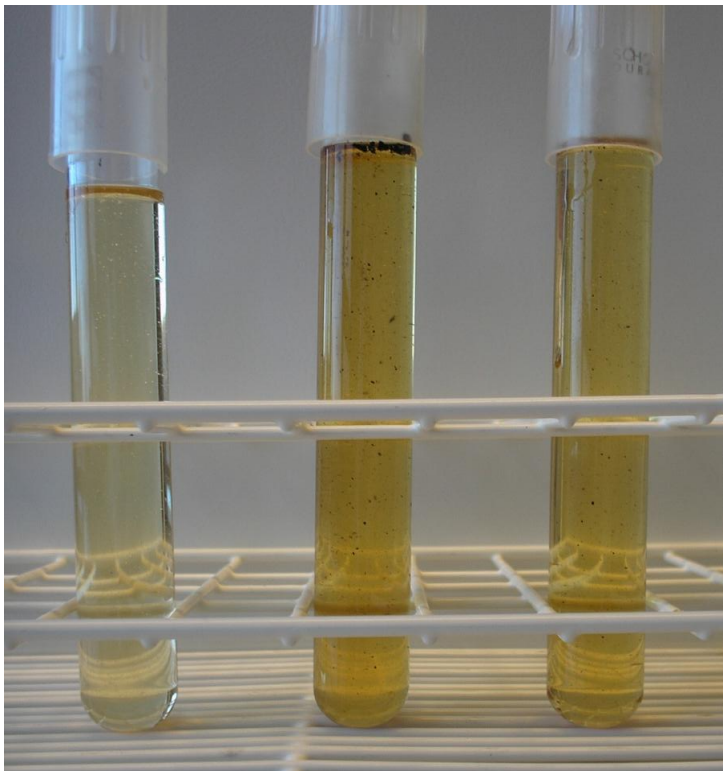

Figure 3.2: Test tubes with the three different batches of crude glycerol received from Meroco. B1 - Batch 1 (100% rape seed), B2 - batch 2 (90% rape seed with 10% waste cooking oil) and B3 - batch 3 (100% rape seed).

As evident by visual inspection the three batches have different appearance. This prompted a further investigation into the composition of the batches.

The reason for the interest in analyzing the batches further is that some microbial species namely *Clostridium spp.* are sensitive to inhibitory compound present in the crude glycerol. This means that certain batches from a manufacturer or certain manufacturers supply crude glycerol which is unsuitable for growth with the particular microorganisms. Since batch variations were detected in the GLYFINERY processes a decision was made to look into the composition.

It should be mentioned that the selection of microorganisms and processes for the GLYFINERY project have been done with regards to the tolerance towards inhibitors present in the crude glycerol provided by Meroco.

All though there is still more work to be done characterizing the contents of the crude glycerol a summary of the mainobservations are:

- Chloride and citric acid were present in fairly large amounts
- 1 peak identified in sample B2 (cooking oil) which was not present in the other samples: Molecular mass of 262. It was present only under negative ionization only (not pos.) indicative of it containing an acid group (-COOH)
- Samples B2 and B3 are more ”complex” in the area of 20-24 min. of HPLC. Further analysis is needed to determine the identity of compounds eluting in this region.
- There seems to be a fair amount of variance within the batches of glycerol received from Meroco although later batches (second and third batch) are more similar than the initial batch received.
- Supplementation of activated charcoal was found to release the toxicity of the crude glycerol significantly. Enabling the wild type strain of *C. pasteurianum* to utilize this crude glycerol

Glycerol from biodiesel produced from 100% rapeseed oil was chosen as the substrate for the work of the GLYFINERY project and the baseline substrate for all calculations and assessment reports.

Discovery of micro-organisms

The main objective of this work package was to screen the strains available in culture collections and isolate new, glycerol-fermenting micro-organisms from complex, natural- and man-made environments. Several selection strategies were implemented. The pure cultures were examined for glycerol-feedstock tolerance, spectrum of fermentation products and tolerance to the products. The purpose was to select the best performing strains with formation of the desired target products. The selected strains were characterized completely at the physiological level and at the molecular level. Strain performance was improved in some cases by genetic modification.

Biological conversion of glycerol

The main objective of this work task was to develop the concepts for biological conversion of glycerol employing integrated production of biofuels/bioenergy, green chemicals/bioenergy, or solely the bioenergy. The goal was to develop the concepts based on the glycerol feedstock

fermentation by a variety of micro-organisms. Appropriate combination of glycerol-feedstock and other co-substrates were found for meeting the demand of microbial strains for macro- and micronutrients, through extensive studies on media composition. Different bioprocess set-ups operating with wild-type strains and mutants were tested for finding the optimal process configuration with highest yields of the products desired. Process optimization was a considerable part of this work area.

Work in the research areas on **discovery of micro-organisms** and **biological conversion of glycerol** resulted in optimized processes for the main products listed below. These processes were further developed in the scale-up stage and evaluated in the integrated assessment:

- Ethanol production
- Butanol production
- 1,3-propanediol production
- Biogas production

Production of ethanol

An ethanol production process has been developed and optimized at DTU based on the non-conventional yeast *Pachysolen tannophilus*. This organism is capable of growing on glycerol, and has been shown to produce ethanol on this substrate in previous studies (ethanol production levels of 4g/L). However, until now, this process has not been optimized to allow for ethanol production levels which could be considered relevant for larger scale production. An ethanol producing process with *P. tannophilus* has been optimized based on knowledge we have gained on the physiology of this organism during the GLYFINERY project. The current process produces 28g/L ethanol (56% of the theoretical yield). Further improvements in production levels would be possible through evolutionary engineering to produce strains which are more ethanol tolerant.

