RESTRUCTURING RENEWABLE ENERGY SOURCES FOR MORE EFFICIENT BIOFUELS PRODUCTION WITH EXTREMOPHILIC MICROORGANISMS

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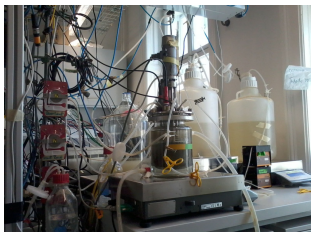
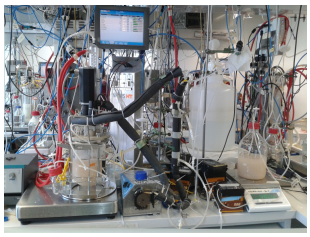
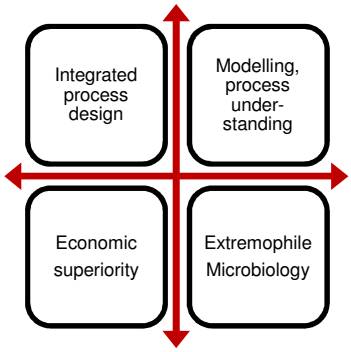
Abstract:

Our mission is to contribute to new biofuel generations such as biological methanogenesis and biohydrogen production. We emphasize especially the bioprocess sustainability side by the mean of:

- Design of integrated biological systems
- Maintaining CO_2 neutrality
- Achievement of process intensification by coupling of waste streams
- "Waste to value" principles: biomaterials production on waste streams

Interdisciplinary approach

I. Biological methanogenesis

Background

Biological methanogenesis is a promising technology for the production of biomethane and for renewable electricity storage**,** a "Power to gas" solution.

Technology

- Anaerobic fermentations
- Liquid or gas limited culture conditions
- Intermittent production profiles for a "Power to gas" approach

Advantages

- Very fast kinetic
- Fast responding physiology
- High selectivity and conversion towards the main product
- Low contamination risks
- Extremely stable and reproducible bioprocesses

Potential applications

Biological methanogenesis is one of the most promising technologies for the production of biomethane in the field of renewable electricity storage. Peak of irregularly generated electric energy needs to be efficiently stored. For this purpose the utilization of hydrogenotrophic methanogens seems to be a very promising candidate for the development of biological gas conversion processes.

II. Biohydrogen production

Background

Nowadays, biohydrogen is considered the ideal alternative energy source. It can be combusted with water as the only oxidative emission or integrated into coupled bioprocesses systems. Biohydrogen production via dark fermentation with hyperthermophilic strains has reported not only high hydrogen to substrate yields, but also high hydrogen to carbon dioxide yields. This last key physiological parameter plays one of the main important roles considering future bioprocess integrated systems under carbon dioxide neutrality.

Technology / Methodology

- Dark fermentative biohydrogen production.
- Medium optimization for biomass and biohydrogen productivity increases.

Advantages

- No contamination at high working temperatures.
- Use of pentoses (xylose) as substrate, considered otherwise as waste.
- Further use of organic acids and alcohols, by-products of the fermentation, for energy substrate recovery.

Potential applications

- Two-stage biohydrogen production process. Coupling with photofermentation systems.
- Two-stage system for biohydrogen and biomethane production.
- Integrated biohydrogen and bioethanol production system for biomethane production under carbon dioxide neutrality.

III. Biological conversion of waste streams to high value added products

Background

Extreme halophilic microorganisms can grow in conditions with up to saturated NaCl concentrations. The pink-red halophilic microorganisms are potential sources of carotenoids that are natural antioxidants and also used as food colorants. Halophiles are able to consume a wide variety of organic material; sugars, alcohols, etc. Biological reduction of organic carbon contents in waste streams with NaCl is a novel industrially applicable biological alternative, a “Waste to value” solution.

Technology

- Recycling waste streams, e.g. from biohydrogen production with NaCl by halophiles
- Bioprocess with extreme halophiles in a corrosion resistant bioreactor
- Production of valuable biomaterials: carotenoids, biopolymers

Advantages

- Process intensification by coupling process streams

- "Waste to value"
- Cost-effective non-sterile bioprocess
- Sustainable waste water treatment alternative

Potential applications

The technology is suitable for saline and non-saline industrial waste streams with organic carbon content, additional NaCl can be required. For instance, the halophilic bioprocess can be coupled with diverse fermentation broths rich in small metabolites.

