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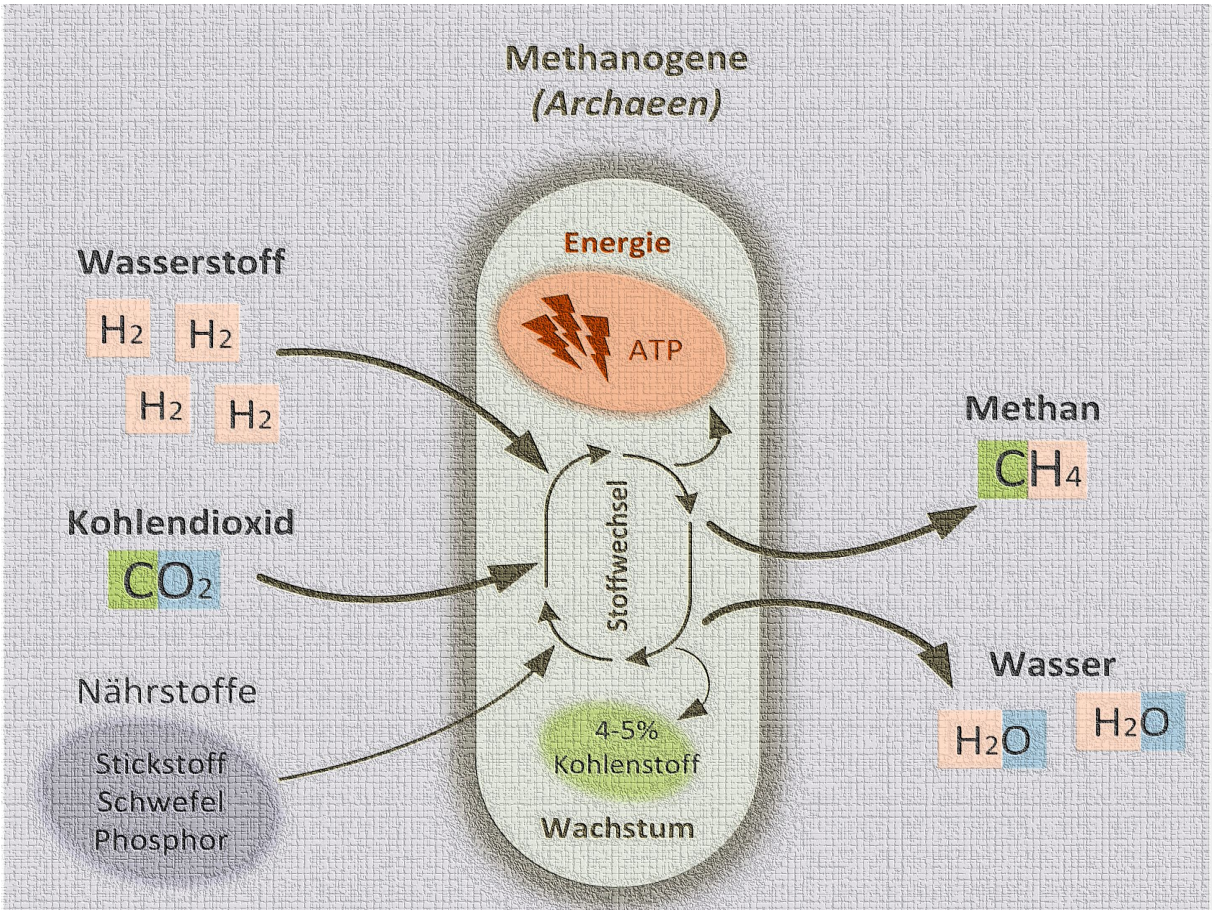
Project CarbonATE

Development of an Enzymatic CO_2 Sequestration Strategy for an Optimized Biological Methanation



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Zusammenfassung

Die Erzeugung von Strom aus erneuerbaren Energiequellen wie Wind- und Sonnenenergie wird in Europa stetig ausgebaut. Viele Mitgliedsstaaten nutzen diese Wege als eine Lösung zur Dekarbonisierung ihrer Primärenergiequellen. Laut dem letzten Fortschrittsbericht zu erneuerbaren Energien werden 26% des Stroms in der EU aus erneuerbaren Energien erzeugt, und etwa 10% des gesamten EU-Stroms stammt aus fluktuierenden erneuerbaren Energiequellen wie Wind- und Solarkraftwerken. In den letzten Jahren wurde die diskontinuierliche Erzeugung von Strom als Hindernis für die Etablierung dieser Systeme zur Energieerzeugung gesehen. Das Power-to-Gas-Konzept (P2G) bietet eine Möglichkeit, diese diskontinuierlich erzeugte Energie in Form von Wasserstoff (H_2) durch Elektrolyse von Wasser (H_2O) zu speichern. Für die Umwandlung der Gase können sowohl katalytische als auch biologische Verfahren eingesetzt werden. In einem weiteren Schritt wird dieser aus der Elektrolyse erzeugte H_2 für die Umwandlung von CO_2 zu Methan (CH_4) genutzt. Bei der biologischen Methanisierung erfolgt die Umwandlung von CO_2 und H_2 zu CH_4 im anaeroben Vergärungsprozesses über den Schritt der hydrogenotrophen Methanogenese, was einer der letzten Schritte im anaeroben Vergärungsprozess darstellt.

Das Verbundprojekt CarbonATE mit österreichischen und schweizerischen Projektpartnern umfasst verschiedene Zielsetzungen, um das Gesamtziel, der Erzeugung von nachhaltigen und erneuerbaren Energieträgern auf der Basis verschiedener Kohlenstoffquellen, zu erreichen. Das Projekt untersucht die enzymatische CO_2 Abscheidung mittels Formiatdehydrogenase und carbonischer Anhydrase zu flüssigen Produkten und die anschliessende Nutzung der Kohlenstoffverbindungen zu Methan durch methanogene Archaeen. Damit soll nicht nur das Rohstoffportfolio für die biotechnologische Nutzung erweitert werden, sondern auch das Zwischenprodukt für Konversionsprozesse bereitgestellt werden. In verschiedenen Reaktortypen und auch Skalierungsstufen werden die Untersuchungen mit Mikroorganismen durchgeführt, wobei Rein- und Mischkulturen verwendet werden. Von den Mischkulturen wird die Zusammensetzung des Mikrobioms in Abhängigkeit von verschiedenen Prozessbedingungen untersucht. Abschliessend erfolgt eine wirtschaftliche Analyse des Biomethanisierungsprozesses.

