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Kinetics, multivariate statistical modelling, and physiology of CO_2-based biological methane production

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HIGHLIGHTS

- Gas-to-gas conversion processes are analyzed with respect to bioenergy production.
- CO_2-BMP modeling is performed and model validity is discussed.
- Multivariate data analysis and biological gas conversion mechanistic is integrated.
- Gas limitation and liquid limitation in pure culture biological CH_4 production are highlighted.
- Continuous culture CH_4 bioprocessing from H_2/CO_2 is discussed.

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ABSTRACT

Conversion of surplus electricity to chemical energy is increasingly attracting attention. Thereof, biological energy conversion and storage technologies are one of several viable options. In this work, the inherent challenges faced in analyzing the CO_2-based biological methane production (CO_2-BMP) process for energy conversion and storage are discussed. A comprehensive assessment of key process parameters on several CO_2-BMP process variables was conducted. It was found that literature data often misses important information and/or the required accuracy for resolution of the underlying mechanistic effects, especially when modelling reactor dependent variables. Multivariate dependencies inherently attributable to gas-to-gas conversion bioprocesses are particularly illustrated with respect to CO_2-BMP. It is concluded that CO_2-BMP process modelling requires the application of process analytical technology. The understanding of the CO_2-BMP mechanistic process is discussed to assist with the analysis and modelling of other gas-to-gas conversion processes. The findings presented in this work could aid in establishing a biotechnology-based energy to gas conversion and storage landscape.

1. Introduction

Converting surplus electricity to chemical energy is increasingly attracting attention [1]. In this frame, chemical or biological energy conversion and storage technologies for the power-to-gas concept are one of several viable options [2,3]. Due to decreasing reserves of fossil fuels and growing awareness for global warming, carbon dioxide (CO_2) utilization has become a topic of industrial relevance [4]. An effective reduction of CO_2 emissions will be achieved in the long term if renewable energy production can be linked with power conversion and storage technologies. Furthermore, the production of renewable energy is significantly more carbon neutral when compared to fossil fuel-based energy production [5,6]. Therein, a renewable energy production scenario that consumes CO_2 and produces biofuels could become an integral part of a biorefinery scenario for reducing CO_2 emissions [7]. However, the environmental impact of biofuels production, utilization, and surplus (or excess) energy conversion systems still needs to be evaluated and re-assessed.

Production of 1st generation biofuels would currently be able to compete with fossil fuels in the case where certain energy crops (e.g. *Saccharum officinalis*) are employed in bioethanol production [5]. 2nd generation biofuel production from e.g. lignocellulose could also become competitive to fossil fuels and are already applied on industrial scale for energy production [5,6]. Biofuel production systems of the 3rd and 4th generations have only reached pilot and pre-industrial scales concerning biodiesel production from algae and photo-fermentation of

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molecular hydrogen (H_2) respectively [7]. Recent advances in bioprocess technology [2,3,8,9] and the development of biorefinery concepts favored the development of 5th generation biofuels, which employ microorganisms to convert gaseous substrate(s) to gaseous end products. 5th generation biofuels encompass CO_2-based biological methane (CH_4) production (CO_2-BMP) and H_2 production from C_1 compounds [9,10]. CO_2-BMP and H_2 production from C_1 compounds are known to be the only gaseous biofuel production technologies that have no immediate requirement for photosynthesis. Thus, integrating surplus renewable power conversion with CO_2 capture and storage can be performed by applying the CO_2-BMP process.

The CO_2-BMP process is characterized by utilizing hydrogenotrophic methanogenic archaea (methanogens) for CH_4 production [9]. Because CO_2-BMP is a bioprocess, it encompasses distinct and emergent advantages compared to its chemical counterpart – the Sabatier process. One such advantage is the autocatalytic regeneration of methanogens accompanied by CH_4 production [9,11–14]. In this process, methanogens exhale CH_4 as a metabolic end product of their energy conserving metabolism while fixing a variable part of CO_2 in the form of biomass [14–16]. Therefore, the production of CH_4 is essential for the survival of the organisms. The CO_2-BMP process can be carried out by an enrichment culture [17–23] or pure culture of methanogens [9,24] and benefits from its ability to convert CO_2 and H_2 to CH_4 at very high volumetric methane evolution rates (MERs) while in continuous culture [25,26]. An additional advantage is the mild bioprocessing conditions (e.g. temperatures from approx. 0 °C to 122 °C) that can be applied during CO_2-BMP [27,28].

High purity H_2 and CO_2 can be employed as substrates for the CO_2-BMP process [9,12,26]. It has also been shown that the CO_2 by-product of the anaerobic digestion process can be microbiologically transformed to CH_4 at different conversion efficiencies and MERs [13,21,24,29]. However, it has been noticed that the technology readiness level (TRL) of the different microbiological biogas converting technologies can vary tremendously [24]. Although direct microbiological biogas conversion in anaerobic digesters was shown to be possible, the MER and CH_4 concentration in the offgas remained negligible [20,24]. On the contrary, microbiological biogas conversion by using pure [13] or enrichment cultures [21,23] of methanogens was shown to be efficient. Drawbacks of using enrichment cultures for microbiological biogas conversion are the ambiguous adaptation procedures, the time it takes for the culture to adapt to certain conditions, and unintended side reactions that occur within the enrichment [24,30]. Eventually, pure cultures of methanogens were not only applied in microbiological biogas upgrading [13,31], but were also utilized for conversion of CO_2 from industrial flue gases [13]. While pure cultures have been used for the conversion of chemical species, it should be noted that the CO_2-BMP process results in a different product formation kinetic [32,33] when compared to liquid-based continuous culture bioprocessing [6,34]. Therefore, many challenges in the analysis of production kinetics, physiology, scale-up, and modelling of the CO_2-BMP process have emerged [8].

The first aim of this study was to comprehensively assess the effects of key process parameters (KPP) on several CO_2-BMP process variables, which were obtained from literature, on continuous culture bioprocessing. Second, this study discusses the multivariate dependencies inherently attributable to CO_2-BMP gas-to-gas conversion bioprocesses. Third, it is shown that the presented models possess limits that prevent a simple analysis of the CO_2-BMP process. Fourth, the application of multivariate data analysis and modelling CO_2-BMP process is thoroughly discussed. It was of great interest to review and refine the understanding of the kinetic aspects involved in gas converting bioprocess technologies and to better control and avoid undesired or uncontrolled limitations of the CO_2-BMP kinetics.

The novelty of this contribution goes beyond bioprocess modelling. Here, a critical analysis of literature on CO_2-BMP in pure culture was performed. It is shown that both liquid and gas limitations need to be carefully considered when attempting CO_2-BMP bioprocessing. Examples on how to model the CO_2-BMP processes are given and it is shown that wrong conclusions have often been drawn due to an application of erroneous results. It is discussed that during CO_2-BMP modelling an in depth understanding of the biology and the process is required and that the physiology of the target organism must be carefully considered to cope with the multivariate nature of this process. Finally, it is shown that biological gas-to-gas conversion and energy storage processes must be scaled by linking kinetics, modelling, and physiology.

2. Material and methods

First, the existing literature of pure culture CO_2-BMP, independent of bioreactor conditions and scale, was reviewed with an in depth examination of methanogenic strains, bioprocess setup, and growth conditions. Second, pure culture CO_2-BMP data was extracted from literature [11,12,25,26,32,35–44]. Third, the data was applied for qualitative and quantitative assessment and subsequent modelling. A list of comprehensively extracted results from literature is provided in Supplementary Material 1. From all literature reports on pure culture CO_2-BMP, only the data on continuous culture experiments were analyzed as the stability of process variables in steady state allowed for a precise quantification. Closed batch and fed-batch CO_2-BMP experiments were not considered.

2.1. Definition of parameters and units

The following variables and KPPs were extracted or calculated based on the information provided in literature: the gassing rate per working volume per minute (vvm [$L\,L^{-1}\,min^{-1}$]), temperature [°C], the pH, oxidation reduction potential (ORP [mV]), agitation [rpm], sulphide dilution rate (DS [d^{-1}]), trace element concentration (TE), medium dilution rate (D [h^{-1}]), the gassing ratio, and the reactor pressure [barg]. Additionally, the following variables relating to production and/or yield were extracted from literature: methane evolution rate (MER [$mmol\,L^{-1}h^{-1}$]), the specific CH_4 evolution rate (qCH_4 [$mmol\,g^{-1}$ (gram cell dry weight) h^{-1}]), the CH_4 offgas concentration [Vol.-%], biomass concentration (x [g (gram cell dry weight) L^{-1}]), the specific growth rate (μ [h^{-1}]), and the growth yield (Y_{CH4} [g (gram cell dry weight) mol^{-1}]), or, where attainable, the growth to product yield ($Y_{(x/CH4)}$ [C-mol mol^{-1}]). $Y_{(x/CH4)}$ was used to assess the flux of the carbon into biomass and into CH_4 on a C-molar level for all the cultivations performed with *Methanothermobacter marburgensis* [11,12,26]. Although the analysis of $Y_{(x/CH4)}$ was possible for experiments reported before [11,12,26,35], $Y_{(x/CH4)}$ could not be retrieved or calculated from all of the experiments presented in Supplementary Material 1 because C-molar biomass productivity ($r_{(x)}$) [C-mmol $L^{-1}\,h^{-1}$] had not been reported. However, Y_{CH4} that was defined as the quotient of μ to qCH_4 [15] could be retrieved from literature. Most KPPs and variables could be directly extracted from literature without the necessity to convert results [11,12,26,35]. In some cases the conversion of extracted literature data into aforementioned molar units was performed.