Benchmarking ethanol production from glycerol

It has been shown that a number of (typically anaerobic) bacteria are capable of growing on glycerol as the sole carbon and energy source. Glycerol can be converted to a wide range of biochemicals and biofuels such as ethanol, butanol, 1, 3-propanediol, succinate, dihydroxyacetone, propionic acid and pigments. The newly isolated bacterium, *Kluyvera cryocrescens* can produce up to 27g/L ethanol from crude glycerol under microaerobic batch fermentation. *Eschericia coli* has

been investigated to be an ethanol production platform on glycerol, with up to 10g/L achievable by engineered *E.coli* growing on 22g/L crude glycerol and with hydrogen and formate as byproducts under anaerobic condition. An engineered *Klebsiella pneumonia* strain has been shown to achieve 25g/L ethanol on crude glycerol. However, these processes require a controlled anaerobic environment, maintained through sparing with nitrogen.

For ethanol production from glycerol, only two genetically engineered yeasts have been reported which can convert glycerol into ethanol. The industrial work horse *Saccharomyces cerevisiae* has been genetically engineered to produce ethanol from glycerol and the several rounds of genetic engineering, the production level achieved was only 3.1g/L highest production level in the modified strain reached 4.4g/L. The methylotrophic yeast *Hansenula polymorpha* was engineered to improve ethanol production by expression of varied genes from bacteria, however after. Results of previous studies are summarized in Table 3.1.

Table 3.1: Comparison of ethanol production from glycerol by different bacteria and yeasts

Organism	Fermentation method	Ethanol production (g/L)	Vol. Ethanol productivity (g/L/h)	Reference
Escherichia coli EH05	Batch	20.7	0.22	Durnin et al., 2009
Klebsiella pneumoniae GEM167/pBR-pdc-adh	Fed-batch	25.0	0.78	Oh et al., 2011
Kluyvera cryocrescens S26	Batch	27.0	0.61	Choi et al., 2011
Hansenula polymorpha HpDL1-L/pYH-pdc-adhB- dhaDKLM	Batch	3.1	0.02	Hong et al., 2010
Saccharomyces cerevisiae YPH499fps1Δgpd2	Batch	4.4	0.04	(Yu et al., 2010)
Pachysolen tannophilus CBS4044	Staged-Batch			Present study
	Phase I	18.7	0.16	
	Phase II	27.5	0.18	
	Phase III	28.1	0.06	

Pachysolen tannophilus was the first yeast shown to be capable of fermenting xylose sugars to ethanol and the xylose utilisation pathway has been extensively studied in this organism. In a previous study, it was reported that *P. tannophilus* could accumulate 4g/L ethanol on glycerol under aerobic growth, however, the conditions for ethanol production were not precisely defined or controlled and the physiology during growth on glycerol has not been extensively studied in this organism. The possibility for studying the physiology of glycerol conversion to ethanol in this organism provides an interesting prospect for the future production of biofuels.

This studies performed in the Glyfinery project show that crude glycerol can be utilized as a potential low cost substrate for producing fuel ethanol for transportation by *P.tannophilus* (CBS4044). After a series of batch experiments for fermentation optimization, the highest yield obtained was 0.28±0.03 g ethanol g^{-1} glycerol which corresponds to 56% of the theoretical yield. The maximum production achieved was 28.1 g/L ethanol in a staged-batch process. This is the highest value for glycerol conversion to ethanol reported to date. The process could be further optimized through fed-batch design and employment of a more ethanol tolerant strain. This strain could then be cultivated in a fed-batch process which could further optimize productivity and yields.

Production of butanol

Microbial-production of butanol has been studied very intensively for many years. Louis Pasteur was the first (in 1862) to describe the production of butanol by microbes [4]. Around the 1900, research was conducted in isolating and describing solvent producing bacteria. At the same time considerable interest in synthetic rubber started (butanol was used as a precursor for butadiene, the starting material for synthetic rubber production). Around 1912, Chaim Weizmann isolated an acetone-butanol producing strain. This strain was later named *C. acetobutylicum* and has been one of the most widespread acetone-butanol-ethanol producers (ABE-producers). The process evolved (also boosted by the World Wars demand for acetone) until the 1950's where the price of substrate (molasses) increased and the cheap crude oil was available, consequent closure of many plant. Production only continued in countries that were cut off international supplies for political or monetary reasons, such as South Africa where ABE fermentation persisted until 1982).

As focus on sustainable energy is increasing interest in the microbial production of butanol is rising. New plants are planned and built. The table below (3.2) lists some of the companies operating with butanol production in US and Europe. None of the companies are using glycerol as substrate, but

are focused on a sugar platform. In the table it is pronounced that in situ removal of butanol is applied in all processes. However, different strategies may be used.

Table 3.2: A list of companies in US and in EU working on butanol production.

Company	Organism	Fermentation process	Separation strategy	Development status	Additional notes
Butamax DuPont/BP	1. *Clostridium* 2. *E. coli*	Semi batch	Continuous *in situ* removal followed by distillation trains	2013 Commercial Additional Feedstocks 2013+	Formed in 2009
Green Biologics (UK)	*Clostridium*. Mixed populations	Continuous fermentation	*In situ* removal Unknown	Building demo in India. Consulting with Chinese firms	
Metex (FR)	"Well known bacteria"	Unknown	*In situ* removal Unknown	Unknown	Produces also 1,3-PDO 1,3-PDO in pilot scale
Butalco Switzerland	Yeast	Unknown	*In situ* removal Unknown	Unknown	Developing an integrated lignocellulose-based bioethanol/ biobutanol production process.
Gevo (Isobutanol)	Yeast	Semi batch	Vacuum flash *in situ* removal followed by distillation trains	2010 Operating pilot in St. Johns, MO. 2011 Commercial	Technology designed to retrofit existing ethanol plants
Cobalt Biofuels	*Clostridium*	Continuous	Vapor compression distillation	2010 Pilot 2011 demo 2012 commercial	Plan to launch cellulosic plant in April 2012
Tetra Vitae	*Clostridium beijerinckii*	Semi batch	Carbondioxide - stripping continuous *in situ* followed by distillation trains	2009 300 l bench 2010 10,000 l pilot	Focused on butanol and acetone production.