Die Ergebnisse der österreichischen Projektpartner einschliesslich der Erkenntnisse aus den Experimenten zur enzymatischen CO_2 Abscheidung durch Formiatdehydrogenase und carbonischer Anhydrase im Labormaßstab sind in einem separaten Abschlussbericht des österreichischen Konsortiums zu finden.

Die erste Aufgabe des Schweizer Konsortiums war die Auswahl von Medien für die Kultivierung von Archaeen. Dazu wurde eine Literaturstudie zur Kultivierung von Mischkulturen und Minimalmedien durchgeführt. Vier verschiedene Nährmedien wurden auf ihre Eignung für verschiedene Experimente untersucht. Von den vier getesteten Minimalmedien schnitt das Medium von Gerhard et al. (1993) bei allen fünf verschiedenen Inokula (zwei aus Biogasanlagen, zwei aus Kläranlagen und die Reinkultur *Methanobacterium formicicum*) am besten ab. Die Analyse der Gas- und Säureproduktion zeigte ähnliche Ergebnisse, während die Amplikon-Sequenzierung auf eine hohe Präsenz von Archaeen hinwies. Aus diesem Grund wurde dieses Wachstumsmedium anschliessend für alle kontinuierlichen Experimente in diesem Projekt verwendet.

Zur Charakterisierung des Bioprozesses wurden mehrere Versuche zur Kultivierung von Mischkulturen in kontinuierlichen Rührkesselreaktoren (CSTRs) und einem Rieselbettreaktor im Labormaßstab durchgeführt. Die Auswertung der chemischen Daten (Gaszusammensetzung, pH-Wert, flüchtige Fettsäuren) aus den CSTR-Versuchen zeigte, dass sich je nach Betriebsweise Inokulum aus Klärschlamm oder Gärrest schneller an die Betriebsbedingungen in den CSTRs anpassten. Darüber hinaus deutet die Produktion höherer Säurekonzentrationen auf eine stärkere Konkurrenz zwischen Archaeen und säurebildenden Bakterien hin, wenn Gärrest als Inokula verwendet wird. Die Aufrechterhaltung eines pH-Werts von 7 oder mehr ist für die Methanproduktion von entscheidender Bedeutung. Die ersten Versuche, die im Rieselbettreaktor durchgeführt wurden, zeigten vielversprechende Ergebnisse. Ohne

grossen Optimierungsaufwand konnten Methankonzentrationen von bis zu 90% erreicht werden. Aufgrund der langen Bauphase war es nicht möglich, den Prozess im Rieselbettreaktor zu optimieren. Der Einfluss von Temperatur und Druck auf den Methangehalt und die CH_4-Produktionsrate muss noch weiter evaluiert und durch zusätzliche Experimente gezeigt werden, nachdem sich ein stabiles Mischkulturkonsortium im Rieselbett etabliert hat.

Das wichtigste Ergebnis der mikrobiellen Untersuchungen in den Systemen ist, dass die Herkunft des Inokulums einen großen Einfluss auf das Mikrobiom hat, das sich in einem System etablieren kann. Es zeigte sich aber auch, dass sich die Kulturen in gleichen Systemen nicht identisch entwickeln. Anhand der 16S rRNA-Genanalysen konnte kein Konsens über die bakteriellen Konsortien in den verschiedenen Reaktortypen gefunden werden. Dies zeigt, dass der im Experiment verwendete Reaktortyp ein eigenes Ökosystem bildet, das durch die verschiedenen in den Reaktoren verwendeten Parameter beeinflusst wird. Im Gegensatz dazu, zeigte sich bei den Archaeen ein anderes Bild. Die Zusammensetzung der Archaeen wurde nach kurzer Zeit des Betriebs hauptsächlich von einer einzigen Gattung (*Methanobacterium*) oder von wenigen Gattungen (*Methanobacterium, Methanobrevibacter, Methanospirillum* und/oder *Methanoculleus*) dominiert, was auf den hohen Selektionsdruck zurückzuführen ist, wenn nur H_2 und CO_2 als einzige Energiequellen zur Verfügung stehen. Da die im Rahmen dieses Projekts gewählten Wachstumsmedien spezifisch für Methananogene waren, ist der Verlust anderer Archaeen-Gattungen zu erwarten. Diese Ergebnisse zeigen, dass die Art des Inokulums (Biogasanlage oder Kläranlage) für den Betrieb der biologischen Methanisierung im grossen Massstab entscheidend sein wird, da sie die Bakterien- und Archaeengemeinschaft in unterschiedlichem Masse beeinflusst.

Abschliessend zeigten die Ergebnisse der technisch-wirtschaftlichen Analyse auf der Grundlage der aktuellen wirtschaftlichen (vor 2022) und technologischen Bedingungen, dass die CO_2-Methanisierung mit der katalytischen Wirbelschichttechnologie im Vergleich zu biologischen Methanisierung in Rührkesseln am kostengünstigsten ist. Ausgehend von Stromkosten von 5 €-ct/kWh_{el} und einer Anlagengröße von 6 MW_{el} konnten Biomethan-Produktionskosten von 13.95 €-ct/kWh für die katalytische und 17.30 €-ct/kWh für die biologische Methanisierung ermittelt werden, jeweils einschließlich PEM-Elektrolyse. Bei der katalytischen Methanisierung wird im Vergleich zur biologischen Methanisierung in einem Rührkessel weniger als ein Drittel des Reaktorvolumens benötigt. Ein wichtiger Kostenfaktor bei der biologischen Methanisierung sind die Kosten für die Nährstoffzufuhr, wenn keine Nährstoffrückgewinnung eingesetzt wird. Die Verwendung natürlicher Medien aus dem Gärrest von Biogasanlagen oder dem anaeroben Schlamm von Kläranlagen könnte eine Möglichkeit sein, diese Kosten in Zukunft zu senken. Weitere Untersuchungen sollten durchgeführt werden, um Daten aus der biologischen Methanisierung mit einem im Pilotmaßstab betriebenen Rieselbettreaktorsystem zu erhalten. Die in der technisch-wirtschaftliche Analyse für die biologische Methanisierung verwendeten Daten basierten ausschliesslich auf Rührkessel-Pilotanlagen und nicht auf Rieselbettreaktorsystemen.

