EXPRESSION OF DSR MESSENGER RNA BY SULFATE REDUCING BACTERIA WITH VARYING SUBSTRATE AND TEMPERATURE

BY

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THESIS

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ABSTRACT

Recent studies raised the possibility of estimating the activity of sulfate reducing bacteria by measuring the number of messenger RNA (mRNA) transcripts of *dsr*, a critical gene in the sulfate reduction pathway, in a cell. Because conditions in aquifers can be quite different from those maintained in laboratory experiments, it is unknown whether the results of such studies can be applied to field settings. We test whether *dsr* expression is dependent on electron donor and incubation temperature, commonly observed variables known to affect reaction rates. We compare results using formate, an electron donor found in pristine aquifers, to lactate and ethanol, substrates commonly used in laboratory experiments and field biostimulation projects, but largely absent in nature. Incubation temperatures of 18°C, 23°C, and 30°C were used to determine the effect of temperature on *dsr* expression. From reactors in which we fixed rates of sulfate reduction, we extracted nucleic acids and measured biomass, then used quantitative PCR to measure the number of transcripts of *dsr* mRNA per cell. We find a clear relationship between *dsr* expression and the rate of sulfate reduction per cell. Considering the difficulty involved in determining sulfate reduction rates in aquifers using other techniques, *dsr* expression may provide a useful estimate of activity under a range of environmental conditions.

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INTRODUCTION

Microorganisms are known to catalyze many of the chemical reactions occurring naturally in groundwater. These environments can support diverse microbial communities wherever sufficient chemical energy exists to drive their metabolisms (Bethke et al., 2011; Flynn, 2011). In anoxic environments, including pristine and contaminated aquifers, anaerobic microorganisms such as sulfate reducing bacteria are among the most important functional groups present (Chapelle and Lovley, 1990; Jorgensen, 1982). Sulfate reducers respire by catalyzing the transfer of electrons from reduced species, such as dihydrogen, fatty acids, or alcohols, to sulfate (Table 1) (Dworkin et al., 2006). Their catabolic reaction affects redox state, acid-base chemistry, the precipitation and dissolution of minerals, and the fate of heavy metals, radionuclides, and organic contaminants (Edwards et al., 1992; Kirk et al., 2004; Lovley et al., 1995; Lovley and Phillips, 1992; Park et al., 2006). Geochemists have long appreciated the importance of sulfate reducers in the environment and as such would like to be able to measure rates of sulfate reduction in situ (Chapelle and Lovley, 1990; Detmers et al., 2001; Fossing and Jørgensen, 1989; Habicht and Canfield, 1997).

Recently, tools from molecular biology have found increased application in studies of aquifer microbiology and biogeochemistry (Barns and Nierzwicki-Bauer, 1997; Fredrickson et al., 1995). Variability in the 16S rRNA gene has revealed the diversity of Bacterial and Archaeal life, and functional gene analysis has revealed similar levels of diversity within specific microbial populations, including sulfate reducers (DeLong and Pace, 2001; Klein et al., 2001; Wagner et al., 1998; Woese, 1987). These techniques can identify microorganisms sampled from an environment and allow scientists to attempt to infer metabolic capabilities from phylogenetic relationships. DNA based surveys are limited, however, because they do not distinguish between active and inactive populations, nor do they reveal how active a particular species or functional group may be.

Analysis of messenger RNA (mRNA) transcripts of functional genes has emerged as a potentially useful measure of microbial activity (Holmes et al., 2004; Neretin et al., 2003). Chin et al. (2004), for

example, found transcription of the *frdA* and *omcB* genes to correlate with rates of fumarate and iron citrate reduction, respectively. Neretin et al. (2003) used a similar approach to study sulfate reduction via transcription of the *dsrA* gene. Chin et al. (2008) concluded from their field study that *dsr* expression was not a useful indicator of the rate of sulfate reduction in petroleum contaminated aquifers. Villanueva et al. (2008) demonstrated that *dsr* expression cannot be used to estimate the overall rate of sulfate reduction, only the rate per cell. They did find, however, that *dsr* expression can be estimated under both electron donor and electron acceptor limited conditions – important considerations for any field study. Strattan (2010) refined this technique by normalizing *dsr* mRNA transcripts to copies of the *dsr* gene, eliminating the uncertainty arising from the highly variable amounts of ribosomal RNA found in the environment. Furthermore, they conducted their experiments using an environmentally relevant electron donor, formate, and used very low rates of sulfate reduction (less than 90 fmol/ cell/ day). This technical approach is still new and must be tested further before successful application in complex field settings.

