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ORIGINAL PAPER

Phototrophic bacteria dominate consortia, potentially to remove CO_2 and H_2S from biogas under microaerophilic conditions

M. Quiroz[1] · J. Orlando[2] · M. Carú[2]

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Abstract The use of microbial consortia to remove contaminants in industrial systems and in natural environments could be an alternative to the use of unique strains of microorganisms, since microbial consortia have greater robustness to environmental fluctuations. However, it is necessary to evaluate the relationship between the genetic structure and functionality of the consortia. In this work, the functional and structural stability over time of two bacterial consortia (C5 and C6) with the potential to remove CO_2 and H_2S from biogas was evaluated. Both consortia decreased the dissolved CO_2 by over 30% at the end of the incubation period, but C5 presented shorter removal kinetics (3.9 days) than C6 (6.4 days). Additionally, a chemical oxidation of H_2S could have occurred in the microcosms. Moreover, both consortia presented a stable genetic structure, measured by terminal restriction fragment length polymorphism profiles of the 16S rRNA gene, characterized by high homogeneity and prevalence of the genus *Rhodopseudomonas* throughout the incubation period, and an increasing abundance of *Xanthobacter* during the exponential phase of the growth curve in C5, which would account for the functionality of the consortia.

Keywords Fingerprinting · Microcosms · Purple non-sulfur bacteria · *Rhodopseudomonas*

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✉ M. Quiroz
madelaine.quiroz@usach.cl

[1] Facultad Tecnológica, Universidad de Santiago de Chile, Santiago, Chile

[2] Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Introduction

Biogas is a renewable energy source since CH_4, obtained as a gaseous product of anaerobic digestion from organic matter, offers an economical attractive alternative to fossil fuels (Weiland 2010). However, despite its attractiveness in terms of generating heat and electricity, it requires a treatment process prior to its use because in addition to CH_4, which determines the calorific value, it also contains CO_2 and H_2S, which are considered pollutants (Ryckebosch et al. 2011). In recent decades, biological methods for removing these contaminants have been explored, based primarily on the use of microorganisms either as single strains or consortia to metabolize the pollutants from biogas.

Among the reported single-strain strategies, bacterial phototrophs which metabolize H_2S, such as green sulfur bacteria (*Chlorobium* sp.) (Cork et al. 1985; Henshaw and Zhu 2001) or purple sulfur bacteria (*Allochromatium* sp.) (Borkenstein and Fischer 2006), have been utilized; chemolithotrophs, such as *Thiobacillus* sp. and *Acidithiobacillus* sp. (Ramirez et al. 2009; Tang et al. 2009), have also been employed; and even *Actinobacillus succinogenes*, which metabolizes CO_2 in the presence of glucose (Gunnarsson et al. 2014), has been used. Archaea, such as *Methanobacterium thermoautotrophicum* which uses CO_2 and H_2 to produce CH_4 (Strevett et al. 1995), and microalgae, such as *Chlorella* sp. which fixes CO_2 (Demirbas 2011; Cuellar-Bermudez et al. 2015), have also been utilized in such studies.

On the other hand, some studies have used bacterial consortia that extract H_2S from biogas using biofilters, in these studies the predominant microorganisms were sulfur-oxidizing bacteria such as *Bosea thiooxidans* and *Thiobacillus* sp. (Garcia-Peña et al. 2012), *Bacillus,*

Pseudomonas and *Xanthomonadaceae* (Omri et al. 2011) and *Xanthomonadaceae* and *Hydrogenophilaceae* (Chouari et al. 2015). In other studies, the transformation of CO_2 to CH_4 was favored by methanogens present in sludge, increasing the proportion of CH_4 while simultaneously decreasing the proportion of CO_2 in the gaseous product of the system (Alimahmoodi and Mulligan 2008). More recently, the use of a consortium of bacteria and algae to remove both CO_2 and H_2S from biogas has been reported (Sun et al. 2016).

When consortia are used, the members of the consortium interact, with each performing specific functions that contribute to the overall functionality of the system. An essential aspect that characterizes these types of microbial relationships is that they have greater robustness against environmental fluctuations when compared to populations formed by only one type of organism (Brenner et al. 2008). Control and regulation of pollutant removal depend on the type and activity of the main bacterial populations, as they determine the behavior of the process. However, it is necessary to understand the relationship between the genetic structure and dynamics of microorganisms which provide the consortia's physiological properties in order to establish the conditions necessary for the removal of contaminants from biogas (Maestre et al. 2009).

Previously, two bacterial consortia with the potential to remove CO_2 and H_2S from biogas in microaerophilic conditions (C5 and C6) were selected from the leachate of a sanitary landfill (Quiroz 2014). These leachates were drawn from six of the sanitary landfill's wells, constituting six inocula, which were grown separately and subjected to a selection process, through which the two bacterial consortia evaluated in the present work were obtained. The composition of the bacterial consortia selected was determined using clone libraries of the 16S ribosomal RNA gene. The clone libraries showed that *Rhodopseudomonas* dominated in both consortia, and in one of them (C5), members of the genus *Xhantobacter* were also found in high proportion (Quiroz 2014). In the present work, the structural and functional stability of these two bacterial consortia, which have the potential to remove CO_2 and H_2S from biogas in microaerophilic conditions, was evaluated at the Microbial Ecology Laboratory of the Universidad de Chile during the year 2013.