2.2. Data validation procedure

Data was curated according to the degree of reduction balance (DoR-balance) and carbon balance (C-balance) by applying manual data quality control steps. These mass balance curation steps could only be performed were the relevant information was provided in literature. The relevant bioprocess and physiological parameters were then presented after a data quality assessment based on published methodologies [9,45]. Data curation also involved a thorough qualitative selection procedure where an assessment step analyzing the data by using the MER/MER_{max} concept was implemented. The MER/MER_{max} ratio presented is the dimensionless quotient of MER to the maximum possible

volumetric CH_4 production rate (MER_{max} [mmol L^{-1} h^{-1}]) according to the reaction stoichiometry and experimental settings neglecting biomass formation [11,12,26]. The MER/MER_{max} concept for apparent gas conversion to maximum theoretical gas conversion has been previously introduced [9,26]. The MER/MER_{max} concept was used to identify outliers according to the percentage of MER in relation to MER_{max}. The resulting quotient is referred to as MER/MER_{max} and is plotted against the CH_4 offgas content in Vol.-%. This characteristic graph changes with different H_2 to CO_2 gas inflow ratios. This is due to the fact that, based on the presented assumption(s), full gas conversion can only be achieved when a H_2 to CO_2 gassing ratio of 4:1 is applied [9]. Even though the data was extracted from literature for CO_2-BMP modelling purposes, re-calculation of the data was necessary to be able to equalize the entries for subsequent qualitative and quantitative analyses. This method overestimates MER for all other data that were not calculated based on the r_{inert} correction factor [9,26]. The r_{inert} correction factor accounts for the fact that stoichiometric gas contraction occurs during conversion. It is needed to calculate the MER based on the educt gas inflow and the CH_4 offgas composition [12,26]. However, if $Y_{(X/CH4)}$ is assumed to be 1–5% of the total carbon flux into the biomass, the error on MER quantification is relatively small [11,12,15,26,36,45]. This approach was applied to reject data with highly deviating DoR- or C-balances.

2.3. Multivariate statistical analyses

Principal component analysis (PCA) was used to cluster the KPPs and variables and to visualize the variability of the CO_2-BMP data. Subsequently, the data will be treated using multiple linear regressions (MLR) to obtain models and describe the MER and qCH_4 for different parameter spaces and reactor configurations. PCA and MLR modelling was conducted by using DataLab (Ipina GmbH, Pressbaum, Austria (www.datalab.com)) and Design Expert 8.0.7.1 (Stat-Ease, Inc., Minneapolis, USA). Data imputation was performed according to the DataLab data imputation routine using mean fill functions only for columns where 15% empty cells or less of the cells were missing data (values shown in Supplementary Material 1). PCA was performed on a qualitatively curated data set from CO_2-BMP continuous culture (Supplementary Material 1). After removing erroneous data, the CO_2-BMP data set used for PCA consisted of 172 continuous culture conditions (n = 172). Data extracted from literature did not provide enough information to accurately sort the data sets according to the following conditions: ammonia concentration, titration liquids, sulphur concentration, liquid dilution rates, gas flow rates, and ORP. Data size for multivariate analyzes was comfortably high (10 data points per KPP or variable, [46,47]) to allow data substantiation concerning multifactorial dependencies. The data set used for multivariate analyses of *M. marburgensis* CO_2-BMP comprised 159 continuous culture conditions (n = 159, Supplementary Material 1). In general, data was checked for multinormality and skewness as well as for linearity of individual variables to individual process factors. All PCA analyses were performed based on a correlation matrix obtained though standardization of data. From a PCA bi-plot, which is based on a correlation matrix, the cosine of the angle between the loadings represents the correlation between process factors and/or dependent variables. Loadings are the sum of the eigenvector multiplied by the square root of the eigenvalue. For PCA, the data set was differentiated according to pre-defined classes. The differentiation into pre-defined classes was necessary due to the related variables being setup dependent, especially for data on gas transfer-related variables. Mixing data from different setup specific CO_2-BMP cultivations would generate inaccurate models. The pre-defined classes of the data set were introduced based on the various setup conditions and associated bioreactor volumes. Experiments previously performed for *M. marburgensis* that vary these conditions were carried out as follows: continuous culture in a 2L bioreactor [12], design of experiments (DoE) in a 2L bioreactor [11], continuous culture in a 10 L bioreactor [26], cell retention in a 10 L bioreactor [26], and DoE in a 10 L bioreactor [35]. From Supplementary Material 1 data subsets for qCH_4 vs D were established by segregating the kinetic limitation faced by $Y_{(x/CH4)}$. The data subsets were used to identify and subsequently demonstrate novel insights into mechanistically inherent aspects of the kinetic limitations occurring during CO_2-BMP. Prior to PCA and MLR analyses the data was analyzed for homoscedasticity by visual data inspection of the corresponding graphs that are provided in Supplementary Material 2. Multicolinearity was assessed using the variance inflation factor (DataLab, Ipina GmbH, Pressbaum, Austria).

3. Results

Since the publication of the simple unstructured mathematical model for a continuous pure culture CO_2-BMP process [32], new approaches have been reported for the cultivation of methanogenic archaea converting CO_2. The model in Schill et al. [32] describes growth and productivity of *M. thermoautotrophicus* in a gas-limited state as function of KPPs such as D or gassing rate. In general, many studies on CO_2-BMP focused on fed-batch or continuous culture modes [26,32,33,36]. The primary goal of these studies was to induce gas-limited or liquid-limited conditions and derive quantitative physiological variables. In some cases, these studies also examined the underlying thermodynamic and metabolic constraints of biological methanogenesis [13,26,33,35,36,48,45]. However, the dual nature of limitations (gas transfer-based or liquid-based) or inhibitions that can be faced upon biomass growth pose challenges for the development of a robust and scalable technology [8].

Continuous culture CO_2-BMP data are shown in Fig. 1. These data were plotted according to the quotient of MER/MER_{max} to CH_4 offgas. *M. marburgensis* continuous culture data fits the MER/MER_{max} to CH_4 offgas relationship. This is a consequence of the method applied for the calculation of MER via r_{inert} gas flow as described previously [9,12,26]. Although the r_{inert} correction factor was shown to be fairly correct for low biomass concentrations between 1 and 5% [15,36], and the DoR-balances were shown to not vary greatly [11,12,26], the calculation could become more erroneous if the $Y_{(x/CH4)}$ is higher, as could be the case for other methanogenic archaea such as *Methanosarcina barkeri* [15]. In addition, it poses limitations in terms of quantification accuracy and the ability to identify physiologic effects during a process operation. Furthermore, if MER and MER_{max} values are calculated by neglecting $r_{(x)}$, the proportion should be true. If MER is measured, but

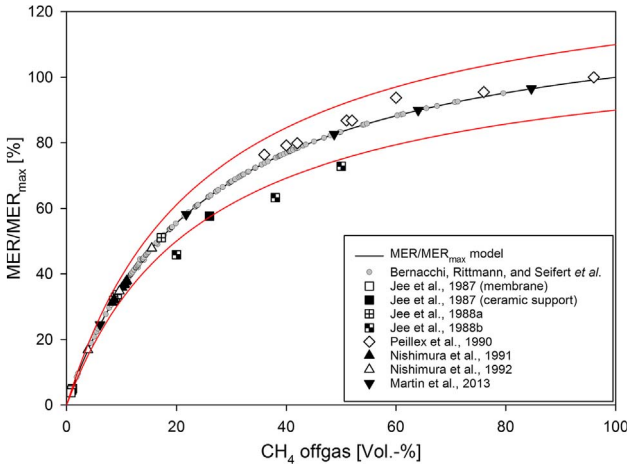

Fig. 1. Data on CO_2-BMP is shown as a function of MER/MER_{max} to CH_4 offgas. The MER/MER_{max} model follows the continuous graph. The continuous red lines denote a 10% deviation from the MER/MER_{max} model. As indicated in the legend, the individual data points were directly extracted from literature or calculated from literature data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

MER_{max} is calculated from literature data neglecting biomass formation, then the ratio is underestimated because the calculated MER_{max} is higher than the real MER_{max}. Therefore, it must be noted, whether or not the MER was calculated from literature data or taken directly from the publication.