Aquifers are physically, chemically, and microbiologically complex, and ambient conditions can differ markedly from those maintained in laboratory experiments. Because reaction kinetics and energetics are controlled by solution chemistry (Jin and Bethke, 2005) it is important to understand how *dsr* expression, an indicator of sulfate reduction, varies. Despite the fact that substrates such as formate, acetate, and dihydrogen are the electron donors most likely to be found in pristine aquifers (McMahon and Chapelle, 1991), lactate has been described as the "classic" substrate for sulfate reducers and has been utilized frequently in laboratory studies (Dworkin et al., 2006). BTEX compounds can be utilized by sulfate reducers in petroleum contaminated sites (Edwards et al., 1992), whereas electron donors such as ethanol, lactate, and acetate are injected into contaminated aquifers to stimulate the reduction of heavy metals or chlorinated compounds by indigenous microbial populations (Anderson et al., 2003; DeWeerd et al., 1990; Finneran et al., 2002; Vishnivetskaya et al., 2010). Temperature, which is also known to affect reaction rates (Lasaga, 1981), can vary both spatially and temporally in an aquifer, depending on climate, depth, and rate of recharge (Anderson, 2005). Laboratory experiments which purport to simulate

in situ conditions are commonly incubated at temperatures higher than are relevant to shallow aquifers to promote rapid growth.

Variations in *dsr* expression due to temperature, if existent, would be straightforward to account for. Calibration experiments could simply be performed at the temperature measured in the aquifer for each sampling point. Where electron donor varies, or where a combination of donors are coupled to sulfate reduction, complications arise. Still, formate and dihydrogen are commonly thought to be physiologically similar (Heidelberg et al., 2004) and may reasonably be expected to affect *dsr* expression similarly. Where a single electron donor is injected into the subsurface, its concentration would dwarf that of any other electron donors present, except where lactate or ethanol is incompletely oxidized to acetate.

In order for *dsr* expression to be useful as an indicator of microbial sulfate reduction, we must first determine the variables which influence *dsr* mRNA transcription. If a relatively constant relationship is found to exist between *dsr* expression and the rate of sulfate reduction per cell in laboratory calibration experiments, it is feasible that rates could easily be estimated in field settings under a wide range of geochemical conditions. If parameters such as temperature or the electron donor being coupled to sulfate reduction lead to variable *dsr* expression, the task of interpreting field *dsr* signals becomes much more difficult. Where multiple electron donors are utilized simultaneously by sulfate reducers, or where the electron donor or temperature varies spatially in an aquifer, it is desirable to know whether *dsr* expression is broadly applicable to estimating rates of sulfate reduction in the field. We attempt here to discern what effect, if any, the choice of electron donor and incubation temperature have on expression of the *dsr* gene as an indicator of microbial sulfate reduction.

METHODS

We grew *Desulfovibrio vulgaris* str. Hildenborough (DZMZ 644, ATCC 29579) anaerobically in 160 ml serum bottles capped with butyl rubber stoppers in a medium defined in He and Sanford (2004). At the start of fed-batch experiments fresh media bottles were inoculated with 10 mls of active culture and supplied with approximately 2mM sulfate, 0.2 % yeast extract, and 2 mM acetate as a carbon source for growth, except where it is produced as a byproduct of incomplete lactate or ethanol oxidation. We attached 2.5 ml gas tight syringes to a 10 channel syringe pump which continuously fed stock solutions of electron donors into the reactors. Different bulk rates of sulfate reduction were established by supplying reactors with different concentrations of electron donor (Tables 1, 2). Substrates were supplied stoichiometrically in terms of equal amount of sulfate reduction (Table 1). 5 ml samples were taken from the reactors after 72 and 96 hours of continuous injection. A 1 ml aliquot of cells was preserved for cell counting in 2% formaldehyde (final concentration), and the remaining 4 mls passed through a 0.22 μm nylon filter, which was subsequently rinsed with 2 mls of RNAlater solution (Ambion, Austin, TX) to preserve RNA. Filters were stored at 4 °C for 24 hours to allow RNAlater to permeate the cells, then frozen at -80 °C. The filtrate was frozen at -20 °C until analysis of volatile fatty acids (or ethanol) and sulfate.