Materials and methods

Sample source

Bacterial consortia C5 and C6 were selected from leachate samples obtained at a sanitary landfill, located in the San Bernardo County in the Metropolitan Region of Santiago (Quiroz 2014). Consortia C5 and C6 were incubated, in triplicate, in serological bottles of 100 ml with aluminum seals and rubber stoppers, at 25 °C for 13 days with continuous illumination from 25-W incandescent lamps in microaerophilic conditions (the atmosphere of the biogas). The initial biogas composition (*v/v*) was: 76% CH_4, 24.5% CO_2, 0.05% H_2S and 0.3% O_2. Physicochemical variables and T-RFLP profiles were determined from eight samples, taken on days 1, 2, 4, 5, 6, 7, 10 and 13 of the incubation period. Bacterial growth was initiated by adding 6 ml of standardized inoculum ($OD_{600nm} = 0.45$) to 60 ml of medium 2.0 consisting of 0.2 mM $MgSO_4 \cdot 7\ H_2O$, 0.1 mM $CaCl_2 \cdot 2\ H_2O$, 0.02 mM NaEDTA, 10 mM NH_4Cl, 1.5 mg/l $FeSO_4 \cdot 7H_2O$, 5 mM potassium phosphate buffer, 1 ml/l of a micronutrient solution, and 1 ml/l of a vitamin stock solution (Kamp et al. 2006) plus 10 mM of sodium acetate and 50 µg/l of vitamin B12. As in the selection stage of these consortia, differences in the measured variables were detected in the aqueous phase (Quiroz 2014); then, the stability measurements were performed on the culture medium during this stage.

Functionality of the consortia

First, a growth curve for each consortium was performed by monitoring the total protein concentration (*Proteins*) using the Bradford colorimetric method (BIOQUANT® Protein, Merck). In addition, photosynthetic biomass was determined by measuring bacteriochlorophyll a (*Bchlo a*), using the method proposed by Oelze (1985). Both determinations were performed in triplicate. Subsequently, data regarding the concentration of *Proteins* and *Bchlo a* over time were adjusted using the modified Gompertz curve (Zwietering et al. 1990), described by the equation:

$$y = A\ \exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda - t) + 1\right]\right\}$$

where $P = Proteins$ or $Bchlo\ a$ (µg/ml) at time t, A = maximum *Proteins* or *Bchlo a* (µg/ml), μ_m = maximum specific growth rate ((µg/ml)/day), λ = lag period (days) and t = time (days).

In addition, concentrations of dissolved CO_2, sulfate and pH in the culture medium were determined as described in Quiroz (2014) while the concentration of sulfide was determined by the selective ion method using a pH/Ion 510 OAKTON® analyzer (Clesceri et al. 1998).

In order to compare the curves describing the kinetics of CO_2 removal, first the removal rate of dissolved CO_2 was calculated with the experimental data obtained from dissolved CO_2 for each consortium (C5 and C6) and for the control without inoculum according to the following expression:

$$TR = (((C_c - C_m)/C_c) * 100)$$

where TR = rate of accumulated dissolved CO_2 (%) over time, C_c = concentration of dissolved CO_2 (ppm) in the control without inoculum at time t and C_m = concentration of dissolved CO_2 (ppm) in a consortium at time t.

Finally, the percentage changes in the CO_2 concentration were adjusted to a hyperbolic function:

$$RCO_2 = ((B_{\max} * t)/(K_d + t))$$

where R_{CO_2} = removal of accumulated CO_2 (%), $B_{\max}$ = maximum CO_2 (%) consumed by each consortium, K_d = constant representing the time required to reach half of $B_{\max}$ and t = time (days).

Genetic structure of the consortia

The genetic structure of the consortia was determined by T-RFLP profiles of the gene encoding the 16S ribosomal RNA (16S rRNA). For this, the cell pellet was obtained by centrifuging (13,000 rpm for 5 min) and the DNA was purified from the samples using the "Ultra Clean DNA" kit (MoBio Lab, Inc). Purified DNA was used to amplify through PCR the gene 16S rRNA using universal primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′), fluorescently labeled with FAM (6-carboxyfluorescein) and rP2 (5′–ACGGCTACCTTGTTACGACTT-3′) (Weisburg et al. 1991). The reaction mixture (25 µl) contained GoTaq® Green Master Mix (GoTaq® DNA polymerase in 1X Green GoTaq® reaction buffer (pH 8.5), 200 µM of each dNTP and 1.5 mM $MgCl_2$) (Promega) and 10 µM of each primer. The amplification program consisted of an initial denaturation at 94 °C for 3 min, followed by 30 cycles with a denaturation at 94 °C for 1 min, hybridization at 57 °C for 30 s and elongation at 72 °C for 2 min; the final extension occurred at 72 °C for 7 min. The amplicons of the 16S rRNA gene of approximately 1500 bp were analyzed in 1.2% agarose gels dyed with GelRed™.

The amplicon mixtures were digested with 30 units of the restriction enzyme *Alu*I (Fermentas) for 12 h at 37 °C, as recommended by the manufacturer. The enzyme was inactivated at 65 °C for 20 min, and the digested fragments were purified by alcohol precipitation. The fragments were separated by electrophoresis with an ABI3730XL Genetic Analyzer (Applied Biosystems; Macrogen Inc., Korea). The length of the fluorescently labeled terminal restriction fragments (T-RFs) was determined by comparison with internal standards using the GeneScan 3.71 software (Applied Biosystems). An electropherogram (T-RFLP profile) was generated for each consortium at the different incubation times, where the number of T-RFs of each sample (richness) and the fluorescence of each T-RF (abundance) were determined.