Keywords: New biofuel generations, CO_2 neutrality, Power to gas, Waste to value

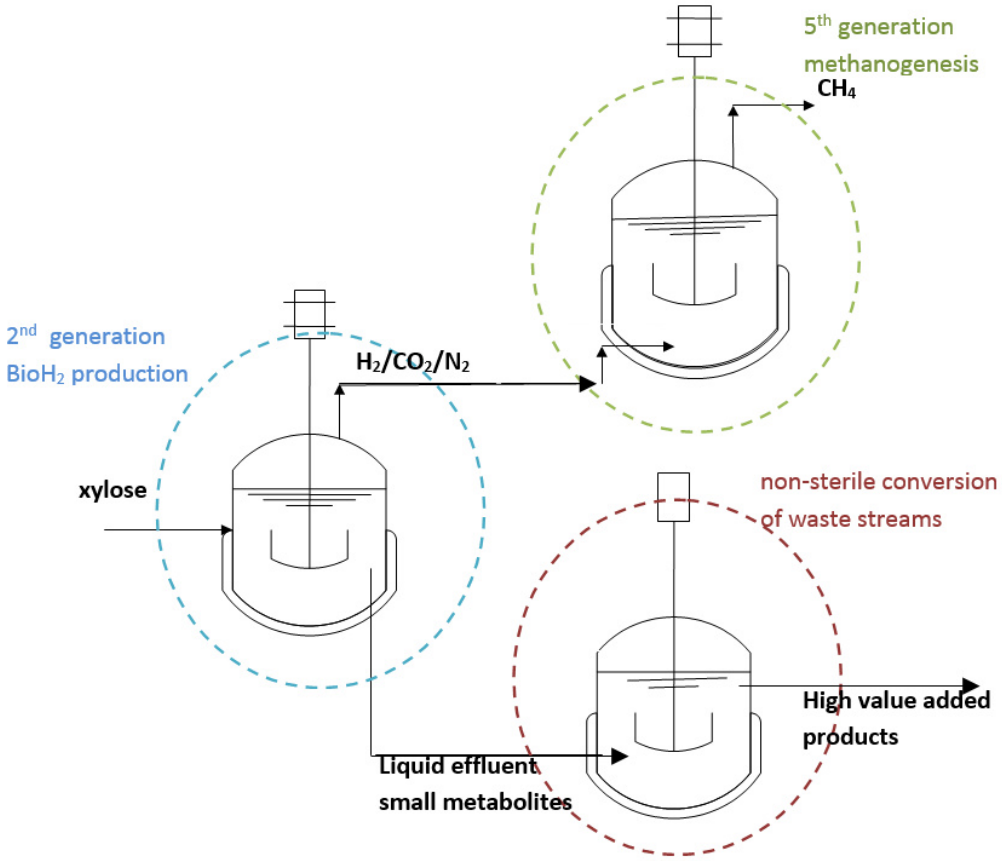
1 Introduction

The future shortage of the fossil fuels imposes an increasing demand for alternative energy sources. Moreover, energy production with fossil sources results in CO_2 release to the atmosphere which is responsible for the endangering and increasing global warming. Hence, the research for alternative energy sources should strive for, in one hand involving more and more renewable resources, and, on the other hand, decreasing the CO_2 impact of energy production. CO_2 neutrality can be realized by using integrated bioprocesses and biofuel production systems [1]; for instance, integration of biohydrogen and biomethane production into the new generations of biofuels within a biorefinery concept, coupling different processes with energy - as well as - stream integration.