Formate, lactate, and acetate concentrations were measured on a Shimadzu Prominence HP-LC (Shimadzu Scientific Instruments, Columbia, MD) equipped with a BioRad Aminex HPx-87H column. Ethanol concentration was measured on an Agilent 7890A GC. A Metrohm Peak Advanced IC fitted with a Metrosep A Supp 7-250/4.0 Anion column (Metrohm Park Inc., Houston, TX) was used to measure sulfate concentrations.

1 μL SYTO BC stain and 10 μL counting beads (Bacteria Counting Kit, Molecular Probes, Eugene, OR) were added to preserved cell suspensions prior to flow cytometry analysis at the Biotechnology Center, UIUC. Cell biomass was determined gravimetrically by passing the remainder of media in the reactors through rinsed, preweighed 0.45 μm glass microfiber filters (Whatman, Springfield

Mill, UK). Filters were dried in a muffle furnace at 110 °C for three hours to drive off water and then baked at 500 °C for one hour to combust cell biomass, with mass measured after each step. Biomass was calculated as the total filtered mass minus the mass of mineral precipitates, which remained after the combustion step. Measured biomass concentrations were converted into yields (g biomass per mole sulfate) by subtracting the mass of inoculum from the final biomass and dividing by the amount of electron donor added (sulfate reduced) over the course of the experiment.

Nucleic acids were extracted using a procedure similar to that used by Flynn et al. (2008) and Strattan et al.(2010), except only the protocol for RNA extraction was carried out, as RNA and DNA are in fact simultaneously extracted (Tsai and Olson, 1991). Briefly, cell-laden filters were incubated at 37 °C in a lysis solution (0.15 M NaCl, 0.1 M EDTA, pH 8, 15 mg/ml lysozyme) for 30 minutes, then STS solution (0.1 M NaCl, 0.48 M Tris, 10% sodium dodecyl sulfate, pH 8) was added and the cells were incubated another 30 min. Cells were then subjected to three cycles of mechanical disruption by freezing in liquid nitrogen and thawing in a 55 °C water bath, after which Proteinase K was added and incubated for another 30 minutes. Proteins were removed by successive extractions in equal amounts of phenol (pH = 4.3), phenol:chloroform:isoamyl alcohol (25:24:1), and chloroform:isoamyl alcohol (24:1), after which nucleic acids were precipitated with 2.5 M ammonium acetate, 50 µg/ml glycogen, and an equal volume of isopropanol. Suspensions were centrifuged at 10,000 RPM at 2 °C to pellet the nucleic acids, which were subsequently rinsed in ethanol and resuspended in molecular grade water. The "RNA" sample was treated with Turbo DNA-free (Ambion, Austin, TX) to remove contaminating DNA, while the "DNA" sample was left untreated. RNase free reagents and plastic ware were used throughout.

dsr mRNA was reverse transcribed to cDNA using the Retroscript Kit (Ambion, Austin, TX) and the reverse primer DSR1R (5'-TTA TCT CAG GTG TCT CTT GCG GT-3'). 2 µl RNA and 5 µl water were added to 5 µl DSR1R primer. The mixture was heated at 80 °C for 3 minutes, followed by 30 seconds at 61 °C, after which the mixture was removed to ice. 2 µl 10X RT buffer, 4 µl dNTPs, 1 µl RNase inhibitor, and 1 µl MMLV-RT were added to the reaction vessel, mixed, and incubated at 43 °C for 1 hour. After 10 minutes at 92 °C, the mixtures were stored at -20 °C.