The peaks with a fluorescence of 30 U and larger than 50 bp were analyzed by peak height. Patterns from different samples were normalized to identical total fluorescence units by an iterative standardization procedure (Dunbar et al. 2001). In addition, manual alignment of T-RF profiles was necessary to prevent misidentification of the T-RFs due to biases of electrophoresis. Then, the relative abundance of T-RFs as a percentage was determined by calculating the ratio between the height of a given peak and the normalized total peak height of each sample.

In addition, in order to compare the obtained T-RFs with the bacteria identity detected in each consortium, the sequences of the clones obtained by Quiroz (2014) from consortia C5 and C6 were digested in silico with the enzyme *Alu*I.

Experimental design and statistical analyses

The experimental design consisted of a CRD (completely randomized design) with two treatments corresponding to consortia C5 and C6 with three repetitions. The experimental units were composed of serological 100-ml bottles.

In order to compare the growth curves between consortia, measured as *Proteins* or *Bchlo a* over time, parameters A (maximum *Proteins* or *Bchlo a*), μ_m (maximum specific growth rate) and λ (lag period) were compared using ANOVA ($p \leq 0.05$) and a Tukey posttest ($p \leq 0.05$). Similarly, for comparing the curves describing the kinetics of CO_2 removal from both consortia, parameters $B_{\max}$ (maximum removal potential) and K_d (removal kinetic constant) were analyzed by ANOVA ($p \leq 0.05$) and a Tukey posttest. Finally, to determine variations in the concentration of the dissolved CO_2 at the beginning and end of the incubation period, a t test for dependent samples was performed for each consortium and for the control without inoculum ($p \leq 0.05$). Statistical analyses were performed with Origin 8 software (OriginLab Corporation).

Results and discussion

Functionality of the consortia C5 and C6

In order to evaluate the performance of the consortia during their growth, stability was evaluated by studying some of their physiological properties and their genetic structure. However, since differences in the measured variables were found in the aqueous phase during the selection stage of these consortia (Quiroz 2014), stability measurements were performed on the culture medium during this stage.

Growth curves of the consortia (C5 and C6), measured as *Protein* or *Bchlo a* concentrations over time, are shown

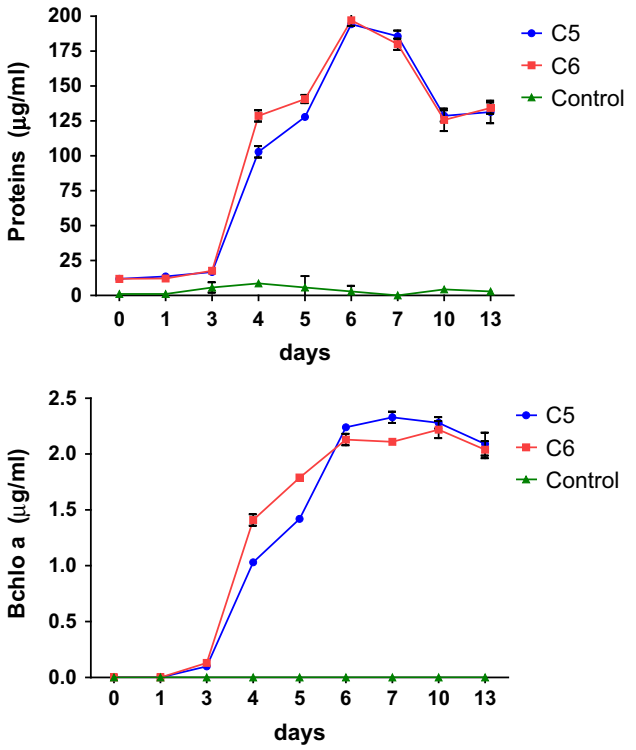

Fig. 1 Growth curves measured as total proteins (*Proteins*; upper panel) or bacteriochlorophyll a (*Bchlo a*; lower panel) throughout incubation. *Bars* indicate standard deviation. Consortia: C5, consortium 5; C6, consortium 6; control: culture without inoculum

in Fig. 1. *Protein* values of the consortia ranged from 13.1 µg/ml on day 1 to 197.1 µg/ml on day 6, starting the exponential phase on day 3 and reaching maximum growth on day 6 of incubation. In the case of photosynthetic biomass, values of *Bchlo a* ranged from 0 µg/ml on day 1 to 2.3 µg/ml on day 7, with the exponential phase initiating on day 3 and reaching the maximum growth on day 7 for C5 and day 6 for C6.

Furthermore, experimental data regarding *Protein* and *Bchlo a* concentrations were adjusted using the modified Gompertz curve (Table 1). When evaluating the parameters of the curves based on the measurement of the *Protein* concentration, significant differences were found in the maximum growth rate (μ_m), which was significantly lower in C5 than in C6. A similar situation was observed when growth was measured as the photosynthetic biomass (*Bchlo a* concentration) (Table 1). These results, together with those obtained in the gene libraries of C5 and C6 and the detection of the molecular marker *PufM* (Quiroz 2014), indicate that the functionality of these consortia could be determined primarily by the activity of bacteria that perform photosynthesis.

In both consortia, an increase in the culture medium's CO_2 consumption coincided with the exponential phase of the growth curve, which is reflected in the decrease in dissolved CO_2 between days 3 and 6 compared to the control without inoculum (Fig. 2). However, a lower concentration of dissolved CO_2 was observed on day 10. Moreover, the results of dissolved CO_2 determined during the stationary phase could be considered as an indicator of consortia stability (Fig. 2), since a sustained decrease was observed in both consortia, especially compared to the control without inoculum. This is consistent with the non-detection of heterotrophic bacteria activity generating CO_2, which could have led to an increase in its concentration in the culture medium.