Extremophilic microorganisms thrive under diverse extreme environmental conditions. Therefore, they can produce some valuable and still unexploited products triggered by their extreme living conditions. Additionally, extreme cultivation conditions can also ensure inherent cultivation selectivity for the microorganisms for cost-effective non-sterile bioprocesses. Using extremophilic microorganisms, process intensification offers economic and ecological rationalisations of chemical and biotechnological processes. Biological methanogenesis is a promising biological alternative for methane production that uses CO_2 and hydrogen for methane production with anaerobic archaea [2]. Moreover, it is also entitled as a "Power to gas" solution for renewable electricity storage [3]. Dark fermentative biohydrogen production with thermophilic microorganisms on the substrate xylose is accompanied by small metabolites, which remain in the fermentation broth [4]. Due to the production of small by-products, the yield on hydrogen is low but maximized. In addition, the xylose, coming from the degradation of the lignocellulosic biomass, is used as a C_5-source in the production of 1st and 2nd generation biofuels, like biohydrogen. Halophilic microorganisms can grow up to saturated NaCl concentrations and are able to grow on different small organic compounds [5]. Halophiles can produce valuable biomaterials such as carotenoids, biopolymers, compatible solutes and halophilic enzymes [6-8]. Due to the high osmotic pressure of the hypersaline cultivation media, the non-sterile re-use of waste streams with small organic by-products implies innovative solutions for turning waste into value added products within a biorefinery concept.

With the presented extremophilic examples, the following items are proposed to use renewable energy sources in a more efficient way for biofuels production:

- Process integration – approaching CO_2 neutrality with introducing new biofuel generations (Figure 1)
- Store electricity with a “Power to gas” principle
- Create extra high added value on waste streams with “Waste to value” solution



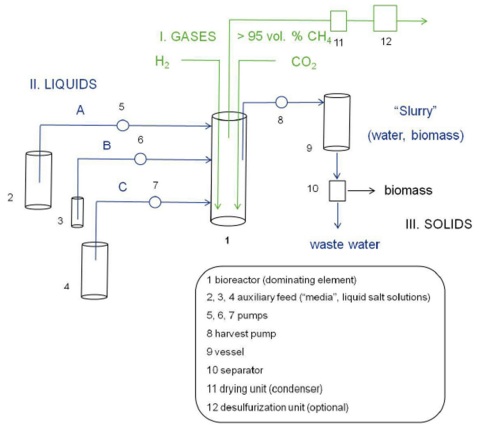
Process integration with extremophilic microorganisms by coupling process streams.

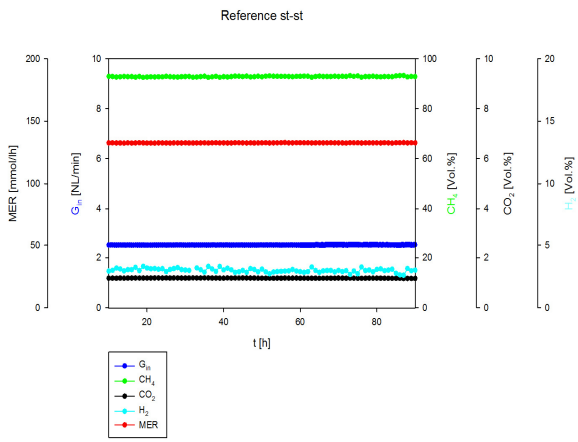
2 Biological Methanogenesis

New generation bio-fuels are a suitable approach to produce energy carriers in an almost CO_2 neutral way. In addition, peaks of irregularly generated energy needs to be efficiently stored because they cannot be absorbed by the existing electricity grid. For this purpose the utilization of hydrogenotrophic methanogens seem to be very promising candidates for the development of biological gas conversion processes. The chemical storage of energy in form of methane generated from renewable resources transforming H_2 to CH_4, by CO_2 fixation is a widely discussed topic as the storage of H_2 at an appropriate density is difficult [21]. The introduced biological methanogenesis enables to gain an energy carrier with a high energy content that can be introduced to existing natural gas infrastructures.

2.1 High quality methane

The basic biological requirements of this anaerobic strain were already investigated and the composition of the feeds and basal medium where set sufficiently high in order to guarantee a gaseous substrate limitation. Cultures limited by gaseous substrate have a different behavior than usual liquid limited culture. Thus the culture can be assumed as a three-phase catalytic system in which the mass transfer of hydrogen from gas to the liquid phase is limiting and where the microorganism is the solid catalyst, which can have different activity depending on physiological conditions applied to the process. In addition, biological methanogenesis offers very high specific activities which allows to have volumetric productivities of [22 $m^3_{CH4}/m^3_{suspension}$*h] which specific production rate of 115 [mmol/g*h] [22-23]. In order to reduce the costs of industrial methanation usually named “SABATIER” process with temperature between 200 and 400 °C and pressures of 5 to 50 bars, biology uses mild conditions with temperature between 35 and 70°C and pressures between 1 up to 100 bar for the strain *Methanocaldococcus jannaschii*. The process flow diagram is found underneath as well as a long lasting and stable methane production performed in a single unit Sartorius 15 L C+ bioreactor enriching pure CO_2 and H_2 to CH_4 at grid quality. The quantification is based on the measurement of the *methane evolution rate (*MER in mmol methane per liter of suspension volume and time.