For qPCR analysis, we added 1 µl cDNA or DNA template to 12 µl of Agilent's Brilliant II SYBR Green qPCR Master Mix (Agilent, Santa Clara, CA), along with 7 µl molecular grade water, 2.5 µl each of the forward primer DSR1F (5'-AAG GAA CCC CGC ACC AAC-3' - position 1 to 102, *dsrA* gene) and the reverse primer previously described (Table 3) (Villanueva et al., 2008). Quantitative PCR was performed using two segment cycling in which DNA was melted at 95 °C for 30s, followed by a combined annealing and extension step at 61 °C, as recommended by Agilent. For each sample, *dsr* cDNA and *dsr* DNA were analyzed in triplicate, while single no reverse transcription controls were carried out to ensure no contaminating DNA remained in RNA samples after the DNase treatment. Copies were calculated from the threshold cycle number, Ct, based off of a standard curve of serial dilutions of *D. vulgaris* DNA quantified using the PicoGreen kit (Invitrogen, Carlsbad, CA).

RESULTS

For each experiment, reactors were continuously supplied an electron donor solution for four days, after which reactors were sampled, cells harvested, and biomass measured. Concentrations of electron donor were below detection limit for all experiments at the time of sampling, indicating that the rate of sulfate reduction was proportional to the rate of electron donor input.

Within a single experiment, increased rates of electron donor addition led to greater biomass accumulation, as seen in Figure 1. The choice of electron donor and temperature, for a given rate of sulfate reduction, also resulted in biomass differences. The three experiments using formate as the electron donor resulted in the lowest accumulation of biomass. Within this experimental subset, those conducted at 18 °C showed the least growth, followed by the experiments conducted at 23 °C and 30 °C. Lactate and ethanol experiments, which were conducted at 30 °C, showed noticeably more growth. Similar results for all but the ethanol experiments were obtained using both gravimetric methods and direct cell counts (flow cytometry), as seen by comparing Figure 1 and Figure 2. Cell counts from the ethanol experiment were considerably lower than expected based upon biomass measurements. Calculated yields (Figure 3) followed similar trends to final biomass concentrations.

Experiments were designed to measure *dsr* expression as a function of the cell specific rate of sulfate reduction (csSRR), which was calculated as the bulk rate normalized to biomass (or cells) in the reactor. Figure 4 gives the results for two experimental conditions, formate at 18 °C and lactate at 30 °C, chosen because of their widely differing biomass yields. Where the csSRR is lower, (i.e. a lower average activity per cell) *dsr* expression is lower. Increasing rates of sulfate reduction per cell correlate with greater expression of the *dsr* gene. This figure further illustrates how experimental setup impacts the nature of our results. Because we established bulk rates of sulfate reduction in our reactors, experiments with higher yielding electron donors accumulated more biomass, resulting in lower csSRR. In figure 4, we see the lactate experiment data cluster close to the origin of the x-axis, indicating a low csSRR. The

formate experiment conducted at 18 °C, on the other hand, accumulated much less biomass - meaning each cell accounted for a larger share of the sulfate reduced, which is reflected by the higher *dsr* expression.

Comparing the three formate experiments at different temperatures (Figure 5), we first note that the 30 °C data plot closest to the origin of the x-axis, followed by the 23 °C and 18 °C experiments, a result of the greater measured biomass at higher temperature. Each experiment shows *dsr* expression increasing with csSRR. Data from all three experiments plot along a similar trajectory, though the lower temperature experiments rise along a slightly higher slope.

The formate, lactate, and ethanol experiments, all conducted at 30 °C, show less uniform results (Figure 6). Data from the formate experiment follow the best linear trend, while lactate and ethanol data points do not show a clear relationship of increasing *dsr* expression at higher csSRR. It is important to note the limited range plotted in this figure, however. When data from all experiments are plotted together on full axes (Figure 7), a general trend of *dsr* expression increasing with csSRR is evident. Figure 8 shows the same data normalized to the number of cells instead of mg biomass.