The concentration of dissolved CO_2 decreased by ($p \leq 0.05$) 34.7% in C5 and 32.5% in C6 at the end of the incubation period; however, in the control without inoculum no significant variation was detected ($p > 0.05$) (Table 2). This suggests that the reduction in dissolved CO_2 in the consortia at the end of the incubation period was most likely due to the consumption of CO_2 by the bacterial biomass constituting the consortia, which is consistent with the increase in the bacterial biomass during the exponential phase of the growth curve. Additionally, it should be considered that before bubbling biogas in the culture medium, no dissolved CO_2 was detected; therefore, the CO_2 detected in the control without inoculum would have come only from the biogas. Thus, the greatest concentration of CO_2 in the control suggests that CO_2 from biogas was dissolved in the culture medium and accumulated as dissolved CO_2 (Fig. 2).

During the incubation period, the pH of both consortia remained in the neutral range between 6.3 and 7.0 (Fig. 3), showing a slight decrease during the first 3 days, which coincides with the lag phase of the growth curve where $CO_{2(g)}$ was dissolved in the culture medium as $CO_{2(aq)}$ and was then dissociated, lowering the pH. Afterward, a slight increase was observed until day 7, a period that coincides approximately with the exponential phase of the growth curve where an increase in photosynthetic biomass and a decrease in the concentration of dissolved CO_2 were evidenced. Finally, pH was relatively constant until the end of the incubation period. In the case of the control without inoculum, pH decreased at the start of incubation until day

Table 1 Kinetic parameters (A, μ_m and λ) obtained by adjusting growth measurements to the modified Gompertz curve

Growth measurement	Consortia	A (μg/ml)	μ_m ((μg/ml)/day)	λ (days)	R^2
Proteins	C5	156.9a	96.2b	2.9a	0.86204
(μg/ml)	C6	156.5a	135.1a	2.9a	0.82531
Bchlo a	C5	2.27a	0.93b	3.0a	0.97342
(μg/ml)	C6	2.09a	1.35a	3.0a	0.98752

Different lowercase letters, arranged vertically, indicate significant differences ($p \leq 0.05$) between consortia for each type of growth measurement (total proteins: *Proteins*; or bacteriochlorophyll a: *Bchlo a*) according to ANOVA and Tukey posttest ($p \leq 0.05$). A: maximum concentration of *Proteins* or *Bchlo a*, μ_m: maximum specific growth rate and λ: lag phase. R^2: correlation coefficient. Consortia: C5, consortium 5; C6, consortium 6

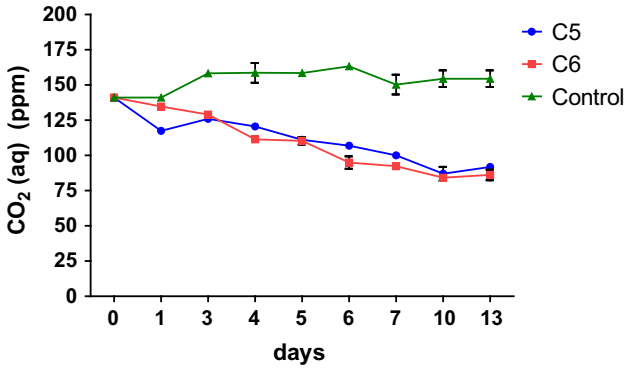

Fig. 2 Kinetic results of dissolved $CO_{2\ (aq)}$ consumption throughout incubation. *Bars* indicate standard deviation. Consortia: C5, consortium 5; C6, consortium 6; Control: culture without inoculum

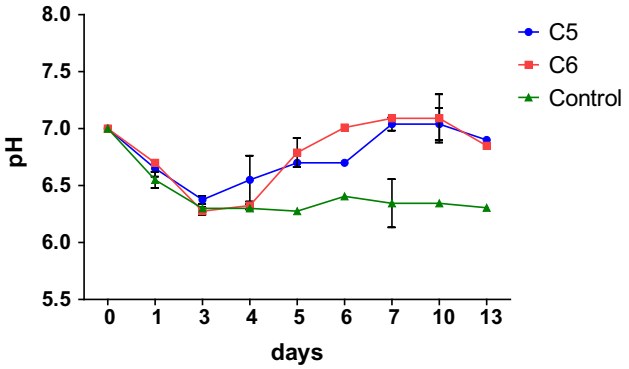

Fig. 3 pH values measured throughout incubation. *Bars* indicate standard deviation. Consortia: C5, consortium 5; C6, consortium 6; Control: culture without inoculum

Table 2 Dissolved CO_2 (ppm) measurements at the initial and final states for each consortium in an incubation period of 13 days

Consortia	Dissolved CO_2 (ppm)		Statistic
	Initial	Final	
C5	140.5 ± 0.7	91.8 ± 0.1	($t = 97.30$; $p = 0.007$)
C6	140.5 ± 0.7	94.9 ± 4.4	($t = 17.70$; $p = 0.036$)
Control	141.3 ± 1.2	154.5 ± 5.9	($t = -2.67$; $p = 0.228$)

Average ± standard deviations of three measurements are shown. Values t and p are given according to the t test for dependent samples calculated for each row. Consortia: C5, consortium 5; C6, consortium 6; Control: culture without inoculum