ButylFuel	*Clostridium sp.*	Continuous two stage dual path anaerobic fermentation	Gas-stripping	Unknown

Benchmarking butanol production from glycerol

The process for producing butanol from glycerol is based on a mutant strain of *C. pasteurianum*. The mutant strain was developed with respect to better crude glycerol tolerance and increase conversion rates. In order facilitate growth for an extended period of time, removal of butanol is necessary. This was done gas-stripping. A medium composition with very low cost was chosen/developed, thus, increasing the feasibility of the process.

The process of pilot scale butanol fermentation was performed in a 30 liters fermentor with the *C.pasteurianum* mutant strain. The process of the butanol fermentation is inhibited by the presence of butanol when its conc. exceeds 10 g/L. Therefore, during the fermentation process the butanol was removed by the stripping method with nitrogen (3.3). The fermentation data and results are provided in 3.3.

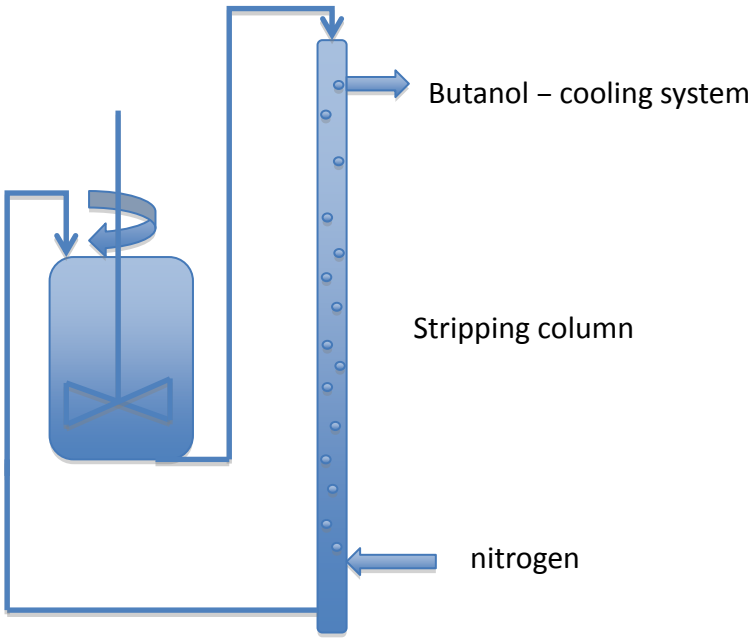

Figure 3.3: A schematic overview of the butanol fermentation system

Table 3.3: Fermentation parameters for the butanol process.

Parameter	Value
Crude glycerol initial conc.	50 g/l
Headspace overpressure	0.2 Bar
pH control level	6.0
Fermentation volume of fermenter A	30 liters
Medium	Biogasol medium
Butanol production efficiency	0.23 g 1,3-PDO / 1g Glycerol
The best observed butanol productivity	0.7 g/l/h
Glycerol uptake	3.0 g/l/h
Final butanol conc.	12.5 g/l
Final glycerol conc.	5.0 g/l
Final biomass content.	2.83 g/l

During the fermentation, the fermentation process system was controlled by pH control, temperature control and headspace overpressure control. The product and substrate content was monitored by HPLC analysis.

There are a limited number of publications dealing with utilization of glycerol as substrate for production of butanol. The widespread ABE producer *C. acetobutylicum*, can metabolize glycerol, but only in the presence of glucose therefore, another strain has been used. *C. pasteurianum* can, however, utilize glycerol as sole carbon source and produce butanol.

In order to produce high amounts of butanol, a high amount of glycerol needs to be converted. It has previously been shown that 63.6 g/l technical grade glycerol could be utilized. The process developed during this project almost doubled the glycerol utilization, even on crude glycerol. In addition, the utilization rates were significantly increased. The maximum utilization rate in batch fermentation reported was 2.62g/l/h, the Glyfinery butanol process was able to increase this rate by more than 2.5 times, still utilizing crude glycerol. This high rate was not achieved by reduced butanol production; the butanol productivity was more than 1.5g/l/h.

The strain developed within the project, tolerates high concentrations of crude glycerol. Never before has initial crude glycerol concentration of 120g/l been reported, emphasizing the robustness of the strain.

By applying gas stripping, circulating the gas-phase of the fermentation, butanol was removed from the fermentation broth continuously assuring non-toxic conditions. As can be seen in table 5 *in situ* removal and especially gas stripping is applied by different industrial research companies (ABE) but it has never been utilized as part of glycerol fermentation. The reason could be that the toxicity of the crude glycerol caused the fermentation to cease before reaching butanol titers critical for the microorganisms. By the development of the butanol producing strain, the butanol toxicity issue became pronounced. Gas-stripping was applied with success assuring non product inhibition.

There are challenges illustrated in previous literature with the conversion of glycerol to butanol. The strain/process developed in this project unambiguously copes with these challenges, bringing the process closer to industrial application.

Production of 1,3-propanediol (1,3-PDO)

The global biodiesel production was over 15 billion liters in 2009 and it is still increasing. The forecast for the worldwide production is over 45 billion liters in 2020. Glycerol is produced as a by-product at a level of 5-10 %. The conversion of glycerol to higher-value products might be the way to decrease the costs of biofuels production. 1,3-propanediol (1,3-PDO) is one of the products that could be produced from the crude glycerol. The main application of 1,3-PDO is a substrate in the polymerization of polytrimethylene terephthalate (PTT), a type of polyester used in the engineering thermoplastics area and in the production of carpets and textile fibers. Biological production of 1,3-propanediol would be a sustainable alternative to the chemical methods. There are several microorganisms which are able to ferment glycerol with the 1,3-PDO as final product. Moreover, the genetically modified *E. coli* strains might be also used.

Table 3.4: Biological methods of 1,3-PDO production.