Physiological effects cannot be quantified if the C-balance variability is too high. In fact, the accuracy required for C-balancing needs to be assessed as a function of the $Y_{(x/CH4)}$ resolution target [8]. Therefore, enhanced C- and DoR-balancing would benefit the modelling of MER and $r_{(x)}$ as a function of KPP. Calculations of MER/MER_{max} from literature data were expected to possess a slight offset from the concept graph line as some systematic differences are inherent to the calculation process. However, even with this in mind, some data could not be closely fitted as can be seen in Fig. 1. An interval around full conversion of reactive gases was set in order to compensate for neglecting $r_{(x)}$. Subsequently, only data fitting within this interval were presented and retained in the final data set. The final data set (Supplementary Material 1) will be used for the modelling of CO_2-BMP and the multivariate data analysis of process variables.

3.1. Gas transfer-limited versus liquid-limited biomass growth

The appearance of dual (gas transfer-based or liquid-based) limitation mechanisms, which are inherent to CO_2-BMP processes pose challenges for process analytical technologies (PAT) and quantification towards the development of a robust, controlled, and automated bioprocess [8,9,35]. Therefore, in order to accurately quantify the kinetics of gas converting bioprocesses, it is important to know the actual limitation at either a given time point or as a function of the process parameters applied to allow for control of the biocatalytic activity [35]. This strategy allows scaled feeding of the organism according to physiologic demand and avoids undesired limitations in the process reaction kinetics. Data from different CO_2-BMP processes [11,12,26,42,43] are shown as individual plots of qCH_4 as a function of D in Fig. 2.

As an example, data extracted from Peillex et al. shows that the data points calculated for qCH_4 have an unusually high variation at the same D [43]. Although data obtained from Peillex et al. fit the MER/MER_{max} concept (Fig. 1, qCH_4 data values were found to be more than 400% above the $qCH_{4,max}$ reported for *M. marburgensis* in continuous culture [12,35]. Although $qCH_{4,max}$ estimation of *M. marburgensis* was performed by using dynamic process conditions [12,48,49] or via a controlled liquid-limited condition [35], it can be considered that a maximum standard deviation of 10% is expected on the reported values. Therefore, qCH_4 values obtained by Peillex et al. are not likely to reflect physiological characteristics of *M. marburgensis* and will therefore not

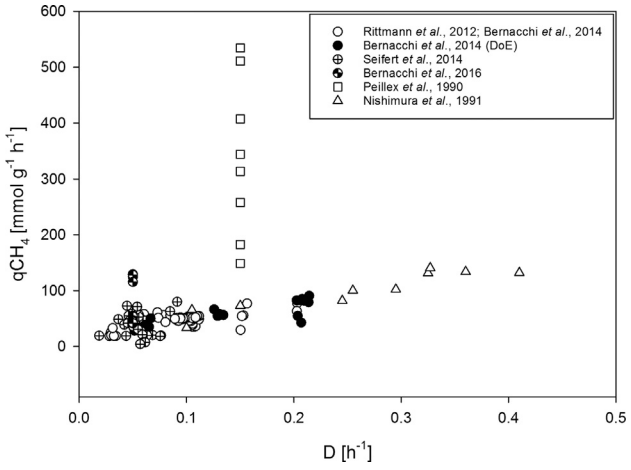

Fig. 2. Data on CO_2-BMP is shown as qCH_4 as a function of D. qCH_4 data from several publications could almost be fitted by using linear regression. However, qCH_4 data from Peillex et al. do not reflect the probable physiological constraints of *M. marburgensis*.

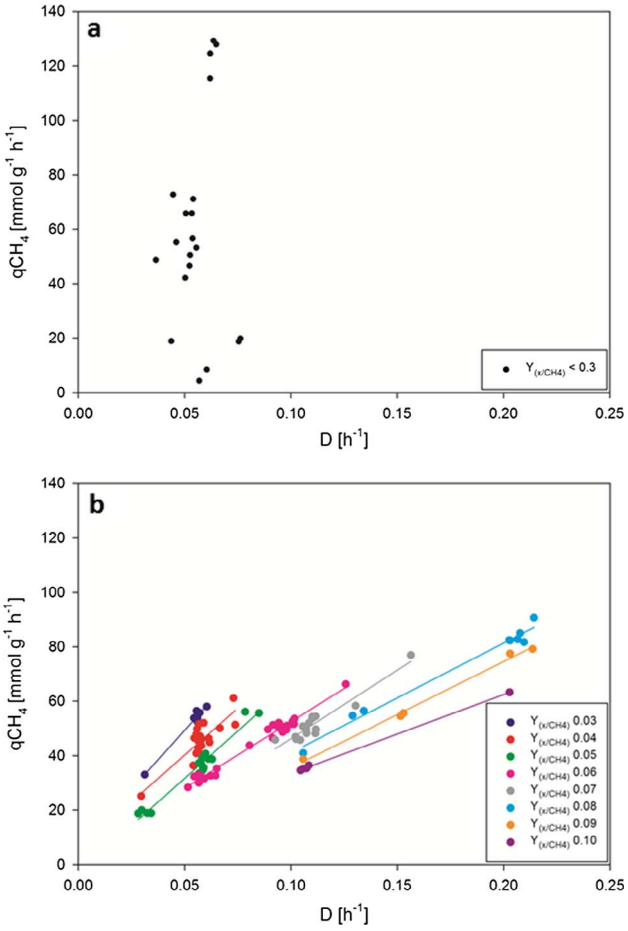

Fig. 3. qCH_4 plotted against D for CO_2-BMP continuous cultures under different types of limitations. The $Y_{(x/CH4)}$-range is indicated. (a) liquid- limited cultures at $Y_{(x/CH4)} < 0.3$ C-mol mol^{-1}, (b) gas-limited cultures. For gas-limited cultures at different $Y_{(x/CH4)}$ only selected steady states from [26,35] were used.

be used for modelling. A possible cause for the qCH_4 deviation in Peillex et al. could be an erroneous determination of biomass. In fact, if biomass concentration was under-evaluated by a factor of ten, then the qCH_4 values would fit results reported elsewhere [11,12,26,35] at the given process conditions. However, higher values of qCH_4 of *M. marburgensis* were reported for fed-batch experiments [48,50]. It is believed that during dynamic fed-batch experiments the quantification challenge (*i.e.* the measurement or calculation of volume compensation) has an impact on the quantification of process responses. This assumption could be verified by analyzing the error propagation over the multiple quantification steps and assumptions reported elsewhere [48,50].

When analyzing the model proposed by Schill et al. during gas-limited CO_2-BMP, a linear relationship between qCH_4 and D is expected. This characteristic is associated to the autobiocatalytic reactions at a fixed $Y_{(x/CH4)}$ and gas transfer rate (GTR). Essentially, a culture will reach different values for x at equilibrium with different applied D (*i.e.* x decreasing with an increasing D as a consequence of wash out) [12,32]. This phenomenon can be observed in Fig. 3, where data from *M. marburgensis* [11,12,26,35] can be dissected into two broad trends for qCH_4 as a function of D depending on the type of limitation affecting biomass growth.

Although simple linear relationships generally describe liquid substrate based bioprocess development [51], the specific product formation with respect to D in a gas- or liquid-limited bioprocess is different [12,32,36,52]. In Fig. 3, it can be seen that linear relationships cannot be trivially applied to CO_2-BMP processes and that this relation depends mostly on the limitation faced by $r_{(x)}$. It is well known that qCH_4 can

vary greatly at a given GTR independent of D when a liquid-limitation or inhibition occurs [11,35]. However, under gas-limited conditions qCH_4 is linearly dependent on D at a slope proportional to $Y_{(x/CH4)}$ [12,32,36]. This is also shown in Fig. 3.

The maintenance energy of a liquid-limited pure culture can be determined by plotting the specific product productivity as a function of D. However, maintenance energy is defined as the energy for metabolic functions not related to growth. Hence, for CO_2-BMP it would correspond to the Gibbs free energy inherent to the material flow used neither for biomass or product formation. A $Y_{(x/CH4)}$ of zero would be experimentally required to allow quantification of the maintenance-related metabolism. Additionally, the kind of limitation (gas or liquid) or inhibition in Fig. 3 could be elucidated when analyzing qCH_4 as a function of D. However, it is extremely challenging to assure that the examined cultures are solely gas- or liquid-limited, since not only proper biomass, vvm, CH_4 offgas quantification, and subsequently analytics must be taken into account, but *a priori* knowledge about the setup of interest is also required.