Das Projekt zeigte, das die enzymatische CO_2-Abscheidung durch Formiatdehydrogenase und carbonischer Anhydrase zu flüssigen Produkten und die anschliessende Verwertung der Kohlenstoffverbindungen zu Methan durch methanogene Archaeen, die Biomethanproduktion optimieren kann. Die vielversprechenden Ergebnisse zeigten, dass diese Technologie ein großes Potenzial für die Erzeugung von hochkalorischem Gas vor Ort auch in kleineren Anlagen zukünftig haben wird. Darüber hinaus könnten die Kosten durch die Verwendung natürlicher Medien in Zukunft erheblich gesenkt werden. Darüber hinaus kann das produzierte Gas in die Netze eingespeist oder im Transportsektor verwendet werden. Zusammenfassend lässt sich sagen, dass die Biomethanisierung sowohl aus ökonomischer als auch aus ökologischer Sicht ein interessantes Verfahren für eine nachhaltige und unabhängige Energieversorgung der Schweiz darstellt.

Résumé

La production d'électricité à partir de sources d'énergie renouvelables, telles que l'énergie éolienne et solaire, est en augmentation constante en Europe. De nombreux États membres utilisent ces technologies pour décarboniser leurs sources d'énergie primaire. Selon le dernier rapport d'avancement sur les énergies renouvelables, 26% de l'électricité de l'UE est produite à partir de sources d'énergie renouvelables. Environ 10% de l'électricité totale de l'UE provient de sources d'énergie renouvelables fluctuantes, telles que les centrales éoliennes et solaires. Ces dernières années, cette production discontinue d'électricité a été considérée comme un obstacle à l'établissement de ces systèmes de production d'énergie renouvelable. Le concept Power-to-Gas (P2G) offre une possibilité de stocker cette énergie sous forme d'hydrogène (H_2) par l'électrolyse de l'eau (H_2O). Pour la conversion des gaz, il est possible d'utiliser des procédés catalytiques et biologiques. Dans une étape ultérieure, ce H_2 produit par l'électrolyse est utilisé pour la transformation du CO_2 en méthane (CH_4). Dans le cas de la méthanisation biologique, la transformation du CO_2 et du H_2 en CH_4 s'effectue dans le processus de fermentation anaérobie par l'intermédiaire de l'étape de méthanogenèse hydrogénotrophe, qui constitue l'une des dernières étapes du processus de fermentation anaérobie.

Le projet CarbonATE, qui réunit des partenaires autrichiens et suisses, poursuit différents objectifs afin d'atteindre l'objectif global de production d'énergie durable et renouvelable à partir de différentes sources de carbone. Le projet étudie la séparation enzymatique de CO_2 au moyen de la formiate déshydrogénase et de l'anhydrase carbonique, générant un produit liquide composé de carbonés qui sert à la production du méthane généré par des archées méthanogènes. Cela doit non seulement permettre d'élargir le portefeuille de matières premières pour une utilisation biotechnologique, mais aussi de fournir le produit intermédiaire pour les processus de conversion. Dans différents types de réacteurs et d'études d'échelle, les analyses sont effectuées avec des microorganismes, en utilisant tant des cultures pures que des cultures mixtes. La composition du microbiome des cultures mixtes sera étudiée en fonction de différentes conditions de processus. Enfin, une analyse économique du processus de biométhanisation sera effectuée.

Les résultats des partenaires autrichiens du projet, y compris les résultats des expériences sur la capture enzymatique du CO_2 par la formiate déshydrogénase et l'anhydrase carbonique à l'échelle du laboratoire, sont présentés dans un rapport final par le consortium autrichien.

La première tâche du consortium suisse était de sélectionner des milieux pour la culture d'archées. Pour ce faire, une étude bibliographique a été menée sur la culture de cultures mixtes et de milieux nutritifs minimaux. Quatre milieux de cultures différents ont été examinés pour déterminer leur aptitude à différentes expériences. Parmi les quatre milieux minimaux testés, le milieu de Gerhard et al. (1993) a obtenu les meilleurs résultats pour les cinq inocula différents (deux provenant d'installations de biogaz, deux provenant de stations d'épuration et la culture pure provenant du *Methanobacterium formicicum*). L'analyse de la production de gaz et d'acides a donné des résultats similaires, tandis que le séquençage des amplicons a révélé une forte présence d'archées. C'est pourquoi ce milieu de croissance a été utilisé pour toutes les expériences continues de ce projet.

Pour caractériser le bio processus, plusieurs expériences de cultures mixtes ont été réalisées à l'échelle du laboratoire dans des réacteurs à cuve agitée en continu (CSTR) et dans un réacteur à lit fluidisé. L'évaluation des données chimiques (composition des gaz, pH, acides gras volatils) des essais CSTR a montré que, selon le mode de fonctionnement, l'inoculum des boues d'épuration ou du digestat s'adaptait plus rapidement aux conditions de fonctionnement dans les CSTRs. En outre, la production de concentrations d'acide plus élevées indique une plus grande concurrence entre les archées et les bactéries acidogènes lorsque les digestats sont utilisés comme inoculum. Le maintien d'un pH de 7 ou plus est essentiel pour la production de méthane. Les premiers essais réalisés dans le réacteur à lit fluidisé ont donné des résultats prometteurs. Des concentrations de méthane jusqu'à 90% ont pu être atteintes

sans grand effort d'optimisation. A cause de la longue phase de construction, il n'a pas été possible d'optimiser le processus dans le réacteur à lit fluidisé. L'influence de la température et de la pression sur la teneur en méthane et le taux de production de CH_4 doit encore être évaluée et démontrée par des expériences supplémentaires, une fois qu'un consortium stable de cultures mixtes a été établi dans le lit fluidisé.