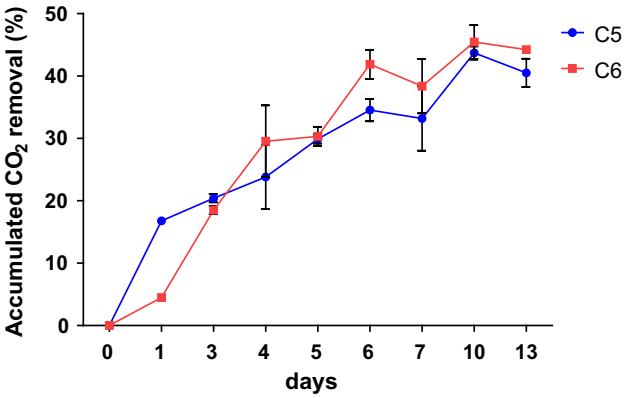

Fig. 4 Kinetics of removal of CO_2 throughout incubation. *Bars* indicate standard deviation. Consortia: C5, consortium 5; C6, consortium 6

3, after which it remained largely unchanged with values close to 6.3.

The maximum removal of dissolved CO_2 was achieved in both consortia on day 10 of the incubation period (Fig. 4). Subsequently, these data were fitted using a hyperbolic curve, and the values of parameters B_{max} and K_d from the adjusted curves for each consortium are shown in Table 3. Significant differences ($p \leq 0.05$) between the consortia for both parameters were detected. C6 presented

Table 3 Parameters (B_{max} and K_d) obtained by adjusting accumulated CO_2 removal percentages to a hyperbolic curve

Consortia	B_{max} (%)	K_d (days)	R^2
C5	53.6c	3.9b	0.9573
C6	68.6a	6.4a	0.9522

Different lowercase letters, arranged vertically, indicate significant differences ($p \leq 0.05$) between consortia for each parameter according to ANOVA and Tukey posttest ($p \leq 0.05$). B_{max}: maximum percentage of CO_2 removed by a consortium, K_d: constant representing the time required to reach half of B_{max}. R^2: correlation coefficient. Consortia: C5, consortium 5; C6, consortium 6

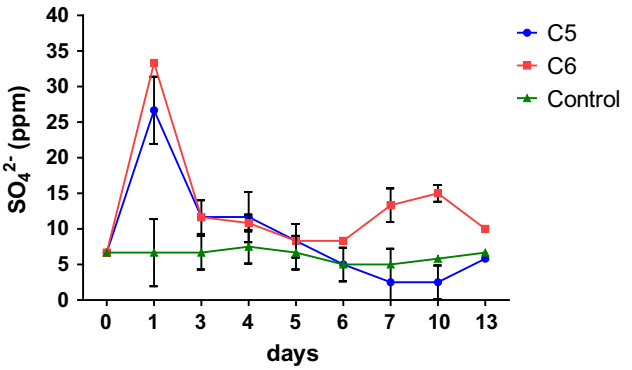

Fig. 5 Sulfate concentration curve throughout incubation. *Bars* indicate standard deviation. Consortia: C5, consortium 5; C6, consortium 6; Control: culture without inoculum

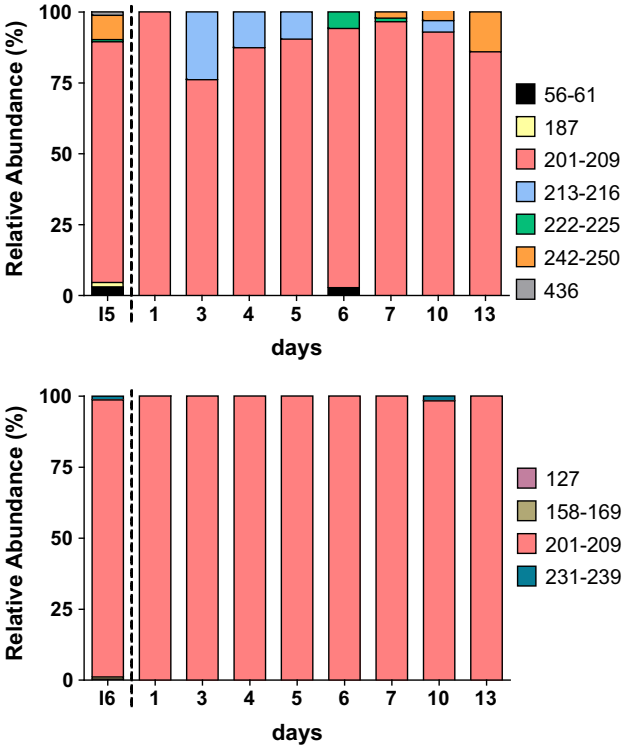

Fig. 6 Relative frequencies of the terminal restriction fragments (T-RFs) of the consortia C5 (*upper panel*) and C6 (*lower panel*) throughout incubation. I5 and I6: inoculum of the consortia C5 and C6, respectively. Different numbers in the legend represent the lengths in base pairs of the T-RFs

a significantly higher ($p \leq 0.05$) maximum CO_2 removal potential (B_{max}) than C5, but C5 presented a significantly higher ($p \leq 0.05$) removal kinetic constant (K_d) than C6, reaching its maximum removal potential in less time. Therefore, C6 could be used in biogas atmospheres with high concentrations of dissolved CO_2, and C5 could be used when a decrease in the time required to reduce the CO_2 concentration is desirable.