3.2. Bioreactor setup – another limitation towards high MER

The correlation of the MER as a function of the volume dependent gassing rate, vvm, is another relationship that is often presented for analyzing CO_2-BMP [12,25,26,42,53]. An analysis of MER as a function of vvm is shown for the data obtained from literature in Fig. 4.

In literature, linear relations were often shown for MER as a function of vvm [26,36]. However, this relation is not only strictly setup dependent, but also only holds true within a limited range of vvm

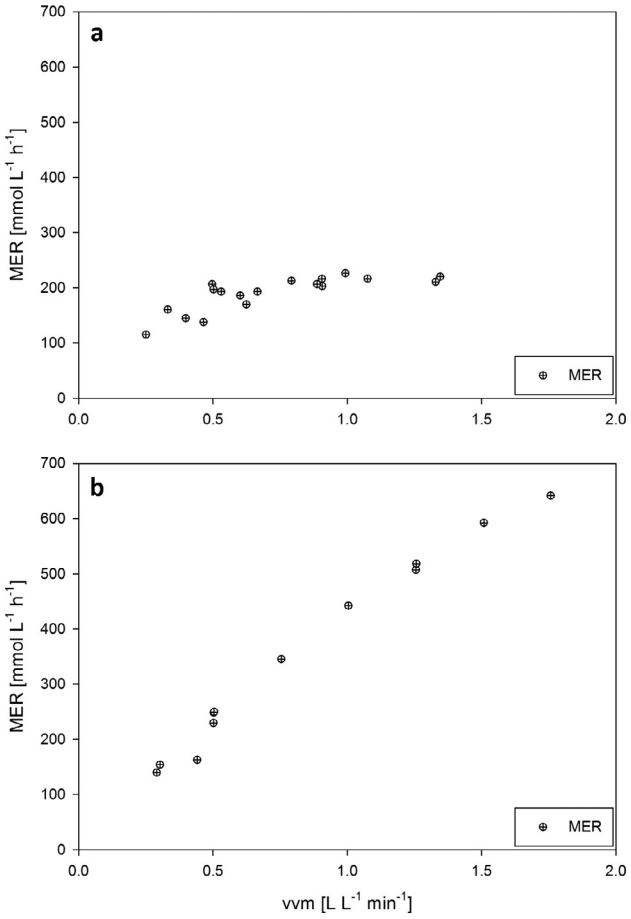

Fig. 4. MER as a function of vvm for CO_2-BMP continuous culture cultivations in two different CSTR setups. (a) setup with 2 rushton impellers at atmospheric pressure. (b) setup using 3 rusthon impellers and pressure above atmospheric. Detailed information can be found in [26,35].

increase and is furthermore known to impact the CH_4 offgas. In a specific CO_2-BMP setup with a specified GTR, a maximum gassing rate can be applied. At higher gassing rates, increases of MER will not occur. In continuously stirred tank reactors (CSTRs), this is caused by flooding of the stirrer [54]. Flooding of the stirrer describes the phenomenon that for a given agitation speed with increasing vvm a set-up specific gas to liquid mass transfer maximum (flooding point) will be reached. Beyond the flooding point additional gas supplied to the bioreactor will not anymore be able to be transferred to the liquid phase. The additionally supplied gas will cause the aggregation of bigger-sized gas bubbles that ascend around the stirrer axis and escape the bioreactor. Fig. 4 shows the comparison of the relation between vvm and MER in two different CSTR setups with deviating mixing systems and therefore different mixing efficiencies. While in one system (Fig. 4a), due to flooding of the stirrer, additional gassing did not contribute to an increase of the MER, the other setup showed a steady increase of MER over vvm as additionally provided gas could also be effectively transferred into the liquid phase (Fig. 4b). However, a trend towards a maximum reachable MER can also be seen here as the curve is flattening with an increasing gassing rate [26].

The gas residence time should also be taken into consideration since at higher vvm the contact time between gas bubbles and liquid is consequently reduced. The mix of these physicochemical limitations implies that for a given reactor setup (with a defined maximum GTR) a maximum MER exists at which a targeted CH_4 offgas quality can be consequently reached. This is because offgas quality is always affected by the interplay of GTR and the average residence time of the reacting gases if sufficient biocatalyst is available [8]. These mechanistic constraints need to be considered and reflected in the models of gas converting bioprocesses particularly in the case of CO_2-BMP. Unfortunately, such trends were not described or translated within the existing models available [11,26,32,36] which are often restricted to a limited operational space and are unsuited for extrapolating knowledge for the purpose of process operation or scale up activities in different reactor setups.

3.3. PCA of CO_2-BMP continuous culture data

PCA is a statistic tool used for multivariate data analysis and that can be utilized to identify correlations and loadings among process parameters and dependent variables [46,47]. In this section PCA was applied to three different data sets. The data sets used for the PCAs are available in Supplementary Material 1. An overview of all process parameters and dependent variables that could be applied in the PCA is shown in Table 1.

The PCA for Fig. 5a comprised 172 independent continuous culture steady state conditions performed with different methanogenic strains. A total of six principal components (PCs) are necessary to explain 77.70% of the total variability (Supplementary Material 3), which renders the cluster challenging to interpret due to the numerous dimensions involved. When analyzing PC 1 and PC 2, only 44.77% of the total variability of the dataset can be explained. This denotes a strong multivariate nature of variables and parameters in the CO_2-BMP processes, which can generally be extended to all gas converting bioprocesses. This is because gas converting bioprocesses are not only dependent on the kinetics of gas to liquid mass transfer but also on the physiology of the biocatalyst. In Fig. 5a, it is difficult to recognize a clear clustering of KPPs with respect to MER or qCH_4. For a CO_2-BMP process performed at the same temperature for a given reactor setup, MER should tendentiously cluster with vvm, pressure, and agitation. Alternatively, qCH_4 should share a correlation with D or $Y_{(x/CH4)}$, as it was shown in Fig. 3.

Such constraints could not be observed in Fig. 5a. The only conclusion that can be drawn from the CO_2-BMP process data reported in literature is that great data variability might occur from the different experimental approaches and setups used by the different authors. This

Table 1
Overview of process parameters and dependent variables partially used for PCA.

#	Process parameter/variable
1	H_2 CO_2 in (vvm)
2	Temperature
3	pH
4	ORP
5	Agitation
6	Gassing ratio
7	DS
8	TE
9	D
10	Pressure
11	MER
12	r(x)
13	x
14	CH_4 offgas
15	μ
16	qCH_4
17	Y_{CH4}
18	$Y_{(x/CH4)}$

led to the intention of retrieving a more compact PCA analysis. To obtain a clustering between the KPP of CO_2-BMP and MER and qCH_4 (please also refer to Fig. 1, a dataset using only data from *M. marburgensis* was applied (Supplementary Material 1). The results of this analysis showing PC1 and PC2 in a bi-plot are presented in Fig. 5b. Therein, three correlating clusters can be identified. Cluster 1 is composed of factors 1, 10, and 11, and therefore represents a combination of MER, H_2/CO_2 gassing rate, and pressure. Cluster 2 is composed of factors 8 and 12, wherein $r_{(x)}$ and TE are correlating. Cluster 3 is composed of factors 4, 7, 9, 16, and 18, where all liquid relevant process factors such as ORP, DS, and D are clustering with the dependent physiological variables qCH_4 and $Y_{(x/CH4)}$. Nevertheless, this case also needs six PCs to explain 78.44% of the total variability. Hence, a detailed analysis would imply examining all of the combinations and permutations of PC1 to PC6. However, this exercise poses a certain challenge for the interpretation of results in such a multi-dimensional space. When considering the contribution of communalities to the individual PCs, it turned out that gas related process parameters (vvm, agitation, pressure, MER, CH_4 offgas) as along with D and TE contribute to the first two PCs.

Finally, a third PCA was performed. This dataset consisted of data from gas-limited *M. marburgensis* cultures only. The relationship of GTR related process factors, aforementioned KPPs, and variables are shown in Fig. 6. In this PCA only two PCs were necessary in order to explain

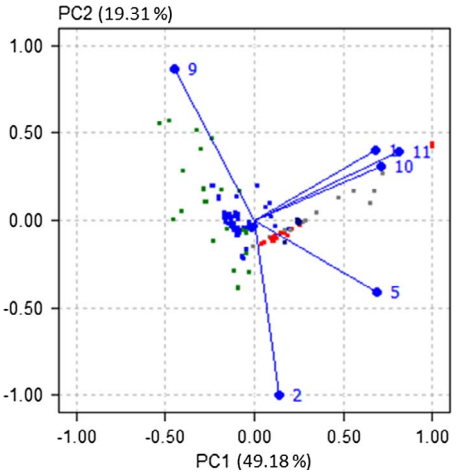

Fig. 6. Bi-plot from PCA of CO_2-BMP showing PC1 and PC2. Only two PCs could explain 68.49% of the total variance of the data.