Le résultat principal des analyses microbiennes dans les systèmes est que l'origine de l'inoculum a une grande influence sur le microbiome qui peut s'établir dans un système. Mais il s'est également avéré que les cultures ne se développent pas de manière identique dans les mêmes systèmes. Les analyses génétiques de l'ARNr 16S n'ont pas permis de trouver un consensus sur les consortiums bactériens dans les différents types de réacteurs. Cela montre que le type de réacteur utilisé dans l'expérience constitue un écosystème propre, qui est influencé par les différents paramètres utilisés dans les réacteurs. En revanche, la situation est différente pour les archées. Après peu de temps, la composition des archées était principalement dominée par un seul genre (*Methanobacterium*) ou par quelques genres (*Methanobacterium*, *Methanobrevibacter*, *Methanospirillum* et/ou *Methanoculleus*), ce qui est attribuéà la forte pression de sélection lorsque les seules sources d'énergie disponibles sont le H_2 et le CO_2. Comme les milieux de croissance choisis dans le cadre de ce projet étaient adaptés aux méthanogènes, on peut s'attendre à la perte d'autres genres d'archées. Ces résultats montrent que le type d'inoculum (installation de biogaz ou station d'épuration) sera déterminant pour le fonctionnement de la méthanisation biologique à grande échelle, car il influence la communauté de bactéries et d'archées dans une mesure différente.

En conclusion, les résultats de l'analyse technico-économique basée sur les conditions économiques (avant 2022) et les technologiques actuelles ont montré que la méthanisation du CO_2 par la technologie du lit fluidisé catalytique est la plus rentable par rapport à la méthanisation biologique dans des réacteurs agitées. En partant d'un coût d'électricité de 5 €/ct/kWh_{el} et d'une taille d'installation de 6 MW_{el}, il a été possible de déterminer un coût de production du biométhane de 13,95 €/ct/kWh pour la méthanisation catalytique et de 17,30 €/ct/kWh pour la méthanisation biologique, y compris l'électrolyse PEM. La méthanisation catalytique nécessite moins d'un tiers du volume du réacteur par rapport à la méthanisation biologique dans une cuve agitée. Un facteur de coût important dans la méthanisation biologique est le coût des nutriments si les nutriments ne sont pas récuperés. L'utilisation de milieux naturels provenant du digestat des installations de biogaz ou des boues anaérobies des stations d'épuration pourrait être une solution pour réduire ces coûts à l'avenir. Des études supplémentaires devraient être menées afin d'obtenir des données sur la méthanisation biologique avec un système de réacteur à lit fluidisé à l'échelle pilote. Les données utilisées dans l'analyse technico-économique pour la méthanisation biologique étaient exclusivement basées sur des installations pilotes à cuve agitée et pas sur des systèmes de réacteur à lit fluidisé.

Le projet a montré que la capture enzymatique du CO_2 par la formiate déshydrogénase et l'anhydrase carbonique générant des produits liquideset l'utilisation de ces composés carbonés par des archées méthanogènes générant du méthane, peut optimiser la production de biométhane. Les résultats prometteurs ont montré que cette technologie aura un grand potentiel pour la production de gaz hautement calorique sur place à l'avenir, même dans de petites installations. En outre, les coûts pourraient être considérablement réduits grâce à l'utilisation de milieux naturels. En outre, le gaz produit peut-être injecté dans les réseaux ou utilisé dans le secteur des transports. En résumé, la biométhanisation représente un procédé intéressant pour un approvisionnement énergétique durable et indépendant de la Suisse, tant du point de vue économique qu'écologique.

Summary

The production of renewable energy from wind and solar energy sources is being continuously expanded in Europe. Many EU member states are already using these types of renewables for the decarbonization of their primary energy consumption. According to the most recent progress report on renewable energy 26% of electrical power in the EU comes from renewable energies, while 10% thereof comes from fluctuating renewable energy sources like wind turbines and photovoltaics. In the past years, the fluctuations and discontinuity of this type of power production was an obstacle for the further implementation of wind and solar power into the energy system. With the Power-to-Gas (P2G) concept, it is possible to store the discontinuously produced energy in the form of hydrogen (H_2) by the use of electrolysis. In a further step, this H_2 generated from electrolysis is used to convert CO_2 to methane (CH_4). For the conversion of the gases, catalytic as well as biological processes can be used. With biological methanization, H_2 and CO_2 is converted to CH_4 by hydrogenotrophic methanogenesis, which is one of the final steps in the anaerobic fermentation process.

The collaborative project CarbonATE with Austrian and Swiss project partners included several objectives to achieve the overall goal of producing sustainable and renewable energy based on different carbon sources. The project investigates the enzymatic CO_2 capture by formate dehydrogenase and carbonic anhydrase to liquid products and the subsequent utilization of the carbon compounds to methane by methanogenic archaea. This is intended not only to expand the raw material portfolio for biotechnological use, but also to provide the intermediate for conversion processes. In different types of reactors and also scaling stages, the studies are carried out with microorganisms, investigating pure and mixed cultures. Of the mixed cultures, the composition of the microbiome is studied depending on different process conditions. Finally, the biomethanation process is highlighted by an economic analysis.

The results of the Austrian project partners including the findings of experiments to enzymatic CO_2 capture by formate dehydrogenase and carbonic anhydrase in lab scale can be found in a separate final report of the Austrian consortium.

The first task for the Swiss consortium was the selection of media for cultivation of archaea. Therefore, a literature study on cultivation of mixed cultures and minimal media was conducted. Four different nutrient media were evaluated to be suitable for different experiments. From the four tested minimal media, the medium of Gerhard et al. (1993) had the best performance with all the five different inocula (two from biogas plants, two from wastewater plants and the pure culture *Methanobacterium formicicum*). The analysis of the gas and acid production showed similar results, whereas amplicon sequencing indicated a good presence of the archaea. It was assumed that this growth medium should be used for all continuous experiments in this project.

For bioprocess characterization, the cultivation of mixed cultures in continuous stirred tank reactors (CSTRs) and a trickle-bed reactor on a laboratory scale were carried out in several experiments. The analysis of the chemical data (gas composition, pH value, volatile fatty acids) from CSTR experiments showed that, depending on the operation mode, WWTP sludge or BGP inocula adapted faster to the operating conditions in the CSTRs. Furthermore, the production of higher acid concentrations indicating higher competition between archaea and acid producing bacteria, using inocula from BGP. The maintenance of the pH value around 7 or more is crucial for methane production. The preliminary experiments, performed in the trickle-bed bioreactor, showed promising results. Methane concentrations up to 90% could be reached without large optimization effort. Due to the long construction phase, it was not possible to optimize the process in the trickle-bed bioreactor. The effect of temperature and pressure on methane content and CH_4 flowrate still must be further evaluated and shown by additional experiments, having a stable mixed culture consortium in the trickle-bed.