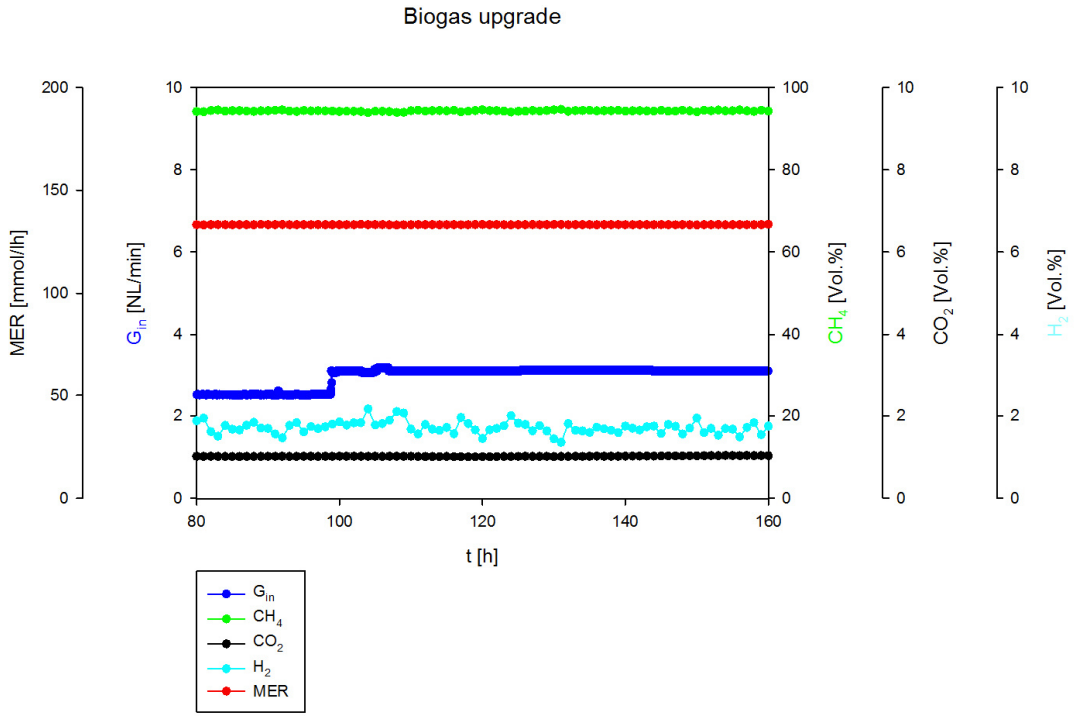




2.2 Biogas upgrade and real gas applications

While in lab scale mostly pure H_2 and CO_2 are used as reactant gasses, an industrial application will, out of economic and environmental reasons, need alternative sources for H_2 and CO_2. This can be all kind of CO_2 and H_2 rich industrial exhaust gasses of chemical and/or biological processes (e.g. syngas, biogas, pyrolysis waste gas, biohydrogen etc.) A suitable replacement of the pure CO_2 source is biogas. In addition, it contributes to the better carbon impact of a biogas plant which will not have to undergo usual pressure swing absorption and desulphurization units as both component, CO_2 and H_2S are raw material for biological methanogenesis and are required for the process performance. Therefore in addition of performing optimization of media components and bioprocess control, we also achieve here feasibility studies of different exhaust gas as possible replacement of the H_2 and CO_2

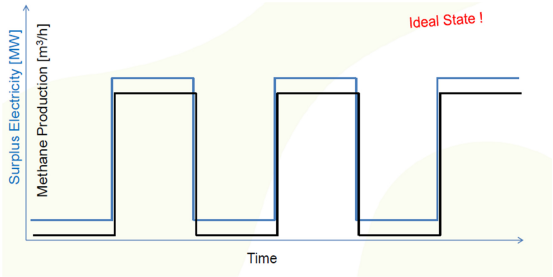
sources which are document elsewhere [24]. Underneath a graph showing a switch to biogas as sole CO_2 source can be found.