68.49% of the total variability (Supplementary Material 3). In Fig. 6, a clustering of factors 1, 10, and 11 can be identified. The results presented in Fig. 6 are supported by findings reported in literature for gas-limited conditions during CO_2-BMP in continuous culture operations [26,36,52]. The results of the PCA presented in Fig. 6 suggest that a proper multivariate model of continuous culture CO_2-BMP could be obtained by applying MLR fitting methods. However, the presented data clearly showed that best results are obtained when using data restricted to a defined physiological state. The more variable the underlying dataset was in terms of physiological states, different reactor setups, or various strains used, the more difficult it was to obtain PCA results that could be set in a logical context to what is found in literature.

3.4. Modelling of MER

To highlight the complex interdependencies between process variables and responses in CO_2-BMP, multivariate models are presented for MER and qCH_4. Based on the results of the PCA analyses and the high number of data available for *M. marburgensis* continuous culture experiments, the latter dataset was subsequently used for MLR. The results are shown in Supplementary Material 2.

MLR analysis lead to the following results: vvm, pH, temperature, agitation, DS, TE, and pressure were found to significantly influence MER (89.3%, $r^2 = 0.8927$). However, it must be noted, that this model

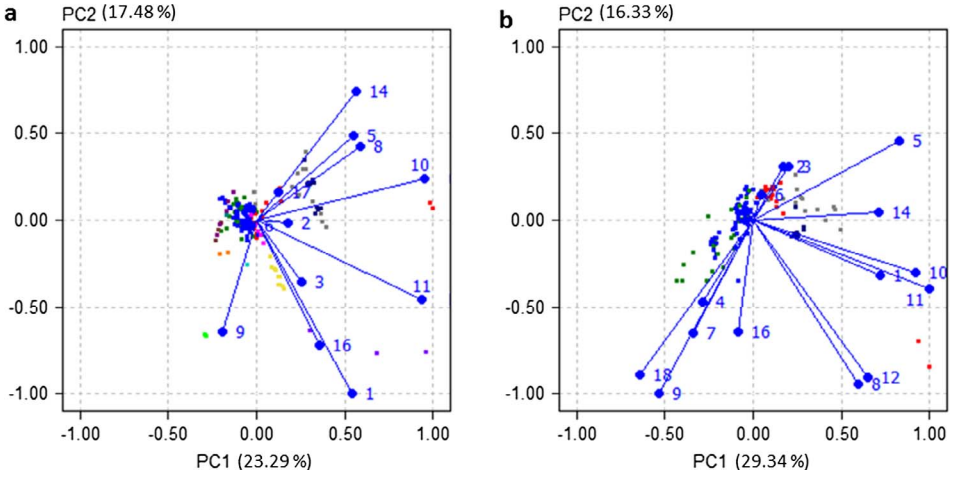

Fig. 5. Bi-plots obtained from correlation PCA of CO_2-BMP showing PC1 and PC2. PC3 – PC6 are not shown. In 5a the bi-plot shows clustering of KPP and variables, which cannot be related to findings published on CO_2-BMP to literature. In 5b the bi-plot illustrates clustering of KPP and variables according to literature published on CO_2-BMP continuous culture.

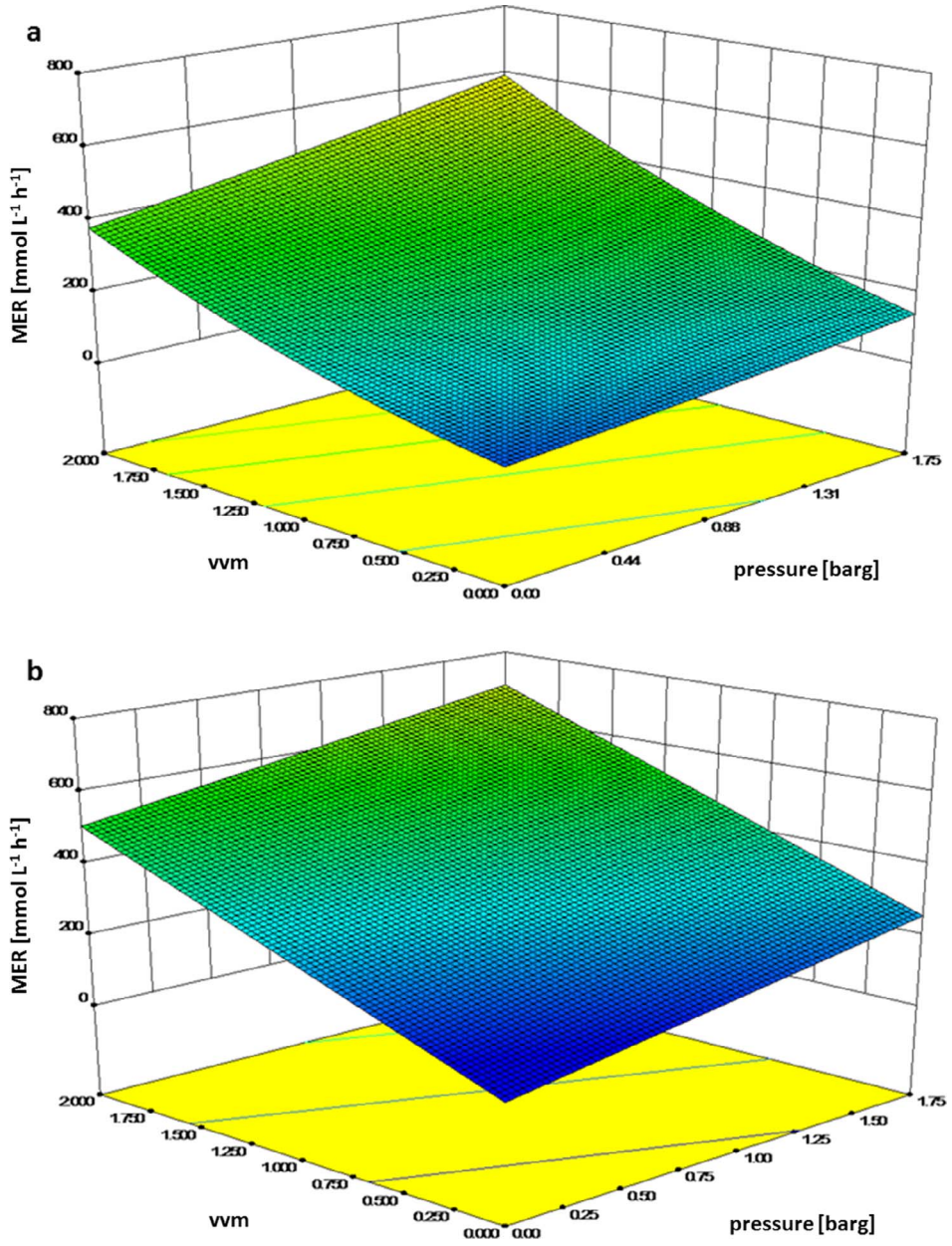

Fig. 7. MLR models for the influence of vvm and reactor pressure on the MER. In 7a, a model based on data from different bioreactor setups is presented. In 7b, a model based on data from a single bioreactor setup is presented.

was established from continuous culture CO_2-BMP results that were based on different bioreactor setups and geometries [11,12,26,35]. Furthermore, the data density towards higher MER values decreases. In Fig. 7a, the MER of an *M. marburgensis* continuous culture utilized for CO_2-BMP is shown as a function of vvm and pressure for gas-limited conditions. The significant ANOVA model of MER of *M. marburgensis* is shown in Supplementary Material 2.

While the overall positive influence of vvm and reactor pressure on the MER is correctly reflected by the model plot presented in Fig. 7a, the exact correlation between vvm and MER is obviously wrong. The presented model predicts an exponential increase of the MER with vvm, while the data collected during experiments with a certain setup (Fig. 4) showed the opposite trend, a flattening of the curve towards higher vvm. As previously explained, this is an inevitable consequence of the flooding phenomena occurring in CSTR reactors at a certain vvm. However, since data from several different CSTR setups with different individual flooding points were used as input for the MLR analysis, the correlation between vvm and MER is erroneously predicted. To overcome this problem, a sub-dataset, consisting of data collected with a single bioreactor setup, was used to perform a new MLR analysis. The outcome is shown in Fig. 7b. In this case, the experimentally determined correlation between vvm and MER is now properly reflected but therefore only valid in the design space. This shows that modelling of gas transfer, and therefore setup dependent variables like MER, should be performed for specific bioreactor setups while taking into account the underlying mechanisms of gas-liquid mass transfer as well as the residence time of reacting species. GTR mechanisms can, among others, be affected by reactor geometry, operation mode, working volume, broth rheology, agitation system, and sparging.