The main finding to the microbial consortia in the systems indicated that the origin of the inoculum has a major influence on the microbiome that can establish in a system. However, it was also shown that the cultures in identical systems afterwards also did not develop similarly. Based on the 16S rRNA gene analyses, no consensus could be found on the bacterial consortia in the different reactor types. This shows that the type of reactor used in the experiment is creating an ecosystem on its own, that is influenced by different parameters used in the reactors. In contrast, a different picture is observed with Archaea, as the Archaeal composition was, after short time during operation, mainly dominated by a single genus (*Methanobacterium*) or by few genera (*Methanobacterium, Methanobrevibacter, Methanospirillum* and/or *Methanoculleus*), based on the selection pressure to grow with H_2 and CO_2 as sole energy sources. As the growth media chosen during this project were specific for methanogens, the loss of other Archaeal genera can be expected. This information shows that the type of inoculum (biogas plant or wastewater treatment plant) will be crucial for operating biological methanation in full scale, as it influences the bacterial and archeal community to different extends.

Finally, the results from the techno-economic analysis based on current economic (before 2022) and technological circumstances, showed CO_2 methanation using the catalytic bubbling fluidized bed (BFB) technology is the most cost effective compared to biological methane ins CSTR systems. Based on electricity costs of 5 €-ct/kWh_{el} and a plant size of 6 MW_{el}, biomethane production costs of 13.95 €-ct./kWh for catalytic and 17.30 €-ct./kWh for biological methanation could be obtained, both including PEM electrolysis. Using a catalytic methanation, less than a third of the reactor volume is required compared to biological methanation in a CSTR. A major cost factor in the biological methanation process could be identified in the expenses related to the nutrients supply, if no nutrient recovery is applied. The use of natural media from biogas plant digestate or wastewater treatment plant anaerobic sludge could be one chance to reduce these costs in future. Further investigation should be conducted to provide data from biological methanation with a trickle-bed reactor system, operated at pilot scale. The used data in the techno-economic analysis for biological methanation were based only on CSTR pilot plants and not on trickle-bed reactor systems.

The project revealed the enzymatic CO_2 capture by formate dehydrogenase and carbonic anhydrase to liquid products and the subsequent utilization of the carbon compounds to methane by methanogenic archaea, optimizing biomethanation production. The promising results showed that this technology has great potential in producing on-site high calorific gas also in smaller units. In addition, the costs might be significantly reduced, due to the usage of natural media in future. Furthermore, the produced gas can be injected into the grids or can be used in the transportation sector. To summarize, the biomethanation is an interesting process for a sustainable and independent energy supply system in Switzerland in both economic and ecological perspectives.

Main findings

In the following there are four bullet points listing the main findings to be obtained from this project and the added value it could generate in terms of Switzerland's energy policy:

- *Media selection for archaea cultivation*

The findings from the literature research and selection of suitable culture media for the cultivation of methanogens (archaea) showed effects on different parameters during the BATCH experiments. From the four tested minimal media, the medium of Gerhard et al. had the best performing with all the inocula. By reducing the volume of required media by using minimal media, the costs for biological methanation can be significant reduced, making it a valuable process for Swiss energy systems in future.

- *Bioprocess characterization*

The cultivation of mixed cultures in stirred tanks (CSTR) and a trickle-bed reactor on a laboratory scale could be proven in several experiments. Especially the findings of the preliminary experiments, performed in the trickle-bed bioreactor, showed great success and a promising technology, reaching methane concentrations of more than 90%. Still, optimization experiments with different temperatures, pressures, flow rates, trickling intensities and media supply are needed for improved operation at pilot scale. With the characterization in lab scale the first step was reached for a promising technology in future Swiss energy systems.

- *Microbial Analysis*

The main finding of the microbial consortia is that one large parameter that influences the microbiome is the origin of the inoculum. This is obvious from the different experiments, as the microbial consortia obtained from the two used inoculation sources were significantly different in its structure. The cultures in the systems afterwards also did not develop similarly. This information shows that, in future, the type of inoculum (biogas plant or wastewater treatment plant) will be crucial for operating biological methanation in full scale in Swiss energy systems.

- *Techno-economic Analysis*

Under current economic and technological circumstances, CO_2 methanation using the catalytic bubbling fluidized bed (BFB) technology is the most cost effective compared to biological methane ins CSTR systems. Based on electricity costs of 5 €-ct/kWh_{el} (before 2022) and a plant size of 6 MW_{el}, biomethane production costs of 13.95 €-ct./kWh for catalytic and 17.30 €-ct./kWh for biological methanation could be obtained, both including PEM electrolysis. A major cost factor in the biological methanation process could be identified in the expenses related to the nutrients supply, if no nutrient recovery is applied. With the use of natural media from biogas plant digestate or wastewater treatment plant anaerobic sludge, the cost can be reduced in future. By using renewable CO_2 and surplus energy, a CO_2 neutral biomethane for injection can be produced, offering the possibility to shift energy from summer into winter, making the Swiss energy supply more independent.

Contents

1 Introduction

1.1 Background information and current situation

The production of renewable energy from wind and solar energy sources is being continuously expanded in Europe. Many EU member states are already using these types of renewables for the decarbonization of their primary energy consumption. According to the most recent progress report on renewable energy 26% of electrical power in the EU comes from renewable energies, while 10% thereof comes from fluctuating renewable energy sources like wind turbines and photovoltaics. In the past years the fluctuations and discontinuity of this type of power production was an obstacle for the further implementation of wind and solar power into the energy system. With the Power-to-Gas (P2G) concept it is possible to store the discontinuously produced energy in the form of hydrogen (H_2) by the use of electrolysis. In recent years technologies were developed for the conversion of H_2 and carbon dioxide (CO_2) into methane (CH_4), as the latter has a higher volumetric energy density and is also easier to handle in the gas grid. These aspects are particularly of great relevance when it comes to a seasonal long-term storage of energy as the expansion of wind turbines and photovoltaics will lead to temporary overproduction of electrical energy in summer. For the conversion of the gases, catalytic as well as biological processes can be used. With biological methanization, H_2 and CO_2 is converted to CH_4 by hydrogenotrophic methanogenesis, which is one of the final steps in the anaerobic fermentation process (Thauer et al., 1977). As the process only works under strict anaerobic conditions, industrial exhaust gas streams, which can potentially be used as a CO_2 source, therefore have a limited applicability due to the possible occurrence of remaining O_2 in the gas. The purification of CO_2 is costly and energy demanding. With the use of an enzymatic CO_2 sequestration process, exhaust gases (e.g., from biomass power plants or CHP units) might be used in the future for the biological methanization while having a low energy demand. With this approach biomass can be used as a source for CO_2 which allows the recycling of the same in the environment. Efficient P2G systems are mandatory for the reduction of fossil fuels and will empower the role of renewable energies in the future European energy system.