Organism	**Carbon source**	**yield ***	**remarks**
Lactobacillus hilgardii	glycerol+glucose or fructose	?	
Citrobacter freundii	Glycerol	0,62 mol/mol	
Clostridium saccharobutylicum	glycerol	0,36 mol/mol	high substrate utilization
Clostridium butyryicum	crude glycerol	68 g/l 0,55 g/g	non-sterile fermentation
Clostridium diolis	glycerol	85 g/l	chemical mutagenesis

			and genome shuffling
Klebsiella HR526	glycerol	42 g/l	D-lactate dehydrogenase inactivation/deletion
Klebsiella pneumoniae	crude glycerol	53 g/l	
Klebsiella pneumoniae	crude glycerol + glucose	63 g/l 0,6 mol/mol	
E. coli	sucrose	3 g/l	genes for the sucrose utilization of another *E. coli* strain
E. coli	glucose	129 g/l 0,34 g/g	genes of dha regulon of *K. pneumoniae*

*molar and mass yields were calculated in relation to the consumed carbon source

Glycerol fermentation by the glycerol-fermenting microorganism is a two-branched pathway. The 1,3-PDO produced in a reductive branch is catalyzed by two enzymes, (i) glycerol dehydratase and (ii)1,3-PDO oxidoreductase, with a 3-hydroxypropionealdehyde as an intermediate. On the other hand, in the oxidative branch, glycerol is dehydrogenated by glycerol dehydrogenase to dihydroxyacetone (DHA). DHA is then phosphorylated by ATP or phosphoenolopyruvate to the phosphohihydroxyacetone which is an intermediate to the pyruvate synthesis.The main microorganisms and methods of the biological 1,3-PDO production were summarized in the table above.

Glyfinery 1,3-PDO process

During the project A&A Biotehcnology developed the process of crude glycerol fermentation and 1,3-PDO production based on the non-GMO mutant strain of *C. butyricum*. The process is continuously performed in two fermenters A and B (Fig. 3.4).

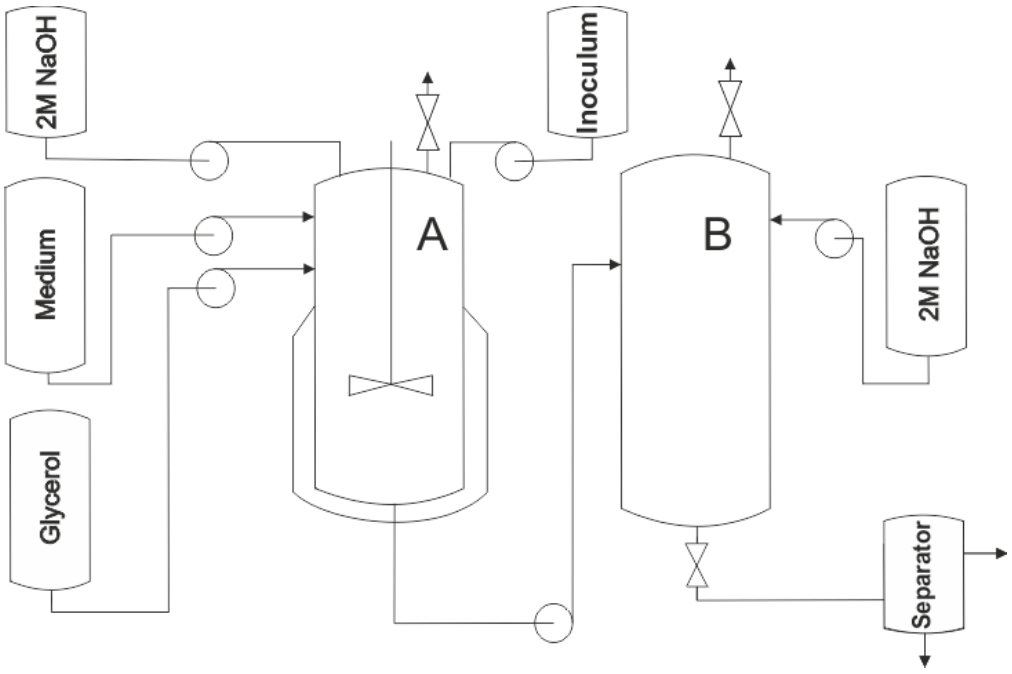

Figure 3.4: Schema of crude glycerol continuously fermentation system.

The fermenter A is highly controlled system where the main fermentation is carried out. The fermenter has the following controlling systems: pH control, level control, temperature control, headspace overpressure control. The first fermentation stage is performed in the steady glycerol concentration and the 1,3-PDO high production efficiency is observed (Table 3.4). The fermenter B is a storage tank with pH control. The second stage of fermentation allows for complete removal of residual glycerol, so the whole used for fermentation glycerol is consumed. The low content of glycerol in the final fermenter is necessary to obtain efficient recovery of 1,3-PDO by extraction.

Table 3.5: Fermentation process parameters for the 1,3-PDO process

Parameter	Value
Crude glycerol initial conc.	60 g/l
Headspace overpressure	0.2 Bar
pH control level	6.5
Fermentation volume of fermenter A	30 liters
Medium	YNB reduced
Glycerol Feeding	0.05 l/h

1,3-PDO production efficiency	0.56 g 1,3-PDO / 1g Glycerol (0.63 g/g theoretical yield)
The best observed 1,3 PDO productivity	0.85 g/l/h
Glycerol uptake	1.31 g/l/h
Final 1,3 –PDO conc.	30.2 g/l
Final glycerol conc.	0.2 g/l
Final biomass	2.13 g/l

Based on the pilot experiment data, the total time and fermentation volume was estimated for 1 ton of glycerol (Table 3.6).

Table 3.6: Fermentation parameters pr. ton of glycerol, based on experimental data.

Parameter	Value
Glycerol	1000 kg
Final 1,3-PDO production	560 kg
Total volume of fermentation media	10 000 liters
Fermenter A	500 liters
Fermenter B	10 000 liters
The total time of fermentation	14-20 days

After the second fermentation in fermenter B, the biomass was separated by pilot scale continuous flow centrifugation (14.000 rpm) with a feed rate of 300 ml/h. Clear supernatant was used for the 1,3-PDO recovery experiments in the pilot scale.