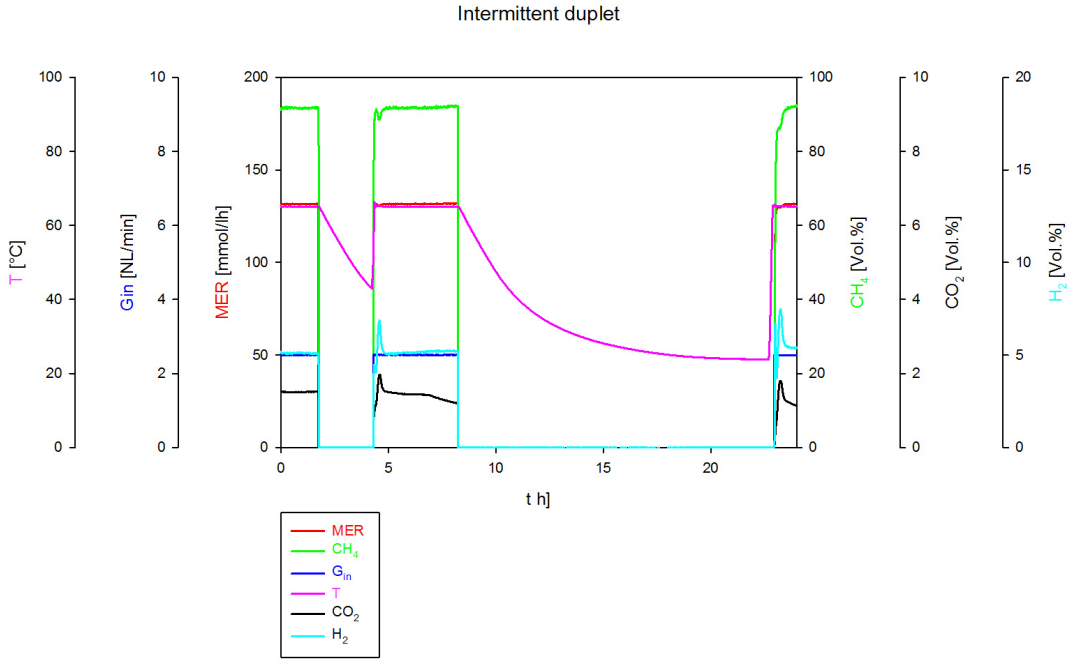
It can clearly be seen that no impact are notifiable compare to a switch to raw biogas as sole CO_2 source of the process. The increase in gassing rate fed to the reactor can be explained by the different volumetric proportion of CO_2 found in the biogas compared to pure CO_2. The methodology involved into those feasibility studies for evaluating the impact of emission gasses on biological methanogenesis can be found in the literature [24].

2.3 Intermittent power storage

The application of biological methanogenesis for storing excess power requires a highly dynamic recovery power towards changing conditions of gas supply. In order to examine the stability and the effects associated with an intermittent production process varying between off-states and fast restart of the supply streams (gaseous and liquid) different set of experiments were performed to evaluate the responsiveness of the strain as well as the process time required towards retrieving a stable methane production after total shutdown of all the equipment's attached to the bioprocess. This guarantees an economic advantage for



biological methanation rather than the usual chemical transformation which cannot benefit of such versatility towards intermittent operating conditions. An example of dynamic situation found in reality with renewable electricity generated from solar or wind sources can be found above. On the other hand, underneath the dynamic response of biological methanogenesis can be seen on the graph which is really high. The physiological “awakening” time was evaluated to be within a couple of minutes.



3 Biohydrogen production

Due to the current energy and environmental problems, related to fossil fuels depletion and greenhouse gas emissions, the interest of searching for different uses of lignocellulosic biomass, a renewable energy source, has increased [1, 9]. The resulting components of the hydrolysis of this lignocellulosic biomass are cellulose and hemicellulose. On the one hand, cellulose is mainly composed of glucose, and it is being widely applied in biofuel production systems. On the other hand, the main component of the hemicellulose is xylose, whose further use in large scale processes is still under research. One of the possibilities of using xylose as a substrate is in the production of biohydrogen via dark fermentation.