1.2 Purpose of the project

1.2.1 Enzymatic CO_2 Sequestration

Carbonic anhydrases (CAs, EC 4.2.1.1, Figure 1) are mostly zinc-dependent metalloenzymes that catalyze the simple but physiologically crucial reversible hydration of gaseous CO_2 to carbonic acid, which dissolves to hydrogen carbonate and protons (Boone et al., 2013). This balance contributes to a number of physiological functions that include the production, transport and consumption of CO_2, H^+ and HCO_3^- The catalytic domain of CAs consists of a tetrahedral Zn^{2+} cation bound to three imidazole residues derived from histidine. CO_2 is a key metabolite in all living organisms. However, CO_2 can freely diffuse into and out of the cell. In order to bind the CO_2 in the cell, it is converted to bicarbonate by carbonic anhydrases. The fast kinetic properties, the high solubility and the ability to overexpress it in E. coli made CAs very attractive for medical and industrial applications (Frost & McKenna, 2014). One of the most promising applications is the CA-mediated sequestration of CO_2 from various sources in the form of carbonate, as aimed in the CarbonATE project.

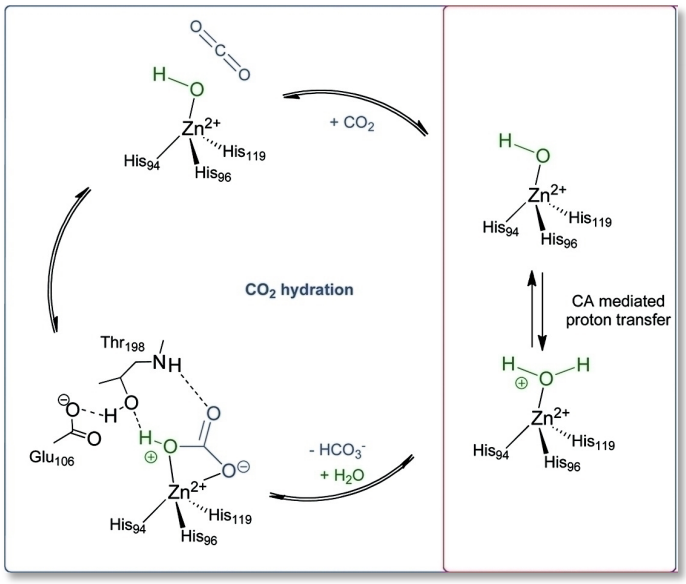

Figure 1: Catalytic function of carbonic anhydrases CA [$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+$] (Lopez et al., 2011).

Formate dehydrogenases (FDHs, *Figure 2*) catalyze the interconversion of CO_2 and formic acid through an oxidoreductive process (Alissandratos et al., 2013). The ability to reduce CO_2 to formic acid has made these enzymes promising candidates for CO_2 sequestration. Acetogens in particular are known to have FDHs that participate in a carbon fixation pathway that produces acetate, the first step being the reduction of CO_2 to formate. Several FDHs are known that catalyze the CO_2 reduction. FDHs from acetogenic and related anaerobic microorganisms are excellent biocatalysts, but very sensitive to oxygen. However, it has recently become possible to express the NAD^+-dependent formate dehydrogenase from *Clostridium carboxidivorans* strain P7T under aerobic conditions by means of autoinduction. The recombinant enzyme was expressed in a soluble form in high yields and showed promising activities for the reduction of CO_2.

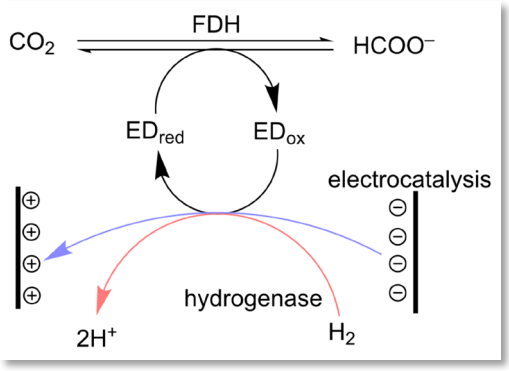

Figure 2: Catalytic function of formate dehydrogenases FDHs (Alissandratos & Easton, 2015).

1.2.2 Microbiological Methanization Process

There are two concepts that can be used for the biological methanization – (a) in-situ methanization and (b) ex-situ methanization. With the in-situ method pure H_2 or hydrogen rich gases is fed into a biogas reactor (Luo & Angelidaki, 2013; Youngsukkasem et al., 2015). CO_2 which is also a side product of the fermentation process can therefore be used directly for the methanization in the reactor, allowing for a higher methane content in the product gas. Disadvantages of this method is the dependency of the available CO_2 from the fermentation process, which poses high demands on controlling the H_2 injection. H_2 can also inhibit the acetogenesis process step which converts propionic and butyric acids into acetic acid, as this process requires a low partial pressure of H_2 to be thermodynamically feasible. With a high partial pressure of H_2 volatile fatty acids can accumulate, leading to instability in the process (Wandrey & Aivasidis, 1983). Ex-situ systems show higher production rates. For the methanization with the ex-situ method, continuous stirred tank reactors (CSTRs) or trickle-bed reactors are used. Rittmann et al., 2015 measured high production rates in a CSTR using monocultures. Trickle-bed reactors were successfully used with mixed cultures (Rachbauer et al., 2017) and present a promising technology for biological methanization.

For the different setups, e.g., pure CO_2, biogas or gasification gas was used as a carbon source. However, the influence of impurities (e.g., tars in the synthesis gas) has not yet been investigated. In addition, the enzymatic pretreatment of the gas and CO_2 fixation have not yet been analyzed.