3.5. Modelling of qCH_4

Another MLR model was established for qCH_4 (Supplementary Material 2) that depicts a coefficient of determination of 65.1% ($r^2 = 0.6148$). Significant factors of the qCH_4 MLR model are vvm, pH, agitation, TE, D, and pressure. A graph for qCH_4 as a function of vvm

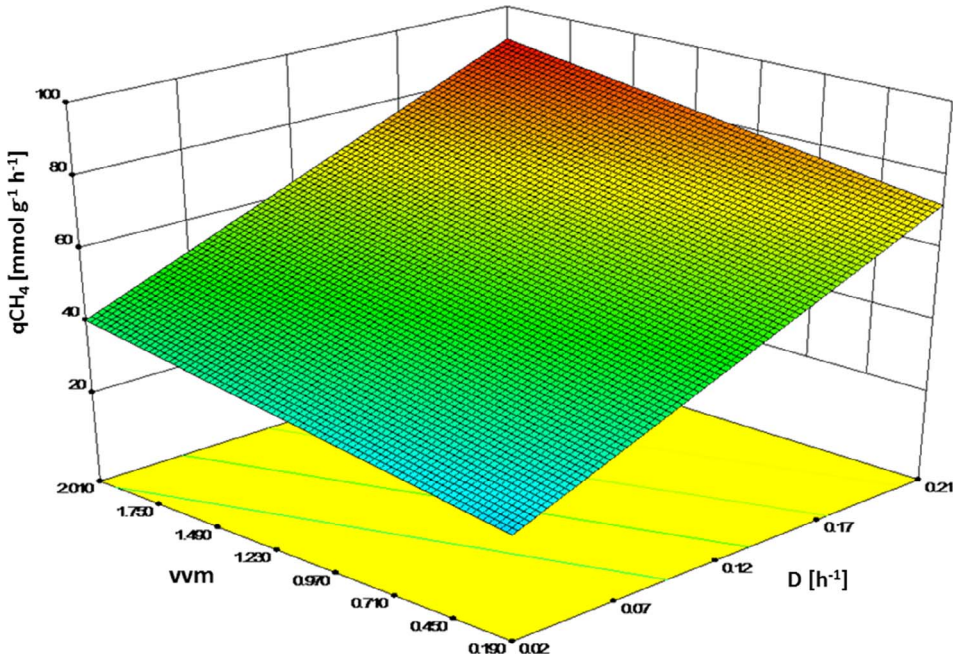

Fig. 8. qCH_4 MLR model showing the interdependency of D and vvm on the catalytic activity of *M. marburgensis*.

and D is shown in Fig. 8 and the ANOVA models are presented in Supplementary Material 2. Temperature, ORP, gassing ratio, and DS were not found to be significant.

The model equation for qCH_4 (Supplementary Material 2) shows that agitation also affected qCH_4 in several ways. Agitation increases the k_La, which influences $r_{(x)}$ and MER. It has been observed that increasing agitation had negative effects on $r_{(x)}$ [11]. These significant qCH_4 model terms in the equation can be explained by the multivariate nature of external influences affecting the physiology of methanogens, e.g. pH, ORP, temperature or pressure [14–16]. Due to the multivariate analysis of existing CO_2-BMP data, it becomes obvious that such influences would require the employment of sensitive analytical methods (e.g. TE analytics) for the liquid phase [55] and fine quantification of gas flow and composition [8] to be able to enhance the overall accuracy of process elemental balancing. This would enable the resolution of small variations of $Y_{(x/CH4)}$ as a function of input parameters and/or to compensate for the eventual lysis of biomass which would significantly affect $r_{(x)}$ determination and subsequent $Y_{(x/CH4)}$ calculation [8].

4. Discussion

The above-mentioned constraints clearly show, that for a gas converting bioprocess, such as CO_2-BMP, the two main kinetic determining limitations, gas- and liquid-limitation, need to be considered for modelling the overall process kinetics. A summary of possible issues, their interpretation and tasks that could occur during analysis and modelling of the CO_2-BMP process is given in Table 2. However, it has to be noted that it is the gas to liquid mass transfer that is limiting MER and not the physiological capacity of the methanogens [11,12,26,32,33,35,56].

In a CO_2-BMP bioprocess, the biomass acts as an autobiocatalyst and needs to be properly handled to exploit the full biocatalytic activity of the organism. Therefore, inhibitory or limiting liquid-based compounds would need to be quantified with sophisticated PAT and methods [12,49,55]. After biomass is grown in CO_2-BMP fed-batch cultures [48,50], the continuous culture CO_2-BMP process will enter a H_2-based gas limitation phase [26]. Therefore, the growth medium for methanogens is generally aimed to be non-liquid limiting, and eventually non-inhibitory, as one of the main goals of CO_2-BMP is to achieve maximum MER for subsequent bioprocess scale-up. Therefore, overfeeding of minerals is often applied to avoid such liquid-based limitations. An example of such a constraint is presented in Fig. 9.

MER of *M. marburgensis* from continuous culture experiments was plotted as a function of feeding ratio with a sulphide flow rate (S_{in}) to $r_{(x)}$. It clearly shows, that the highest MER values were obtained at a $S_{in}/r_{(x)}$ feeding ratio of either > 0.001 and < 0.017 which is close to the elementary composition found in the biomass of methanogens [57]. Higher feeding rates are thus not necessary. This could also be an indication that sulphide overfeeding was affecting the quantification of physiological responses shown for CO_2-BMP in literature. Such findings could also be because of a variation observed in physiologic responses that could explain why none of the models shown above are valid. The equilibrium of sulphide species in aqueous phase and their interaction with TE needs to be dissected as a function of the pH, temperature, vvm, and ORP [58]. However, only one attempt has been made to account for $H_2S/HS^-/S^{2-}$ equilibrium when performing elemental balancing in CO_2-BMP [56]. The negative effect on either MER or $r_{(x)}$ could not be precisely determined for DS even though modelling indicates the possibility that the latter parameter was negatively influencing MER (Supplementary Material 2). Recently, sulphide and TE interactions were determined during CO_2-BMP fed-batch bioprocessing. This was done to avoid physiologically unfavorable KPP settings [48]. Even so, this attempt did not fully dissect the complex sulphide and TE interactions in CO_2-BMP processes. During CO_2-BMP modelling, the gas transfer limitations are also of concern. As it was shown before, gas transfer related variables, such as MER or CH_4 offgas, were found to be strongly setup dependent [59]. Modelling across different reactor setups can consequently lead to erroneous results if the influences on the system are not properly characterized.

Without a combination of PAT and experimental approaches it is difficult to unscramble liquid and gas transfer related influences, which could easily lead to misinterpretation of process factor correlations [11,12,48,49]. Proper modelling for CO_2-BMP therefore requires prior detailed knowledge about both the bioreactor setup and the physiology of the applied strain. This, however, creates the need for analytical tools that allow for the balancing of individual compounds, particularly for carbon and hydrogen molar fluxes, to a very accurate level. The presented results show that models based on literature data often lead to erroneous predictions and conclusions.

Previous approaches for modelling CO_2-BMP neglected parameters such as the influence of liquid limitations on the performance of the

Table 2
Overview of issues, their interpretation and the required action that could be occurring during analysis and/or modelling of CO_2-BMP.