Production of biogas

The interest in biogas is bigger than ever in Europe. The number of biogas plants has increased greatly during the last years. In 2010 the highest number of new installed biogas plants was observed in Germany, Hungary and Czech Republic. Different substrates are used and also the field of application differs between countries in Europe. The biogas production in Germany, Denmark

and Austria takes place mainly on farm based plants, while in for example Sweden and Poland the biogas is for the most part produced at sewage treatment plants . The biogas produced in Europe is mainly used for the production of electricity. Less than 10% of total biogas output was in 2010 upgraded to biomethane quality and injected into the gas grid or used as vehicle fuel. There are only eight countries: Germany, Sweden, Netherlands, Switzerland, Austira, UK, France and Finland, that upgrades the quality of the biogas to a higher standard. In Europe, Sweden was the first county to use biogas as vehicle fuel on larger scale and has today the highest ratio of biogas in the vehicle fuel (51%). Except for electricity production and vehicle fuel, biogas is used for production of heat, steam and cooling, production of chemicals and in fuel cells.

However, the driving forces for the development of biogas in the European counties are different. In Denmark the main purpose of producing biogas from agricultural byproducts is to avoid nitrogen leakage. There is also an economical driving force behind the production of biogas. It can be tax relief on biogas as vehicle fuel which is common in Sweden and Switzerland or governmental support for the produced electricity which is found in Germany, Austria and France.

Future of biogas in Europe

The European Commission has set up a goal where 20% of the European energy demands will come from renewable energy in 2020. Two Danish researchers predict that biogas produced from energy crops, animal manure and industrial organic waste can supply nearly half of the European natural gas consumption in the coming decades and it will represents at least 25% of all bioenergy.

The production of biogas from glycerol was not investigated in the project. The main objective of this work carried out focused on investigating the anaerobic digestion of residual effluent from alcohol and 1,3-propanediol fermentation. Effluents which passed the step of product recovery will be fed into the anaerobic bioreactor producing biogas, containing mainly methane and carbon dioxide. In the first experiment, the gas potential of concentrated cell-biomass of *Pachysolen tannophilus* and *Clostridium butyricum* was investigated at 37 °C. In a second experiment, the effect of pre-treatment on the methane yield was studied. Not only biomass from P. tannophilus and *C. butyricum*, but also from *Clostridium pasteurianum* was included.

Determination of the gas potential and methane production rate was done using a batch method. The gas production in each test bottle was analysed and presented as mean accumulated methane yield in NmL per gram volatile solids (VS) over time. The daily methane production, in NmL CH4/g VS • day, was also calculated. Together with the maximum production per day these values were used as a comparative value between the different substrates and pre-treatments.

In the initial experiment, the biomass of *C. butyricum* showed the highest methane potential, although it did not have the highest maximum methane production rate. At 17 days of incubation the methane yield from *P. tannophilus* was almost the double compared to *C. butyricum*. After 20 days the gas production from the biomass of *C. butyricum* continued to rise, while *P. tannophilus* creased. This difference over time and methane yield is of interest from a production point of view. No difference in methane content was observed between the two biomasses.

In the second experiment, no increase in methane potential was observed for neither of the pre-treated cell-biomasses. The concentrated biomass was diluted in order to facilitate the pre-treatment of the biomass. This dilution and the change of inoculum may be the explanation to the change in the maximum production rate and methane yield compared to experiment 1. Experiment 2 showed much higher production rate and earlier day of the production peak. However, the gas potential from the different untreated biomass was equal between the different samples, except for C. pasteurianum which had a higher value. This level of methane yield can be compared with the methane production from food waste (400-600 m3 CH4/ton VS), which is often used in biogas processes (Jarvis and Schnürer, 2009). The gas production from *P. tannophilus* and *C. butyricum* was measured up to the same gas yields as for distillers waste (300-400 m3 CH4/ton VS).

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Glyfinery glycerol to biodiesel process

During the course of the project the idea of utilizing yeast to convert glycerol back into biodiesel was presented. In the series of experiments, the yeast strains which were genetically engineered to produce fatty acids were tested on various carbon substrates. The basic aim of this invention is to provide a flexible model of biofuel production from variety of carbon sources belonging to first and second generations. The method and the media designed for the growth and production remains same throughout the process. The only variable factor is the carbon substrate. Thus, this is a

technology which enables microbial biodiesel production using genetically engineered yeast strains growing on defined media to produce biodiesel.

Although initial experiments were completed further optimization still needs to be performed for the process to mature into pilot scale. One additional outcome was the filing of a PCT Application:

The Technical University of Denmark (DTU) filed priority founding patent applications before the European Patent Office (EPO) and the United States Patent and Trademark Office (USPTO) on June 18th 2010. The initial search performed by the EPO indicated novelty of the claimed subject matter. Accordingly, DTU filed a PCT application on June 18th 2011 claiming priority of the above-mentioned EPO and USPTO applications. The application was published on December 2011.

The patent application claims:

1. A genetically modified organism (GMO) for the extracellular production of fatty acids wherein said organism is most importantly characterized;

a. By a reduced fatty acyl-CoA synthase activity conferred by a FAA2-gene deletion, an enhanced CoA carboxylase expression by a promoter-optimized ACC1-gene, a transgene encoding a pyruvate formatelyase comprising PflA and PflB or any combination of these.

b. As belonging to a selected set of fungi or yeast strains.

2. A growth medium comprising a subset of components known to stimulate the production of fatty acids by the GMO. And for which the carbon source are any of the listed.

3. Method for extracting the fatty acids and esters produced by the before mentioned GMO.

Product recovery

The objective of this work area was to find an optimal method for recovery of the target products from the fermentation broth at laboratory scale. The post-fermentation broth samples of the most promising producers of alcohols and 1,3-propanediol were subjected to the variety of organic extraction systems. Subsequently the most effective extractions were followed by distillation using either simple distillation in solvent extraction or distillation with steam. The recovery processes were optimized both from the chemical (effectiveness, purity of final target chemicals) and

economical (operational costs and wastes treatment costs) angles to provide the best feedback for pilot-plant and industry scale-up.