Sulfide was not detected in the culture medium during the incubation period. However, in both consortia the sulfate concentration increased during the first 24 h (Fig. 5), which could represent an intracellular accumulation of an intermediary from the oxidation of thiosulfate, followed by oxidation to sulfate (Rolls and Lindstrom 1967). Subsequently, the sulfate concentration decreased in the exponential phase, suggesting sulfate consumption by the biomass (Imhoff 2006; Wiegel 2006). In C6, the sulfate concentration increased between days 8 and 10, and during that same period the sulfate concentration decreased in C5, becoming even lower than in the control without inoculum. These results suggest that part of the biomass contained by that consortium could metabolize more sulfate than C6, due to differences in the bacterial composition of each consortium. Afterward, values close to the sulfate concentration of the control without inoculum were obtained in both consortia, which did not significantly vary during the incubation period. From these monitoring results, it is not possible to confirm the biological oxidation of thiosulfate as proposed in the consortia selection step, which considered a previous chemical oxidation of H_2S from biogas into thiosulfate (Quiroz 2014).

Genetic structure of the consortia C5 and C6

In order to evaluate the stability of the consortia at different sampling times, genetic structure was determined by T-RFLP profiles of the gene coding 16S rRNA. Similarly, the T-RFLP profiles of the inocula of each consortia were determined (Fig. 6). Finally, the identity of each T-RF was inferred by determining potential T-RFs of each bacterial genera previously detected in the gene libraries of these consortia (Quiroz 2014).

In the inoculum of C5 (I5), 87.2% of the T-RFs were related to the genus *Rhodopseudomonas*, while the genera *Xanthobacter* and *Castellaniella* constituted 12.9% of the remaining T-RFs. However, in the inoculum of C6 (I6) the four T-RFs obtained were associated with *Rhodopseudomonas*.

These results show that in the T-RFLP profiles of inocula, fewer genera were found than when compared with those identified in the libraries of each consortium (Quiroz 2014), which could be explained by the elimination of T-RFs which were only slightly represented in the sample (low fluorescence) during the standardization of the method because they were in the discrimination limit of the technique (Dunbar et al. 1999; Schütte et al. 2008). The case of C6 is representative of the genera *Sphingobium* and *Xanthobacter* and the family *Alcaligenaceae*.

The T-RFLP profiles of the consortia over time showed that in both cases they have a stable genetic structure, characterized by high homogeneity during the incubation period (Fig. 6), especially the detection of a predominant T-RF (201–209) associated with *Rhodopseudomonas*. This homogeneity is due to both the enrichment of the culture from which the consortia were obtained and the metabolic versatility of *Rhodopseudomonas* (Larimer et al. 2004; Carlozzi et al. 2006; Karpinets et al. 2009). Therefore, *Rhodopseudomonas,* which belongs to a genus of purple non-sulfur photosynthetic bacteria with a clear dominance during the incubation period, could account for the physiological properties of the consortia.

Since bacteria of the genus *Rhodopseudomonas* have presented high metabolic versatility in terms of the sources of carbon and energy they can use (Larimer et al. 2004), under the consortia culture conditions (microaerophilic and photosynthetic) it is expected that they use CO_2 as a carbon source and acetate as an electron donor, as reported by Butow and Bergstein-Ben Dan (1991) in similar experimental conditions to those in this study. This is consistent with the diminution of CO_2 observed in the exponential phase of the growth curves.

Additionally, in C5, the appearance of T-RF 213–216 was observed on days 3, 4, 5 and 10 of incubation, which reduced its relative abundance with increasing incubation time and is associated with *Xanthobacter*. Therefore, *Rhodopseudomonas* predominated in C5 during the 13-day incubation period, but on days 3, 4 and 5, coinciding with the exponential growth phase, *Xanthobacter* was detected and also reappeared in a low percentage on day 10 of the incubation period. Changes in the abundance of T-RF 242–250, which is associated with the phylum *Bacteroidetes*, were also observed during the stationary phase.

According to the aforementioned observations, bacteria of the genus *Xanthobacter* could contribute to the CO_2 consumption capacity, which were detected in 24.5% in the C5 library (Quiroz 2014) and in the T-RFLP profiles during the exponential phase of the growth curve. Representatives of the genus *Xanthobacter* have an autotrophic metabolism, fixing CO_2 and using various sources of energy (Meijer et al. 1990; Padden et al. 1998). Furthermore, they could have a heterotrophic growth (Meijer et al. 1990), or even a mixotrophic growth, using acetic acid and other organic compounds as carbon sources (Padden et al. 1998).

On the other hand, *Bacteroidetes* is a bacterial phylum that is poorly represented in the libraries of C5 and C6, but did show an increasing abundance in the T-RFLP profiles of C5 in the stationary phase of the growth curve. The representatives of this phylum have a flexible metabolism that allows them to colonize aerobic, microaerophilic and anaerobic environments. These bacteria have been described as related to the degradation of organic matter (Thomas et al. 2011) and to monosaccharides as glucose (Ito et al. 2012) which, when fermented, produce fatty acids such as propionate, succinate and acetate. Members of this phylum need high concentrations of CO_2 either because they must fix it, or because under these conditions they compete more successfully for resources (Fischbach and Sonnenburg 2011). Therefore, depending on the genus of *Bacteroidetes* present in the consortium, it could contribute to CO_2 fixation or could generate fatty acids by fermentation that may in turn be metabolized by *Rhodopseudomonas*,

helping to maintain the functionality of the consortium over time.

Generally, a microbial community constituted by species or functional groups capable of a differential response can maintain or increase their stability over time; conversely, specialized communities with few dominant microbial species might be more fragile and require longer recovery times (McCann 2000).