Challenge	Interpretation	Solution in CO_2-BMP
In order to validate the quantification approach for both, physiology and kinetics, of a gas converting bioprocess. Process balances (elementary, mass, ORP, energy etc.) must be closing	Proper process balancing allows to demonstrate that the different transformations involved in the bioprocess are known and that the quantification methods used are accurate	To close the C- and DoR- balances it is recommended to implement elementary analysis of biomass as well as an accurate control and quantification of the gas flows and their composition
The dual nature of the qCH_4 dependence that is observed during steady-state experiments can originate from both, the variable capacity to transfer the gas limiting substrate or by physiologic variations influencing $Y_{(X/CH4)}$	qCH_4 is strongly influenced by liquid and/or gas limitation	To resolute and statistically validate the variability of qCH_4 in a given set of experiment, it is suggested to first be able to control the process in either a liquid limited or a gas limited state. It is of importance that the culture is facing only one type of kinetic limitation
MER is not strictly linearly dependent on vvm	Like many scientific phenomena, linearity is only valid for a defined interval of values	The linearity between MER and vvm can be a valid first approximation if other influences, such as stirrer flooding or liquid limited states are avoided within design space
CH_4 offgas is a process variable which is influenced by different input process parameters	On one hand CH_4 offgas will be very much dependent on the physical chemistry involved in the transfer of the limiting gaseous substrate and on the other hand on the biocatalysis and proportion of carbon fixed in biomass	Gas transfer estimation is a challenging task in bioprocess quantification. Furthermore, during CO_2-BMP the conversion of H_2/CO_2 to CH_4 leads to a gas volume contraction. Hence, traditional methods for k_La determination have their limits. A strict control of the physico-chemical properties of the reaction broth is needed to be able to acquire comparable results. All assumptions are based on the prerequisite that physiological influences are negligible
More than 2 PCs are necessary to explain a min. 75% of the full CO_2-BMP data variability	The clustering and interpretation of data variability in an unstructured CO_2-BMP dataset requires the use of several dimensions or PCs in order to depict the dual nature of existing limitations	The number of necessary PCs to explain the variability of a given CO_2-BMP data set can be reduced if the data originates only from one of the limiting states (gas limitation or liquid limitation) as shown in Figure 6
Multivariate models can be mathematically valid but not necessarily statistically verified	Several factors can influence the outcome of a modelling endeavor. Either the data set is inappropriate, or the methods are not suitable for a given task	We suggest the readers to enhance preliminary statistical testing procedures of the dataset before selecting an appropriate method, a data transformation tool or removing outliers (Figure 1)
The mathematically valid models predict an exponential increase of the MER with increased vvm. Experiments where only one variable is change depict a logarithmic trend	Although the presented models exhibit a high validity coefficient, the correlations in proximity to the end of the design space need to be treated with care	The resulting trend obtained from data fitting depends much on the repartition of data points in a given space and the fitting method applied. If sufficient data was not available, *i.e.* at the borders of the design space, and the variability of reproduced experiments is high, one has to be very careful with the model trend line depiction (Figure 4 or Figure 7)
Due to the high variability no valid model for $Y_{(X/CH4)}$ as a function of process parameters could be established.	The physiology of a biocatalyst employed in a CO_2-BMP process is influenced by multiple parameters and the $Y_{(X/CH4)}$ is generally low	During CO_2-BMP using *M. marburgensis* $Y_{(X/CH4)}$ is shown to be between 0.01 and 0.07 C-mol mol^{-1}. Hence, in order to resolute the influence of varied process parameters on this variable it is suggested that the C-balance is closing between 99.5 to 100.5 % for modelling activities since CO_2 is assumed to only be transformed to either biomass or CH_4. Furthermore, carbonate scrubbing and subsequent carbon loss through the harvest also needs to be considered

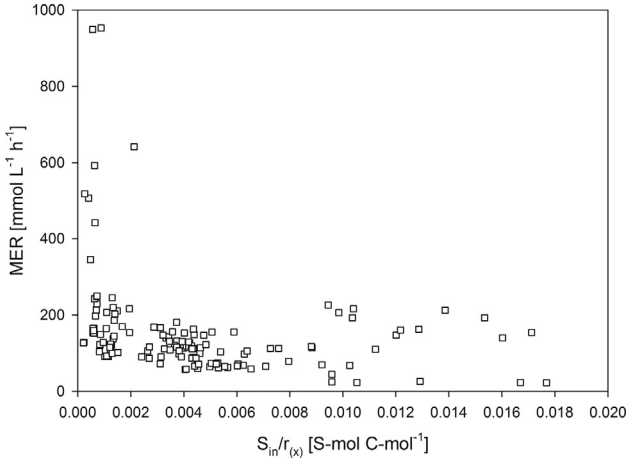

Fig. 9. MER values of CO_2-BMP continuous culture cultivations of *M. marburgensis* as function of the quotient of $S_{in}/r_{(x)}$ at different process conditions.

system, assuming the culture to be solely gas-limited [32]. While this approach can deliver valuable results, it is very limited in applicability, since the constraints that need to be made to keep the assumptions valid are narrow and are difficult to achieve. This is especially true as several studies have shown a strict separation of gas transfer limitations and liquid limitation. This interdependency adds a great deal of complexity to the modelling of any gas converting bioprocess and is particularly true for CO_2-BMP.

5. Conclusions

This work shows the inherent challenges faced in modelling CO_2-BMP. The most important aspect is the dependency of the performance on both, gas transfer limitations and liquid-based limitations. Utilizing PAT is inevitable in order to discriminate between these two factors. Implementing a real-time biomass sensor to correct the r_{inert} calculation method for the MER, where biomass formation is currently neglected, could result in an improved C- and DoR-balancing and would allow performing accurate and timely k_La determinations. Literature data often misses important information and/or the required accuracy for resolution of the underlying mechanistic effects, especially when modelling reactor dependent variables. Modelling can only be based on a mechanistic understanding of a particular process. Otherwise, modelling misinterpretation might occur. Understanding the mechanistic effects of CO_2-BMP could therefore assist the analysis and modelling of other gas-to-gas conversion bioprocesses.

Competing interests

AHS and SB declare to have competing interests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apenergy.2018.01.075.

References

[1] Sternberg A, Bardow A. Power-to-what? – Environmental assessment of energy storage systems. Energy Environ Sci 2015;8:389–400.
[2] Götz M, Lefebvre J, Mörs F, McDaniel Koch A, Graf F, Bajohr S, et al. Renewable power-to-gas: a technological and economic review. Renew. Energy 2016;85:1371–90.
[3] Rönsch S, Schneider J, Matthischke S, Schlüter M, Götz M, Lefebvre J, et al. Review on methanation – from fundamentals to current projects. Fuel 2016;166:276–96.
[4] Aresta M, Dibenedetto A, Angelini A. Catalysis for the valorization of exhaust