Results

Due to increasing price of petrochemical feedstocks and extensive oil consumption, a considerable effort has been made t oadvance the production of biofuels. Among these, butan-1-ol and propane-1,3-diol (1,3-PDO) were targeted as very promising. In case of butanol besides pervaporation and traditional distillation, other solvent recovery techniques have been developed, i.eg. gas-stripping. The separation techniques studied for 1,3-PDO include ion-exchange chromatography, evaporation, distillation, pervaporation, solvent and reactive extraction.

State-of-art butanol recovery process

Recent publications concern mainly ABE (acetone-butanol-ethanol) fermentation performed by *Clostridia* strains. In the ABE fermentations where butanol is usually the main product, the maximum achievable butanol concentration in the fermentation broth is ~20g/L. The final ABE composition depends on product inhibition and butanol toxicity. [1-4] With regards to above mentioned facts all synthesis approaches have focused on in situ separation of butanol from fermentation broth.

Distillation is the traditional technique of product recovery for the ABE fermentation process. Due to high boiling point of water, most of energy requirement during distillation originates from the water evaporation in the fermentation broth. Distillation efficiency is related to the energy integration applied, as the energy requirement determines the operational costs [5].

Pervaporation is a well-described method of butanol recovery. It is a combination of membrane filtration and solvent evaporation from fermentation broth [6-8]. The process is based on volatiles diffusion through a solid membrane and remaining the nutrients, macromolecules and microbial cells in the feed. Selectivity of product recovery and velocity of membrane penetration depends on the membrane properties, its thickness, composition of liquid and gas-phase, process temperature and pressure[9-13.]

Gas-stripping has been described as the most important industrial technique of butanol recovery in fermentation-integrated systems. The method allows for selective separation of volatile products from the feed with no membrane usage. The process is based on product concentration difference in liquid and gas-phase. The gas-phase is sparged into the fermentor and butanol is condensed and

recovered from the condenser. After product removal gas is recycled to continue gas-stripping. During gas-stripping it is possible to maintain the anaerobic conditions by using oxygen-free gas (nitrogen, carbon dioxide, hydrogen).

Application of gas-stripping in butanol fermentation using *C. acetobutylicum* was first described by Ennis et. al. [14] Butanol recovery method has many advantages over other removal processes, for example, it is simple and inexpensive to perform. Integrated system of gas-stripping and fermentation leads to decreased toxicity and increased butanol production [15]. The list of butanol separation techniques and companies operating with butanol in Europe and US are shown in the section 5.2 „Production of butanol" (Table 5).

Glyfinery 1-butanol recovery process

Based on WP 3,4,5 interactions the final WP6 system proposed for recovery of 1-butanol is a three stages integrated process which combines following steps: gas-stripping, liquid-liquid extraction, distillation and solvent recovery.

Gas stripping is the most important technique for removal of 1-butanol from fermentation broth. The 1-butanol volatile properties allows for selective in situ product removal from fermentation broth without using any membranes. The gas stripping process has many advantages, e.g. it is simple and inexpensive to operate. Moreover, integrated fermentation process involving gas stripping allows to avoid the inhibitory effect of 1-butanol on the culture during fermentation and obtain high concentration of target product. The 1-butanol toxicity can be kept below the inhibitory levels by feeding the reactor at a slow and controlled rate, while the product-removal technique is applied simultaneously to remove the 1-butanol being produced. It is widely known method as described in the state-of-art section.

The post-stripping aqueous solution of 1-butanol is then subjected to liquid-liquid extraction (LLE) performed by means of the most efficient organic solvent. Main advantage of the process is high efficiency (99.5%) and low energy requirement (0.5MJ/kg of product).

Subsequent operation step is distillation of post-extraction solution of 1-butanol organic solution at yield reaching 95%. The target final product is finally obtained at very high purity (99.90%).

Solvent recovery is the side step in proposed separation process of 1-butanol from fermentation broth. Due to economical and environmental reasons stripping is the most viable technique.

Recycled solvent can be successfully reused for 1-butanol extraction from fermentation broth. Regarding to low toxicity of selected solvents even some traces of solvent remaining in the raffinate would be environmentally acceptable as it is commonly utillized in biological treatment systems.

Table 1: Summary of results of the integrated 1-butanol recovery system

Recovery process efficiency [%]	99
Total energy requirement [MJ/kg of product]	57.4
Product purity [%]	99.90

According to available data and publications the proposed system has never been utillized before. It offers an obvious advantage of lower energy requirement due to liquid-liquid extraction stage and resulting reduced volume of 1-butanol containing stream subjected to distillation process.

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State-of-art 1,3-PDO recovery process

Several methods for the separation and purification o f 1,3-propanediol (1,3-PDO) from fermentation broth or similar processes have been reported in many previous studies and patents.

One of the 1,3-PDO recovery techniques was based on the reactive extraction (Malinowski 2000). Malinowski (2000) proposed the formation of 2-methyl -1,3-dioxane (2-MD), a product of reaction of acetic aldehydes with 1,3-propanediol catalyzed by Dowex or Amberlite ion-exchange resin with

simultaneous extraction of the product (2-MD) by organic solvents. In another method, propionaldehyde, butyraldehyde, and isobutyraldehyde were used as reactants as well as extractants to form substituted 1,3-dioxane (Hao et al . 2005, 2006). Fang and Zhou (2006) proposed the kinetic study of formation of 2 –MD by 1,3-propanediol and acetaldehyde catalyzed by cation exchange resin HD-8. All these processes are complicated, and besides the additional need to regenerate 1,3-propanediol from its dioxolane derivative, the complexity, and the cost of the chemicals used make the extraction process quite prohibitive. Moreover, if this process is used for real fermentation broth, then acetaldehyde can react with other by-products and proteins, making this process inefficient.