In the case of the consortia studied here, the existence of a dominant bacterial type over time could theoretically suggest the establishment of a highly unstable system. However, the metabolic versatility of *Rhodopseudomonas* could explain their rapid acclimation to abiotic changes during the incubation period, reflected in the dominant T-RFs (202–209; 222–225; and 231–239) associated with this bacterial genus, which also further explains the homogeneity found in the structure of the consortia, especially in C6. Further studies are necessary in order to evaluate the behavior of the consortia under modified incubation conditions, by increasing, for instance, the acetate concentration in the culture medium and/or by increasing the partial pressure of CO_2 in the biogas input by increasing the total pressure of biogas, which would increase the solubility of CO_2 and its availability for bacterial consortium. This situation was reported by Gunnarsson et al. (2014), who by increasing the total pressure of biogas from 101.3 kPa to 140 kPa (equivalent to a CO_2 partial pressure of 56 kPa) increased the rate of consumption of CO_2 by *Actinobacillus succinogenes* 130Z, thereby decreasing CO_2 in the exit gas as well as increasing the proportion of CH_4, by decreasing the proportion of CO_2 in the gas. Thus, it seeks to promote the reduction of CO_2 dissolved in the aqueous phase and the CO_2 in the gas phase at the end of the incubation period.

Finally, according to the results of this study, it is yet unclear whether dissolved sulfide in the culture medium is metabolized directly by the main members of the consortia. However, the advantage of using microaerophilic culture media, as in the case of this study, is that it allows for the use of a type of biogas with a higher concentration of H_2S (e.g., from wastewater), since this would be chemically oxidized by the oxygen present in the culture medium, reducing its concentration to levels tolerated by purple non-sulfur bacteria such as *Rhodopseudomonas* (0.5 mM) (Imhoff 2006). Gonzalez-Sanchez and Revah (2007) found that a chemoautotrophic consortium grew on intermediate concentrations of sulfide oxidation, six times higher than the inhibitory sulfide concentration for that consortium (1.19 mM), allowing higher sulfide loading rates. This would be a way to compensate for the greater sulfide tolerance present in green sulfur bacteria (4 mM) (Overmann and Schubert 2002) and purple sulfur bacteria (2 mM) when grown in strictly anaerobic conditions (Zehnder 1988; Borkenstein and Fischer 2006), and studied for this purpose (Cork et al. 1985; Syed and Henshaw 2003; Borkenstein and Fischer 2006).

Conclusion

Rhodopseudomonas, a genus of purple non-sulfur photosynthetic bacteria, had a clear dominance during the incubation period of the consortia and could account for their physiological properties. The presence of a dominant bacterial type in consortia could result in a highly unstable system; however, the metabolic versatility of *Rhodopseudomonas* allowed it to respond to abiotic changes during the incubation period. Both consortia had a stable genetic structure over time, characterized by a high homogeneity during the incubation period. This homogeneity could be due to both the enrichment of the culture from which the consortia were obtained and the metabolic versatility of *Rhodopseudomonas.*

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References

Alimahmoodi M, Mulligan C (2008) Anaerobic bioconversion of carbon dioxide to biogas in an upflow anaerobic sludge blanket reactor. J Air Waste Manag Assoc 58:95–103

Borkenstein C, Fischer U (2006) Sulfide removal and elemental sulfur recycling from a sulfide-polluted medium by *Allochromatium vinosum* strain 21D. Int Microbiol 9:253–258

Brenner K, You L, Arnold H (2008) Engineering microbial consortia: a new frontier in synthetic biology. Rev Trends Biotechnol 26:483–489

Butow B, Bergstein-Ben Dan T (1991) Effects of growth conditions on acetate utilization by *Rhodopseudomonas palustris* isolated from a freshwater lake. Microb Ecol 22:317–328

Carlozzi P, Pushparaj B, DeQl'Innocenti A, Capperucci A (2006) Growth characteristics of *Rhodopseudomonas palustris* cultured outdoors, in an underwater tubular photobioreactor, and

investigation on photosynthetic efficiency. Appl Microbiol Biotechnol 73:789–795

Chouari R, Dardouri W, Sallami F, Rais B, Le Paslier D, Sghir A (2015) Microbial analysis and efficiency of biofiltration packing systems for hydrogen sulfide removal from wastewater off gas. Environ Eng Sci 32:121–128

Clesceri LS, Greenberg AE, Eaton AD (1998) Standard methods for the examination of water and wastewater. APHA, 20th edn. American Public Health Association, Washington

Cork D, Mathers J, Maka A, Srnak A (1985) Control of oxidative sulfur metabolism of *Chlorobium limicola* forma *thiosulfatophilum*. Appl Environ Microbiol 49:269–272

Cuellar-Bermudez S, Garcia-Perez J, Rittmann B, Parra-Saldivar R (2015) Photosynthetic bioenergy utilizing CO_2: an approach on flue gases utilization for third generation biofuels. J Clean Prod 98:53–65

Demirbas M (2011) Biofuels from algae for sustainable development. Appl Energy 88:3473–3480

Dunbar J, Takala S, Barns S, Davis J, Kuske CH (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl Environ Microbiol 65:1662–1669

Dunbar J, Ticknor L, Kuske C (2001) Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Appl Environ Microbiol 67:190–197

Fischbach M, Sonnenburg J (2011) Eating for two: how metabolism establishes interspecies interactions in the gut. Cell Host Microbe 10:336–347

Garcia-Peña E, Nakauma-González A, Zarate-Segura P (2012) Biogas production and cleanup by biofiltration for a potential use as an alternative energy source. In: Kumar S (ed) Biogas. Croatia, InTech, pp 113–134