1.2.3 Analysis of the Microbiomes

Little is currently known about the microbial communities in both reactor types, in contrast to CSTR with polymeric material (Weiland, 2010). There are already various studies regarding the potential or the use of pure cultures of methanogens (Lecker et al., 2017), but an analysis of the community structure is necessary. The use of next generation sequencing (NGS) technologies, either as 16S rRNA gene amplicon sequencing or as total DNA metagenomics in combination with deep sequencing technologies, enables a detailed community analysis.

1.3 Objectives

The collaborative project with Austrian and Swiss project partners included several objectives to achieve the overall goal of producing sustainable and renewable energy based on different carbon sources.

The objectives of the Swiss project partners of the ERA-NET project CarbonATE were as following:

Subgoal 1: *Media selection for archaea cultivation*

Literature research and selection of suitable culture media for the cultivation of methanogens (archaea). Characterization of biomass growth, monitoring of substrate consumption and product formation when using the different culture media.

Subgoal 2**:** *Bioprocess characterization*

Cultivation of mixed cultures in stirred tanks (CSTR) and a trickle-bed reactor on a laboratory scale. Evaluation of key bioprocess parameters and growth conditions. Definition of parameters for the technical up-scaling.

Subgoal 3: *Microbiome analysis*

A detailed knowledge of the microbial consortia performing the methanation process is crucial to determine the key players. For this purpose, a 16S rRNA gene amplicon sequencing approach is used. To understand the biochemical pathways involved in methanation, a total DNA metagenome analysis will

allow the observation of microbial potential and the identification of key microbes that are particularly abundant.

Subgoal 4: *Process evaluation*

Techno-economic benchmarking and comparison of the desired process with state-of-the-art concepts for methane production from renewable electricity and CO_2 (e.g., catalytic methanation) is targeted.

2 Main conclusions

2.1 Media selection for archaea cultivation

The findings in the literature research and selection of suitable culture media for the cultivation of methanogens (archaea) showed effects on different parameters during the BATCH experiments. From the four tested minimal media, the medium of Gerhard et al. had the best performance with all inocula. The analysis of the gas and acid production had similar results, as well as the amplicon sequencing observed a good growth of the archaea during enrichment cultivation run (L1) and sub cultivation run (L2). It was assumed that this growth medium should be used for all continuous experiments in this project.

2.2 Bioprocess characterization

The cultivation of mixed cultures in continuous stirred tank reactors (CSTRs) and a trickle-bed reactor on a laboratory scale could be proven in several experiments.

Unfortunately, the first experiment with the CSTRs (KIDDIEs) was too short to draw any meaningful conclusions. Additionally, since the gas was distributed from a dosing unit (MFCs) to three reactors via a pipe, it was attempted to adjust the gas flows in the individual reactors as evenly as possible (as described in chapter 2.4.2). Nevertheless, the reactors KID 1-3 and KID 4-6 were not perfect triplicates, since certain deviations in the gas flow were unavoidable. Due to the same reason, it was also not possible to measure the volume flow of each reactor. Since the use of a bubble column (for the accurate measurement of the volume flow) changed the back pressure in the pipes and the reactor, no corresponding measurements were possible. Therefore, the absolute amount of produced methane could not be determined. Furthermore, the limitation of the usage of H_2 and CO_2 only 8 hours per day, caused limitation in growing for mixed culture, resulting in longer adaption time.

In the second experiment in the CSTRs, all challenges were fixed (equal distribution of the input gases to each reactor, feeding of H_2 and CO_2 24 hours per day) and promising results were achieved.

The BATCH experiments showed the influence of the used media, leading to the usage of the medium from Gerhard et al in all continuous trials. The analysis of the chemical data (gas composition, pH value, volatile fatty acids) from the second experiment showed that BGP inocula adapted faster to the operating conditions (37°C, 250 rpm, H_2: 16 mL/min, CO_2: 4 mL/min) in the CSTR experiments. Furthermore, the production of higher acid concentrations indicating higher competition between archaea and acid producing bacteria, using inocula from BGP. The maintaining of pH value around 7 or more is crucial for methane production.

The preliminary experiments performed in the MyLady trickle-bed bioreactor indicated a great success at the end. Methane concentrations up to 90% could be reached without any large optimization (47°C; 1.03 bar; H_2: 43 mL/min, CO_2: 11 mL/min; pH: 7.5) efforts. Due to the long construction phase, it was not possible to also perform the optimization of the trickle-bed bioreactor. The effect of temperature (33-35°C in 1 and 2; 45-47°C in 3 and 4) and pressure (1.04 bar in 1, 2 and 4; 3 bar in 3) on methane content and CH_4 flowrate still must be further evaluated by additional experiments, in which only one parameter at the time is varied but only after having reached a stable culture consortium in the trickle-bed.

2.3 Metagenome Analysis

2.3.1 Primer Selection

Based on the *in silico* analysis, we have selected the best archaea primer set suited for Illumina sequencing. This primer pair covers the v4 region in the 16S rRNA gene, for which the largest data amount is available (Tahon et al., 2021). The largest challenge was here to reduce the potential of co-amplifying bacterial 16S rRNA genes. Nevertheless, amplification using adapted primers gave the most optimal amplification of archaea but not of bacteria.

The first set of community analysis data showed that, although a thorough selection, the primers for archaea did amplify a low number of bacterial genes as well in three samples. We expect that these samples contain a very low number of archaea. On the other hand, we also observed archaeal reads in the data set for bacteria, indicating that their relative number must be in a similar range as that of the total bacteria. It should be reminded that the primer set used for bacteria is rather universal, also giving the chance to amplify archaeal templates. As in a natural sample, the number of archaea is several orders of magnitude smaller than that of bacteria, one would not observe too many archaeal reads.

2.3.2 Sequencing

Based on the 16S rRNA gene microbiomes, no consensus can be found on the bacterial consortia in the different reactor types. Even between runs in similar systems, differences were observed. This shows that the type of reactor used in the experiment is creating an ecosystem on its own, that is influenced by different parameters used in the reactors.