3.1 Biohydrogen production via dark fermentation

Biohydrogen can be produced via biophotolysis, photofermentation and dark fermentation. Among them, the third bioprocessing route is the most promising one, regarding biohydrogen yields and production rates. In dark fermentative bioprocesses different carbon sources (mono-, di- or polysaccharides) can be used as substrates by a wide range of microorganisms. In our work we focused on the use of the monosaccharide xylose to generate biohydrogen by the anaerobic extreme thermophilic strain *Caldicellulosiruptor saccharolyticus* [10, 11]. To increase the biohydrogen yields and productivities of this strain under predefined conditions, two strategies were carried out:

1) Optimization of the medium composition, in order to induce metabolic shifts in the cells towards the product of interest

2) Implementation of an external cell retention system, in order to increase the microbial biomass productivity in the bioreactor and therewith the biohydrogen productivity.

3.1.1 Medium optimization

Our system consisted on biohydrogen production via dark fermentation on xylose by the strain *C. saccharolyticus*. The optimization of the reference medium used in this system was based on the study of the complex compound and the nitrogen amount present on it [10].

The study of biohydrogen production in a complex or a defined medium on batch mode allowed us to characterize four different H_2-production phases, correlated with the biomass growth, independently of the medium applied. Furthermore, the quantification of the biohydrogen physiological key parameters in these systems showed up the positive effects on hydrogen productivities and yield if growing the strain under the presence of yeast extract. Therefore, this complex compound should be further considered in the medium formulation. Another possibility, if a defined medium is required, would be to carry out a detailed analysis of the yeast extract, in order to replace this complex component by defined quantities of its corresponding compounds.

The characterization of the yeast extract found in the complex medium would be really useful, considering the necessity of working on a defined medium for a further biohydrogen productivities increase in the presented system. This necessity lies in the positive results got working with a double carbon-nitrogen-limiting culture, as N-limiting conditions ended up in higher specific biohydrogen productivities [10].

– **Product yields and volumetric and specific hydrogen production rates at steady states (*D* 0.1 h^{-1}) on xylose at different ammonium feed concentrations (pH 6.70 ± 0.02, 72.5 ± 0.3 °C).**

	Feed NH_4^+ [mM]	NH_4^+ [mM]	Xylose [g/L]	BM [g/L]	HER [mmol/L h]	q_{H_2} [mmol/g/h]	$Y_{(H_2/CO_2)}$ [mol/C-mol]	$Y_{(H_2/s)}$ [mol/C-mol]	$Y_{(NH_4/s)}$ [mol/C-mol]
C-Limitation	13.76 ± 0.18	6.37 ± 0.18	0.0 ± 0	0.46 ± 0.03	6.13 ± 0.12	13.33 ± 1.00	1.36 ± 0.05	0.41 ± 0.01	0.013 ± 0.00
N–C-Limitation	1.55 ± 0.02	0.0 ± 0.0	0.0 ± 0	0.39 ± 0.02	6.21 ± 0.12	15.92 ± 1.19	1.37 ± 0.05	0.42 ± 0.01	0.011 ± 0.00
N-Limitation	1.03 ± 0.01	0.0 ± 0.0	0.09 ± 0	0.38 ± 0.02	6.31 ± 0.13	16.71 ± 1.25	1.39 ± 0.06	0.44 ± 0.02	0.007 ± 0.00

3.1.2 Cell retention system

Another strategy to increase biohydrogen production via dark fermentation was based on the implementation of an external cell retention system. In this case, due to the cellular stress applied on the cells once they left the bioreactor, no biomass concentration increase was observed. That involved no biohydrogen production increase in respect with a standard continuous culture (data under publication). Nevertheless, this strategy was useful to study the effects of process parameters on the metabolic responses of this strain. This fact makes possible the use of this strategy as a general platform to study the behaviour of (high density) pure cultures under different working conditions.