carbon: from CO_2 to chemicals, materials, and fuels. technological use of CO_2. Chem Rev 2014;114:1709–42.
[5] Caspeta L, Buijs NAA, Nielsen J. The role of biofuels in the future energy supply. Energy Environ Sci 2013;6:1077–82.
[6] Liao JC, Mi L, Pontrelli S, Luo S. Fuelling the future: microbial engineering for the production of sustainable biofuels. Nat Rev Microbiol 2016;14:288–304.
[7] Martinez-Porqueras E, Rittmann S, Herwig C. Biofuels and CO_2 neutrality: an opportunity. Biofuels 2012;3:413–26.
[8] Bernacchi S, Herwig C. Challenges and solutions for development of gas limited bioprocesses illustrated by the biological methane production (BMP) process development. Curr Biochem Eng 2016;3:1–12.
[9] Rittmann S, Seifert A, Herwig C. Essential prerequisites for successful bioprocess development of biological CH4 production from CO_2 and H_2. Crit Rev Biotechnol 2015;35:141–51.
[10] Rittmann SK-MR, Lee HS, Lim JK, Kim TW, Lee J-H, Kang SG. One-carbon substrate-based biohydrogen production: microbes, mechanism, and productivity. Biotechnol Adv 2015;33:165–77.
[11] Bernacchi S, Rittmann S, Seifert AH, Krajete A, Herwig C. Experimental methods for screening parameters influencing the growth to product yield (Y(x/CH4)) of a biological methane production (BMP) process performed with Methanothermobacter marburgensis. AIMS Bioeng 2014;1:72–86.
[12] Rittmann S, Seifert A, Herwig C. Quantitative analysis of media dilution rate effects on Methanothermobacter marburgensis grown in continuous culture on H_2 and CO_2. Biomass Bioenergy 2012;36:293–301.
[13] Seifert AH, Rittmann S, Bernacchi S, Herwig C. Method for assessing the impact of emission gasses on physiology and productivity in biological methanogenesis. Bioresour Technol 2013;136:747–51.
[14] Liu Y, Whitman WB. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci 2008;1125:171–89.
[15] Thauer RK, Kaster A-K, Seedorf H, Buckel W, Hedderich R. Methanogenic archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol 2008;6:579–91.
[16] Thauer RK, Kaster A-K, Goenrich M, Schick M, Hiromoto T, Shima S. Hydrogenases from methanogenic archaea, nickel, a novel cofactor, and H_2 storage. Annu Rev Biochem 2010;79:507–36.
[17] Alitalo A, Niskanen M, Aura E. Biocatalytic methanation of hydrogen and carbon dioxide in a fixed bed bioreactor. Bioresour Technol 2015;196:600–5.
[18] Burkhardt M, Busch G. Methanation of hydrogen and carbon dioxide. Appl Energy 2013;111:74–9.
[19] Guneratnam AJ, Ahern E, FitzGerald JA, Jackson SA, Xia A, Dobson ADW, et al. Study of the performance of a thermophilic biological methanation system. Bioresour Technol 2017;225:308–15.
[20] Lecker B, Illi L, Lemmer A, Oechsner H. Biological hydrogen methanation – a review. Bioresour Technol; 2017. doi: 10.1016/j.biortech.2017.08.176 [in press].
[21] Rachbauer L, Voitl G, Bochmann G, Fuchs W. Biological biogas upgrading capacity of a hydrogenotrophic community in a trickle-bed reactor. Appl Energy 2016;180:483–90.
[22] Strübing D, Huber B, Lebuhn M, Drewes JE, Koch K. High performance biological methanation in a thermophilic anaerobic trickle bed reactor. Bioresour Technol; 2017. doi: 10.1016/j.biortech.2017.08.088 [in press].
[23] Wise DL, Cooney CL, Augenstein DC. Biomethanation: Anaerobic fermentation of CO_2, H_2 and CO to methane. Biotechnol Bioeng 1978;20:1153–72.
[24] Rittmann SK-MR. A critical assessment of microbiological biogas to biomethane upgrading systems. Adv Biochem Eng Biotechnol 2015;151:117–35.
[25] Nishimura N, Kitaura S, Mimura A, Takahara Y. Cultivation of thermophilic methanogen KN-15 on H_2-CO_2 under pressurized conditions. J Ferment Bioeng 1992;73:477–80.
[26] Seifert AH, Rittmann S, Herwig C. Analysis of process related factors to increase volumetric productivity and quality of biomethane with methanothermobacter marburgensis. Appl Energy 2014;132:155–62.
[27] Takai K, Nakamura K, Toki T, Tsunogai U, Miyazaki M, Miyazaki J, et al. Cell proliferation at 122 degrees C and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation. Proc Natl Acad Sci USA 2008;105:10949–54.
[28] Taubner R-S, Schleper C, Firneis MG, Rittmann SK-MR. Assessing the ecophysiology of methanogens in the context of recent astrobiological and planetological studies. Life 2015;5:1652–86.
[29] Budzianowski WM, Postawa K. Renewable energy from biogas with reduced carbon dioxide footprint: implications of applying different plant configurations and operating pressures. Renew Sustain Energy Rev 2017;68(Part 2):852–68.
[30] Rachbauer L, Beyer R, Bochmann G, Fuchs W. Characteristics of adapted hydrogenotrophic community during biomethanation. Sci Total Environ 2017;595:912–9.
[31] Strevett KA, Vieth RF, Grasso D. Chemo-autotrophic biogas purification for methane enrichment: mechanism and kinetics. Chem Eng J Biochem Eng J 1995;58:71–9.
[32] Schill N, van Gulik WM, Voisard D, von Stockar U. Continuous cultures limited by a gaseous substrate: Development of a simple, unstructured mathematical model and experimental verification with Methanobacterium thermoautotrophicum. Biotechnol Bioeng 1996;51:645–58.
[33] Schill NA, Liu J-S, von Stockar U. Thermodynamic analysis of growth of methanobacterium thermoautotrophicum. Biotechnol Bioeng 1999;64:74–81.
[34] Kyazze G, Martinez-Perez N, Dinsdale R, Premier GC, Hawkes FR, Guwy AJ, et al. Influence of substrate concentration on the stability and yield of continuous biohydrogen production. Biotechnol Bioeng 2006;93:971–9.
[35] Bernacchi S, Krajete A, Herwig C. Experimental workflow for developing a feed forward strategy to control biomass growth and exploit maximum specific methane productivity of Methanothermobacter marburgensis in a biological methane production process (BMPP). AIMS Microbiol 2016;2:262–77.
[36] de Poorter LMI, Geerts WJ, Keltjens JT. Coupling of Methanothermobacter thermautotrophicus methane formation and growth in fed-batch and continuous cultures under different H_2 gassing regimens. Appl Environ Microbiol 2007;73:740–9.
[37] Jee HS, Nishio N, Nagai S. Continuous CH_4 Production from H_2 and CO_2 by Methanobacterium thermoautotrophicum in a fixed-bed reactor. J Ferment Technol 1988;66:235–8.
[38] Jee HS, Nishio N, Nagai S. CH_4 production from H_2 and CO_2 by Methanobacterium thermoautotrophicum cells fixed on hollow fibers. Biotechnol Lett 1988;10:243–8.
[39] Jee HS, Yano T, Nishio N, Nagai S. Biomethanation of H_2 and CO_2 by Methanobacterium thermoautotrophicum in membrane and ceramic bioreactors. J Ferment Technol 1987;65:413–8.
[40] Martin MR, Fornero JJ, Stark R, Mets L, Angenent LT. A single-culture bioprocess of Methanothermobacter thermautotrophicus to upgrade digester biogas by CO_2-to-CH_4 by conversion with H_2. Archaea 2013;2013:e157529.
[41] Morii H, Koga Y, Nagai S. Energetic analysis of the growth of Methanobrevibacter arboriphilus A2 in hydrogen-limited continuous cultures. Biotechnol Bioeng 1987;29:310–5.
[42] Nishimura N, Kitaura S, Mimura A, Takahara Y. Growth of thermophilic methanogen KN-15 on H_2-CO_2 under batch and continuous conditions. J Ferment Bioeng 1991;72:280–4.
[43] Peillex J-P, Fardeau M-L, Belaich J-P. Growth of Methanobacterium thermoautotrophicum on H_2/CO_2: high CH_4 productivities in continuous culture. Biomass 1990;21:315–21.
[44] Tsao JH, Kaneshiro SM, Yu SS, Clark DS. Continuous culture of Methanococcus jannaschii, an extremely thermophilic methanogen. Biotechnol Bioeng 1994;43:258–61.
[45] Taubner R-S, Rittmann SK-MR. Method for indirect quantification of CH_4 production via H_2O production using hydrogenotrophic methanogens. Microbiotechnol Ecotoxicol Bioremed 2016:532.
[46] Aceves-Lara CA, Latrille E, Buffiere P, Bernet N, Steyer J-P. Experimental determination by principal component analysis of a reaction pathway of biohydrogen production by anaerobic fermentation. Chem Eng Process Process Intensif., SIMO 2006 2008;47:1968–75.
[47] Buttigieg PL, Ramette A. A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. FEMS Microbiol Ecol 2014;90:543–50.
[48] Abdel Azim A, Pruckner C, Kolar P, Taubner R-S, Fino D, Saracco G, et al. The physiology of trace elements in biological methane production. Bioresour Technol 2017;241:775–86.
[49] Spadiut O, Rittmann S, Dietzsch C, Herwig C. Dynamic process conditions in bioprocess development. Eng Life Sci 2013;13:88–101.
[50] Schönheit P, Moll J, Thauer RK. Growth parameters (K s, μmax, Ys) of Methanobacterium thermoautotrophicum. Arch Microbiol 1980;127:59–65.
[51] Takors R. Scale-up of microbial processes: Impacts, tools and open questions. J Biotechnol Genome-based Microbiol -omics Res Syst Synth Biol 2012;160:3–9.
[52] Liu J-S, Schill N, van Gulik WM, Voisard D, Marison IW, von Stockar U. The coupling between catabolism and anabolism of Methanobacterium thermoautotrophicum in H2- and iron-limited continuous cultures. Enzyme Microb Technol 1999;25:784–94.
[53] Fardeau M-L, Peillex J-P, Belaïch J-P. Energetics of the growth of Methanobacterium thermoautotrophicum and Methanococcus thermolithotrophicus on ammonium chloride and dinitrogen. Arch Microbiol 1987;148:128–31.
[54] Chmiel H, Takors R, Weuster-Botz D. Bioprozesstechnik. Springer; 2018.
[55] Nischkauer W, Vanhaecke F, Bernacchi S, Herwig C, Limbeck A. Radial line-scans as representative sampling strategy in dried-droplet laser ablation of liquid samples deposited on pre-cut filter paper disks. Spectrochim Acta Part B At Spectrosc 2014;101:123–9.
[56] Bernacchi S, Weissgram M, Wukovits W, Herwig C. Process efficiency simulation for key process parameters in biological methanogenesis. AIMS Bioeng 2014;1:53–71.
[57] Duboc P, Schill N, Menoud L, van Gulik W, von Stockar U. Measurements of sulfur, phosphorus and other ions in microbial biomass: influence on correct determination of elemental composition and degree of reduction. J Biotechnol 1995;43:145–58.
[58] O'Flaherty V, Mahony T, O'Kennedy R, Colleran E. Effect of pH on growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic and sulphate-reducing bacteria. Process Biochem 1998;33:555–69.
[59] Inkeri E, Tynjälä T, Laari A, Hyppänen T. Dynamic one-dimensional model for biological methanation in a stirred tank reactor. Appl Energy 2018;209:95–107.