González-Sánchez A, Revah S (2007) The effect of chemical oxidation on the biological sulfide oxidation by an alkaliphilic sulfoxidizing bacterial consortium. Enzyme Microb Technol 40:292–298

Gunnarsson I, Alvarado-Morales M, Angelidaki I (2014) Utilization of CO_2 fixating bacterium *Actinobacillus succinogenes* 130Z for simultaneous biogas upgrading and biosuccinic acid production. Environ Sci Technol 48:12464–12468

Henshaw P, Zhu W (2001) Biological conversion of hydrogen sulphide to elemental sulphur in a fixed-film continuous flow photo-reactor. Water Res 35:3605–3610

Imhoff J (2006) The phototrophic alpha-ptroteobacteria. In: Dworkin M (ed) Prokaryotes, vol 5, 3rd edn. Springer, New York, pp 41–64

Ito T, Yoshiguchi K, Ariesyady HD, Okabe S (2012) Identification and quantification of key microbial trophic groups of methanogenic glucose degradation in an anaerobic digester sludge. Bioresour Technol 123:599–607

Kamp A, Stief P, Schulz-Vogt H (2006) Anaerobic sulfide oxidation with nitrate by a freshwater *Beggiatoa* enrichment culture. Appl Environ Microbiol 7:4755–4760

Karpinets T, Pelletier D, Pan C, Uberbacher E, Melnichenko G, Hettich R et al (2009) Phenotype fingerprinting suggests the involvement of single-genotype consortia in degradation of aromatic compounds by *Rhodopseudomonas palustris*. PLoS One 4:e4615

Larimer F, Chain P, Auser L, Lamerdin J, Malfatti S, Do L et al (2004) Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. Nat Biotechnol 22:55–61

Maestre JP, Rovira R, Gamisans X, Kinney KA, Kirisits MJ, Lafuente J et al (2009) Characterization of the bacterial community in a biotrickling filter treating high loads of H_2S by molecular biology tools. Water Sci Technol 59:1331–1337

McCann K (2000) The diversity–stability debate. Nature 405:228–233

Meijer W, Croes L, Jenni B, Lehmicke L, Lidstrom M, Dijkhuizen L (1990) Characterization of *Xanthobacter strains* H4-14 and 25a and enzyme profiles after growth under autotrophic and heterotrophic conditions. Arch Microbiol 153:360–367

Oelze J (1985) Analysis of Bacteriochlorophylls. In: Gottschalk G (ed) Methods in microbiology, vol 18. Academic Press Inc, Florida, pp 261–262

Omri I, Bouallagui H, Aouidi F, Godon JJ, Hamdi M (2011) H_2S gas biological removal efficiency and bacterial community diversity in biofilter treating wastewater odor. Bioresour Technol 102:10202–10209

Overmann J, Schubert K (2002) Phototrophic consortia: model systems for symbiotic interrelations between prokaryotes. Arch Microbiol 177:201–208

Padden A, Kelly D, Wood A (1998) Chemolithoautotrophy and mixotropy in the thiophene-2-carboxylic acid-utilizing *Xanthobacter tagetidis*. Arch Microbiol 169:249–256

Quiroz M (2014) Selecting and characterizing bacterial consortia with the potential of fixing CO_2 and removing H_2S in a biogas atmosphere. Water Air Soil Pollut 225:1934

Ramirez M, Gómez JM, Aroca G, Cantero D (2009) Removal of hydrogen sulfide by immobilized *Thiobacillus thioparus* in a biotrickling filter packed with polyurethane foam. Bioresour Technol 21:4989–4995

Rolls J, Lindstrom E (1967) Effect of thiosulfate on the photosynthetic growth of *Rhodopseudomonas palustris*. J Bacteriol 94:860–866

Ryckebosch E, Drouillon M, Vervaeren H (2011) Techniques for transformation of biogas to biomethane. Biomass Bioenergy 35:1633–1645

Schütte UME, Abdo Z, Bent SJ, Shyu C, Williams CJ, Pierson JD, Forney LJ (2008) Advances in the use of terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA genes to characterize microbial communities. Appl Microbiol Biotechnol 80(3):365–380

Strevett K, Vieth R, Grasso D (1995) Chemo-autotrophic biogas purification for methane enrichment: mechanism and kinetics. Chem Eng J 58:71–79

Sun S, Ge Z, Zhao Y, Hu C, Zhang H, Ping L (2016) Performance of CO_2 concentrations on nutrient removal and biogas upgrading by integrating microalgal strains cultivation with activated sludge. Energy 97:229–237

Syed M, Henshaw P (2003) Effect of tube size on performance of a fixed-film tubular bioreactor for conversion of hydrogen sulfide to elemental sulfur. Water Res 37:1932–1938

Tang K, Baskaran V, Nemati M (2009) Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem Eng J 44:73–94

Thomas F, Hehemann J, Rebuffet E, Czjzek M, Michel G (2011) Environmental and gut *Bacteroidetes*: the food connection. Front Microbiol 93:1–16

Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 85:849–860

Weisburg W, Barns S, Pelletier D, Lane D (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Wiegel J (2006) The genus *Xanthobacter*. In: Dworkin M (ed) Prokaryotes, vol 5, 3rd edn. Springer, New York, pp 290–314

Zehnder A (1988) Biology of anaerobic microorganisms. Wiley, New York

Zwietering M, Jongenburger I, Rombouts F, Van't Riet K (1990) Modeling of the bacterial growth curve. Appl Environ Microbiol 56:1875–1881