4 Biological conversion of waste streams to high value added products

The habitats of Halophiles, solar salterns and salt lakes, often turn bright pink or red due to halophilic microbial blooms due to their C_{40} and C_{50} carotenoid contents [12]. The industrial and commercial relevance of pure carotenoid compounds of natural origin is very high, according to the initiative to avoid the side-effects of synthetic food colorants. Carotenoids can be used not only as food colorants, but also as precursors for vitamin A synthesis. Moreover, carotenoids are playing an important role in the prevention of human diseases like cardiovascular diseases, osteoporosis and diabetes; moreover they are anticancer materials due to their protective function against oxidative stress [13]. Furthermore, human dietary guidelines recommend the consumption of fruits and vegetables based on their antioxidant phytochemicals for health prevention [14]. Some Archaea are even able to cope with high salinity and high alkalinity at the same time, due to their two extreme capacities, they were named Haloalkalophiles. Halophiles are able to grow on a wide variety of carbon sources and can survive up to saturated NaCl concentrations [15], which ensures inherent cultivation selectivity. The high salt concentration ensures low risk of contamination and the feasibility for cost-effective non-sterile bioprocesses for process intensification by coupling industrial streams and by converting the organic by-products in several kinds of waste streams to valuable halophilic bioproducts. Hence, non-sterile bioprocesses with Halophiles can exhibit a large potential for biotechnology. The biotechnological potentials of Halophiles are however still not entirely exploited, since reproducible as well as quantitative bioprocess development with Halophiles has to face the difficulties of the extremities of the highly saline environments.

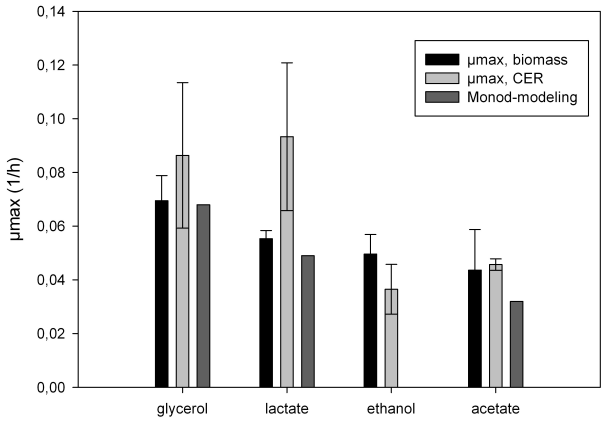
4.1 Methodological basis for bioprocess quantification with extreme halophiles

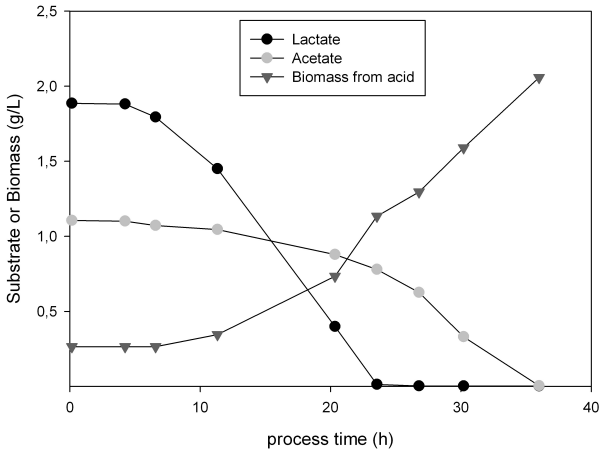
Our work was to establish on one hand a methodological basis for quantitative bioprocess analysis of extreme halophilic Archaea with an extreme halophilic strain as a generic platform [16, 17]. As a novel usage, firstly, a corrosion resistant bioreactor setup for extreme halophiles has been implemented. Then, on the other hand, with special attention to the total bioprocess quantification approaches, an indirect method for biomass quantification using on-line process signals was developed. Subsequently, providing defined and controlled cultivation conditions in the bioreactor and therefore obtaining suitable quality of on-line as well as off-line datasets, robust quantitative data evaluation methods for halophiles could be developed.

4.2 Physiological characterization of extreme halophiles in bioreactor

Based on the quantitative methodological tools, new physiological results of extreme halophiles in bioreactor have been also obtained in the corrosion resistant bioreactor [16]. For the first time, quantitative data on stoichiometry and the kinetics were collected and evaluated on different carbon sources. The used carbon sources may also have relevance since they are common residues in industrial waste streams. Batch and continuous experiments were carried out to investigate the stoichiometry and the kinetics on different carbon sources. With proposing metabolic mechanisms, the results on various substrates were interpreted by linking to the reported primary carbon metabolism of extreme halophilic