Malinowski (1999) proposed liquid – liquid extraction where the distribution of 1,3-propanediol into extraction solvents appeared to be not good enough to make simple extraction efficient. Another attempt to separate 1,3-propanediol from a dilute solution by normal physical or complex extraction was also not successful (Xiang et al. 2001). Although many solvent extractants are given in a patent, the hydrophilic 1,3-propanediol in diluted broth fails to enter into hydrophobic solvents, except when adding a large amount of solvents into a concentrated broth (Baniel et al. 2004). Similarly, ethyl acetate was used in phase separation of 1,3-propanediol where the ethyl acetate phase which contained 1,3-propanediol and 1,2-propanediol was subsequently used for chromatographic purification . In addition, the partition coefficient of the target product was below 1.9 (Cho et al. 2006). However, this process has low separation efficiency and also requires the handling of large quantities of solvents.

The pervaporation method based on the ZSM -5 zeolite membrane had drawbacks such as a low flux and selectivity (Li et al. 2001).

Vacuum distillation is preferred over traditional distillation as it saves energy due to the decline of boiling point. Ames (2002) and Kelsey (1996) in their patents and Sanz et al. (2001) evaluated the vacuum distillation- based separation process. However, desalination and deproteinization are required before evaporation which makes the entire process complicated and non-profitable. Gong et al. (2004) and Hao and Liu (2005) evaluated the potential of electrodialysis before evaporation, but low product yield and membrane pollution make this process undesirable.

The available methods for separation of 1,3-PDO from fermentation broths are summarized in the table 10.

Table 2: Comparison of different separation techniques for 1,3-propanediol.

Separation methods or unit operation	Application / investigation	Drawbacks or problems	References
Evaporation / distillation or vacuum distillation	Evaporation was applied for the removal of water from the fermentation broth. Distillation was applied for the final purification of 1,3-PDO	Evaporation and distillation suffer from a large amount of energy consumption. Moreover, desalination and deproteinization are required before evaporation which makes the entire process complicated and non-profitable.	Kelsey 1996; Sanz et al. 2001; Ames 2002;
Pervaporation	Na-ZSM-5 and X-type zeolite membranes were used to separate 1,3-PDO from an aqueous mixture by pervaporation. The high 1,3-PDO /glycerol selectivity was due to referential adsorption of 1,3-PDO Zeolites combined with a cross-flow filtration module were applied to separate the biomass and enrch 13-PDO in fermentation broth,	The performance of pervaporation needs to be verified by using real fermentative broth in the presence of impurities, e.g., proteins and salts	Li et al.2001a, b, c, 2002; Corbin and Norton 2005

	respectively.		
Electrodialysis	Electrodialysis has been used for desalination before evaporation	Low product yield due to loss of 1,3-PDO during electrodialysis. Membrane pollution can be very serious. High energy input for further removal of water.	Gong et al. 2004; Hao and Liu 2005
Chromatography	Combined strongly acidic cationic and weakly basic anionic resins were used to desalinate in the fermentation broth. A cationic exchange resin was used for recovery of 1,3-PDO. Adsorption of 1,3-PD on hydrophobic zeolites or active charcoal was investigated for separation of 1,3-PDO. A preparative silica gel liquid chromatography was used to separate 1,3-PDO after phase separation or concentration of protein-free broth.	Although high overall purity and yield of 1,3-PDO could be obtained, the 1,3-PDO solution was not concentrated but diluted because of the low selectivity and capacity of resin or adsorbent. This method consumed more energy than the simple evaporation and distillation. In addition, the chromatographic matrix had to be regenerated frequently if the feed was not desalinated or deproteinized. This situation also occurred for ion-exchange resins used to desalinate due to	Roturier et al. 2002; Hilaly and Binder2002; Corbin and Norton 2003; Wilkins and Lowe2004; Adkesson et al.2005; Cho et al. 2006 Roturier et al. 2007; Anand et al. 2011

		high salt concentrations.	
Solvent extraction / liquid –liquid extraction	Many extractants have been investigated for the recovery of 1,3-PDO from dilute broth. It is partly partitioned into the solvent phase only when adding a large amount of solvent into a concentrated broth	No effective extractant has been so far found for liquid –liquid extraction of 1,3-PDO. Major problem is because 1,3-PDO is hydrophilic	Malinowski 1999; Xiang et al. 2001; Baniel et al. 2004; Cho et al. 2006
Reactive extraction	Reactive extraction includes three key steps: reaction, extraction, and hydrolysis. A reversible reaction between 1,3-PDO and aldehyde was used to form a dioxolane derivative (e.g., 2-MD). 2-MD is then extracted into an organic solvent and finally hydrolyzed into 1,3-PDO	This process is quite complicated. The removal of proteins and ethanol as well as salts is necessary before reaction. Additionally, the trace amount of aldehyde in 1,3-PDO is prohibitive for polymerization of PTT	Broekhuis et al. 1994, 1996; Malinowski 2000; Hao et al. 2005, 2006 Fang and Zhou 2006

So far, no economically feasible strategy for recovery of 1,3-PDO from fermentation broth based on the glycerol has been developed and published.

Glyfinery 1,3-PDO recovery process

The optimal procedure of isolation of 1,3-PDO from fermentation broth, developed in WP6, is based on the following steps:

-extraction of fermentation broth

-recovery of solvent (from extract) by distillation

-vacuum distillation
-recovery of solvent (from raffinate) by stripping

Liquid-liquid extraction is complex and always requires some type of pilot plant experiments to generate the necessary data for process design. This is especially true in the case of biotechnological applications. The fermentation broth can often vary in composition and contain trace quantities of other materials that affect the phase separation or efficiency of the process. Any pilot plant testing should be performed with actual fermentation broth, as synthetic blends will not reveal any problems. There are many types of devices available to accomplish the liquid-liquid extraction process, including mixer-settlers, packed columns, sieve tray columns, agitated columns, and centrifugal units. Two types of agitated column were tested. Liquid-liquid extraction efficiency is 96%.

The solvent recovery step is the critical aspect of any liquid-liquid extraction process design. Efficient solvent recycling greatly affects the economics of the process. In the proposed process solvent recycling is being recovered by distillation at 90% efficiency. The recovered solvent can be returned directly to extraction step without any further purification. Vacuum distillation is a final purification stage of 1,3-PDO recovery. The yield of distillation is 99% with 99.99% purity of target product. This process requires a low energy input due to extremely low volumes being processed.