One large parameter that influences the microbiome is the origin of different inocula. This is obvious from the experiments with the BATCH or KIDDIES, as the consortia obtained from the two used inoculation sources were significantly different in its structure. The cultures in these systems afterwards also did not develop similarly. It is thus expected that the composition with individual members of the microbiome in the inoculation source is not overlapping, not allowing a similar development. Equally large were the differences between samples from Austria or Switzerland. A different picture is observed with Archaea, as the Archaeal composition was, after short time during operation, mainly dominated by a single genus (*Methanobacterium*) or by few genera (*Methanobacterium, Methanobrevibacter, Methanospirillum* and/or *Methanoculleus*). As the growth media chosen during this project were specific for methanogens, the loss of other Archaeal genera can be expected.

The whole metagenome analysis delivered up to now similar indications as obtained from the amplicon sequencing. Although there is still a lot to be done on the functional analysis of the metagenome data, the first conclusions show that there may be a link between function and obtained sequences, although this has still to be proven (Dyksma et al., 2020; Li et al., 2022). Additionally, it should be remarked that enrichment experiment like these may yield better results as when soil samples are analyzed by these methods, as soil is a matrix for extreme diverse microbiomes. Sequencing of a full soil microbiome may even not be possible (Nesme et al., 2016). However, it also needs to be stated that the analysis method needs a lot of high-quality data. The sequencing of only one single sample in a full MiSeq run was in this case a strategically well-chosen step to obtain sufficient data.

2.4 Techno-economic Analysis

Under current economic and technological circumstances, CO_2 methanation using the catalytic bubbling fluidized bed (BFB) technology is the most cost effective compared to biological methane ins CSTR systems. Based on electricity costs of 5 €-ct/kWh_{el} and a plant size of 6 MW_{el}, biomethane production costs of 13.95 €-ct./kWh for catalytic and 17.30 €-ct./kWh for biological methanation could be obtained,

both including PEM electrolysis. Using a catalytic methanation, less than a third of the reactor volumes is required compared to biological methanation in a CSTR. A major cost factor in the biological methanation process could be identified in the expenses related to the nutrien supply if no nutrient recovery is applied. The use of natural media from biogas plant digestate or wastewater treatment plant anaerobic sludge could be one potential chance to reduce these costs in future. Further investigation should be done to provide data from a biological methanation with a trickle-bed reactor system, operated at pilot scale. The used data in the techno-economic analysis for biological methanation were based only on CSTR pilot plants and not on trickle-bed reactor systems.

3 Outlook and next steps

3.1 Bioprocess characterization

The trickle-bed bioreactor system will be further investigated in future. By the end of this project the whole measurement equipment was optimized for further research. There are still fields to improve the whole system. Further experiment with different temperatures, pressures, flow rates, trickling intensities and media supply are needed for operating in pilot scale.

3.2 Metagenome Analysis

The whole metagenome data will be further analyzed as described in the pipeline (Pérez-Cobas et al., 2020; Yang et al., 2021). This may still require some major effort. It is planned to summarize the data from BATCH and KIDDIES in a single publication before the end of 2023, while the data from the whole metagenome will represent another publication by the beginning of 2024. Furthermore, results from the trickle-bed experiments are planned to publish by beginning of 2024. For the publications, the collaboration with ZHAW-ICBT is required for the description of the different systems.

4 National and international cooperation

In this ERA-NET project there was a close cooperation between the Swiss and the Austrian project partners. Due to the COVID-19 pandemic situation, the planned meetings in person and personal exchange could only take place to a limited extent and was mainly online. For the experiment different samples were exchanged by the Swiss and Austrian consortia.

The project was presented on several conferences which took place in Switzerland, Germany, Poland and Italy. By this, the network with universities and companies in other countries could be expanded.

Furthermore, an online workshop series on the topic of biocarbon capture was organized in cooperation with the Austrian project partner BOKU (https://biocarboncapture.boku.ac.at/). Lectures were held for seven weeks (Tuesdays) from 20th April 2021 to 1st June 2021. The number of participants ranged from 40 to around 90.

Cooperation with industry took place directly in the project, as industrial partners from Austria were involved in the project. Furthermore, there was exchange and communication via different channels like Task 37.

5 Publications

Publications:

Austrian partners:

Steger, F; Ergal, I; Daubek, A; Loibl, N; Rachbauer, L; Fuchs, W; Rittmann, SKMR; Bochmann, G. (2022): Trickle-Bed Bioreactors for Acetogenic H_2/CO_2 Conversion (2022). FRONT ENERGY RES.; 10, 842284

Steger, F; Reich, J; Fuchs, W; Rittmann, SKMR; Gübnitz, GM.; Ribitsch, D; Bochmann, G. (2022): Comparison of Carbonic Anhydrases for CO2 Sequestration (2022). INT J MOL SCI.; 23(2), 957

Fuchs, W; Steger, F; Reich, J; Ribitsch, D; Rittmann, SKMR; Bochmann, G. (2021): A Simple and Straightforward Method for Activity Measurement of Carbonic Anhydrases. CATALYSTS.; 11(7), 819

Swiss partners:

Gantenbein, A., Kröcher, O., Biollaz, S. M. A., & Schildhauer, T. J. (2022). Techno-Economic Evaluation of Biological and Fluidised-Bed Based Methanation Process Chains for Grid-Ready Bio-methane Production. Frontiers in Energy Research, 9, 775259. https://doi.org/10.3389/fenrg.2021.775259

More publications are in the pipeline.

Conferences:

Merkle, W., Baier, U. (2021). Characterisation and optimisation of ex-situ biological methanation process. Webinar series on biological carbon capture and utilization, Wien, 21.05.2021.

Merkle, W., Baier, U. (2021). CarbonATE: Entwicklung einer enzymatischen CO2-Abtrennungsstrategie für eine optimierte mikrobiologische Methanisierung. Bioener-gieforschungstagung BFE, Bern, 25.05.2021.

Merkle, W. (2021). Characterisation and optimisation of ex-situ biological methanation process. XV Summer School on Advanced Biotechnology, Palermo, 13.-15.09.2021.

Merkle, W., Baier, U. (2021). Charakterisierung und Optimierung der Ex-situ Biologischen Methanisierung. Expertinnen- und Expertengespräche Power-to-X, Rapperswil, 23.09.2021.

Merkle, W. (2021). Characterisation and optimisation of ex-situ biological methanation process. Progress in Biogas V, Stuttgart, 22.-24.09.2021

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Merkle, W. (2022). Development of ex-situ biological methanation process. Great Cycle: Symposium of Rural Soils and Waters Organic Pollution Control, Beijing, 27. 29.09.2022.

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