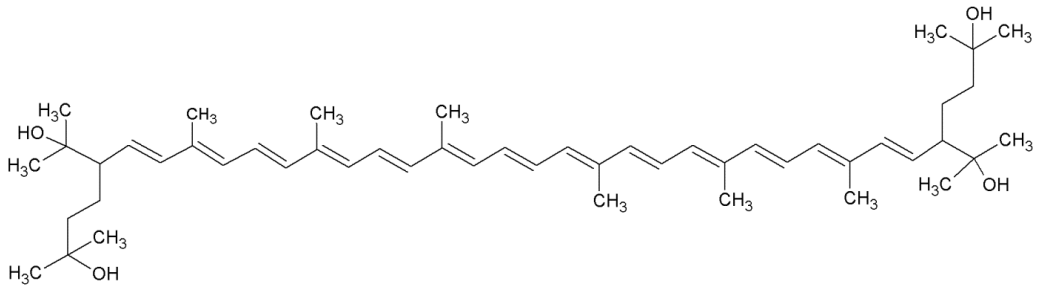
Archaea. Batch cultivations on single carbon sources showed exponential growth, while diauxic growth pattern could be observed on the combination of certain carbon sources. Results from chemostat continuous cultures also demonstrated that extreme halophilic organisms showed Monod-kinetics on different sole carbon sources.





4.3 Bioproduct portfolio analysis

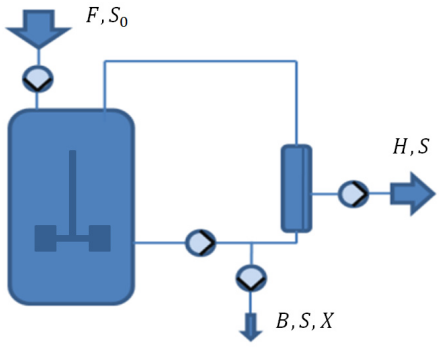
Extreme halophilic Archaea are known to produce a variety of lipophilic compounds which may also be valuable natural products with many possible applications from food colouring agents to anticancer materials. The main compound of the carotenoid content of extreme halophilic Archaea is bacterioruberin, an acyclic C_{50} carotenoid with four hydroxyl groups [18]. A method for identification and downstream processing of potentially valuable bioproducts produced by Archaea was developed [19]. To circumvent extreme salinities during analysis, a direct sample preparation method was established. Halogenated solvents, as used in conventional extraction methods, were omitted due to environmental considerations and potential process scale-up. The HPLC-MS/MS method using atmospheric pressure chemical ionization was developed. Polar lipids, the geometric isomers of the C_{50} carotenoid bacterioruberin and vitamin MK-8 were the most valuable products found in bioreactor samples.



4.4 Productivity increase

High biological activity and volumetric productivity are considered as prerequisites for efficient bioprocesses, extreme halophilic Archaea have, however, lower growth rates, for which reason halophilic Archaea are so far not used in industrial bioprocesses. To overcome this physiological limit and to achieve increased volumetric productivity, the produced

biomass must be retained in a bioreactor, for example equipped with an external cell retention system. In our work, the characterization and parameterization of a bioreactor setup with cell retention was carried out with an extreme halophilic strain. Bioprocess quantification was used to demonstrate the process controllability. Focussing on maximizing the volumetric productivity; 10-fold productivity increase was achieved compared to chemostat continuous cultures [20].



4.5 Studies on real-media

Exploiting the benefits of controlled bioprocessing of extreme Halophiles, two patents have been filed. One of them elaborates the waste water treatment capacity of Halophiles within the framework of an industrial collaboration at TU Wien and has been already registered. The other one, a patent application of TU Wien proposes the use of Halophiles for biological conversion of any waste streams with small metabolites to high value added products. Hence, the potential biotechnological applications with Halophiles can cover wide ranges of intelligent process intensification solutions.

5 Conclusions, Outlook

Although the 1st generation biofuels are well-established in the market, these biofuels derive from a feedstock that could also be used as food; hence, there is a competition between agriculture and fuel. For future sustainable biofuel production systems, the introduction of new biofuel generations is required with rationalizations,

- process intensification by not only performing energy integration but also coupling different streams in the framework of the biorefinery concept with “waste to value” principles
- more cost-effective non-sterile processes with inherent cultivation selectivity for extremophilic microorganisms
- Intelligent and flexible solutions for energy storage, “Power to gas” with biological methanogenesis.

As an outlook, microalgae from the 3rd biofuel generation biofuel are also being integrated as alternative renewable substrate sources within the “waste to value” concept.

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