Recovery of solvent from raffinate can be performed by stripping. The recovered solvent can be successfully reused in liquid-liquid extraction of target product.

The 1,3-PDO integrated recovery system is summarized in the table 11.

Table 3: Summary of 1,3-PDO integrated recovery system.

Recovery process efficiency [%]	90.3
Total energy requirement [MJ/kg of product]	158*
Product purity [%]	99.99

70% of energy can be recovered as heat energy that can be utillized in heat demanding processes (i.e. fermentation)

The proposed 1,3-PDO recovery system integrated with bioconversion of glycerol represents a unique process that can be easily adopted by industry. Clearly there are no existing counterparts to the proposed process that have been applied in industrial scale.

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Process integration at pilot scale

The main objective of this WP is to scale up the biological, glycerol-based conversion process from the laboratory scale to the pilot scale, characterize the process performance under different operational conditions and point out the optimal conditions for the fermentation processes.

Integrated assessment

This project includes an integrated sustainability assessment covering technological, environmental and economic aspects, which are presented here.

The investigated use options for glycerol are:

- Direct material use of glycerol
- Generation of energy by combustion of glycerol or production of biogas out of glycerol
- Biotechnological conversion of glycerol into either ethanol, butanol or PDO (1,3-propanediol, a precursor for the production of bioplastics).

In summary, the conventional **direct material use** is the best of the assessed options from an environmental point of view. This scenario covers that glycerol as a final product functionally substitutes simpler chemicals as an additive to a wide range of products like cosmetics. This is currently the most common way to use glycerol, which can be realised with limited technological efforts and financial expenditures. However, the direct material use of glycerol is a limited market and may lose importance if the biodiesel market and thus the production of glycerol will expand further, especially, if no completely new material use options will be identified.

To the extent to which a direct material use cannot be realised any more because of limited capacities, alternatives such as **biotechnological use options** and the **use for energy production** including biogas can play a bigger role in future. There is no clear winner amongst these options from an environmental perspective although the production of ethanol and the optional refining of biogas to biomethane are clearly disadvantageous compared to the other options. All other processes, especially the production of PDO, butanol or biogas via cofermentation, have different environmental potentials each. It will be essential to realise these individually. Under the underlying conditions of this study, the production of butanol stands out due to its high probability to be economically profitable, whereas the production of ethanol will likely lead to losses. The innovatively produced PDO involves the highest economic chances but also high risks. Next to this,

the choice of the glycerol use option does generally not affect the environmental or economic performance of the whole biodiesel production substantially.

In particular, the **conversion of glycerol to ethanol, butanol or PDO** by means of innovative biotechnological processes is technically demanding and energy consuming, which causes high economic and environmental expenditures. Limited technical risks exist but they are controllable. For these reasons, the biotechnological conversions are mainly environmentally disadvantageous compared to the direct material use of glycerol but comparable to its use for energy generation. From an economic point of view, the higher expenditures for products of higher value can pay off although significant economic risks exist. Generally, the bandwidths of the results are high for these pathways because they are currently only established in a pilot scale. In contrast to the other conversions, the **production of ethanol** is unfavourable from an environmental and economic perspective. The **production of PDO** can lead to the highest possible profits and environmental benefits of the innovative pathways but can also result in significant losses, in part due to uncertain market perspectives, and additional environmental burdens under unfavourable conditions. The **production of butanol**, in which PDO is obtained as a by-product, shows profits under all assessed conditions and additionally offers nearly unlimited market capacities. Environmentally, it performs in tendency slightly worse than the sole production of PDO.

The option to produce heat and / or power from glycerol via **direct combustion** in stationary plants or via **biogas production** can be rated similarly sustainable from an environmental and economic point of view. Depending on the specific design, the assessed processes of energy generation show minor differences: the purification of biogas to biomethane for feeding into the natural gas grid results in environmental disadvantages but can result in economic advantages. Another example is the production of biogas from glycerol without mixing in other substances, which has in tendency less advantages from an environmental and economic perspective. Compared to the direct material use, the energy generation is disadvantageous under environmental and economic perspectives. Only potential synergy effects from a biogas fermentation, in which glycerol is mixed with other substrates, could substantially improve the performance. Nevertheless, the energy generation is not limited in capacity and can be realised with similarly low technological efforts and investments as the direct material use.

The most important **recommendations** for different groups of decision makers, especially from science, industry and politics, are the following ones (more recommendations are listed in the full report):

- From an environmental perspective, further development of the investigated biotechnological conversion processes is recommended, if at all, only for the production of PDO or butanol.
- The further development of the biotechnological conversion processes should focus especially on increasing yields and on a significant reduction of the energy input for product purification. This should also be taken into account for the development of sustainable biotechnological processes in other contexts.
- The further development and field testing of the biogas production from glycerol should focus on synergy effects in the cofermentation of mixed substrates and on the sustainable supplementation of nutrients in case of the separate fermentation of glycerol.
- Other use options for glycerol should be explored besides the ones assessed here. This could be other applications for glycerol without conversion e.g. as a product ingredient, a biotechnological conversion into other chemicals, and also catalytic chemical conversions.

As an **outlook**, other external factors should be considered, which will be important for the future development of the glycerol market and upcoming glycerol use options. Generally, the glycerol market will be influenced on the supply side by the development of the biodiesel production and on the side of the demand by the emergence of new use options. One example is the recent production start of a big chemical plant by Solvay to convert bio-glycerol into a precursor for epoxy resins. Therefore, fluctuations of the glycerol price seem more likely than a constant decline taking the current developments into account. The assessed use options can play an important role if the glycerol supply rises but they represent only a part of all possible alternatives. Furthermore, a politically relevant and comprehensive rating of glycerol use options also has to take other aspects into account like the security of the energy and food supply, social aspects or the progress of knowledge, which is especially important for industrialised countries in Europe. The results, conclusions and recommendations of this study can be of great value for defining the concept and specifications of such assessments.

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