DISCUSSION

Final biomass concentrations in the reactors varied due to the rate of electron donor input and the choice of incubation temperature and electron donor. The rate of electron donor input limited the amount of sulfate that could be metabolized and the biomass which could be synthesized. Because the different experiments were designed to consume identical amounts of sulfate, differences in biomass were likely due to the amount of energy which cells could derive from sulfate reduction (Roden and Jin, 2011). *D. vulgaris* is an incomplete oxidizer, meaning it cannot mineralize to bicarbonate acetate or larger compounds (Dworkin et al., 2006). Formate, which donates two electrons per molecule, can be completely oxidized, unlike lactate and ethanol, which react to form acetate and bicarbonate. Of the twelve electrons per molecule from each, only four then were available for sulfate reduction by *D. vulgaris.* The acetate produced was used within the cell to generate an extra ATP by substrate level phosphorylation (Magee et al., 1978), leading to more growth from lactate or ethanol than from formate.

Differences in final biomass concentrations among the formate experiments were larger than expected. Temperature is well known to affect rates of microbial metabolism (Huser et al., 1982); thermodynamics, not kinetics, though, are thought to control the amount of biosynthesis (Loffler et al., 1999; Roden and Jin, 2011) and differences in free energy between 18 °C and 30 °C are quite small. Growth yields from sulfate reduction have in some cases been shown to vary with temperature, however. Knoblauch and Jørgensen (1999), for example, measured growth yields which decreased with increasing temperature in five psychrophilic sulfate reducers isolated from Arctic sediments. The yields of mesophilic sulfate reducers, on the other hand, were found to increase with temperature up to 28° C (Isaksen and Jorgensen, 1996). Considering that *D. vulgaris* is a mesophilic sulfate reducer, our observation of yield increasing with temperature makes sense.

Gravimetric and direct biomass measurements yielded similar results for all but the ethanol experiments, in which cell counts were much lower than expected and did not increase with higher rates of electron donor input. This was perhaps due to inadequate preservation and subsequent decay of the

cells after sampling. Cell counts measured by flow cytometry were performed several months after sampling, while gravimetric measurements were made immediately after cells were harvested.

If *dsr* expression is to be useful as an indicator of microbial sulfate reduction in the field, we must determine whether its relationship with the rate of sulfate reduction per cell changes under different environmental conditions. The results of Figure 5 indicate that where formate is the electron donor for sulfate reduction, *dsr* expression in *D. vulgaris* increases with higher csSRR, regardless of the incubation temperature. This relationship appears to be somewhat dependent on temperature, however; a steeper slope describes the plot of *dsr* expression vs. csSRR at 18 °C than 30 °C. Calibrating this relationship at a temperature close to that of the aquifer being studied would help to resolve this problem. Where temperature varies, several calibration curves could be generated. This might be inconsequential, though, as the effect is very small. Furthermore, in Figure 8 where the rate of sulfate reduction is normalized to cells in the reactors, we observe less of a temperature dependence on slope.

The effect of electron donor on *dsr* expression is less clear. In Figure 6, data from the lactate experiment plot above the line which fits formate and ethanol experiments fairly well. Furthermore, the data for lactate and ethanol are not well described by a straight line increasing from the origin. This may be related to the different physiological state of *D. vulgaris* when using lactate and ethanol, which yield extra ATP through substrate level phosphorylation. Alternatively, this could be an artifact of the experimental setup. Biomass rapidly accumulates when lactate or ethanol are delivered to the reactors, resulting in a lower rate of sulfate reduction for each cell. The high yield on lactate and ethanol significantly compresses the x-axis, meaning the variability from sampling and analysis might be too large over this limited range to resolve a clearer trend. When the data for each condition are plotted together on wider axes (Figure 7), however, a clear trend of *dsr* expression increasing with csSRR becomes evident. Considering the difficulties and uncertainties of, for example, determining the direction of groundwater flowpaths, the nature of permeability fields and sediment reactivity, and identifying important chemical reactions and microbial populations in an aquifer, *dsr* expression may provide a useful tool for estimating rates of microbial sulfate reduction under a range of environmental conditions.

By itself, *dsr* expression provides a method to infer the rate of sulfate reduction, per cell, for a population of sulfate reducers. The rate per cell, a measure of average cellular activity, could prove useful in many situations. For example, Flynn et al. (2008) found that the populations of bacteria, including sulfate reducers, which colonize aquifer sediments can be quite different from the planktonic community. By sampling each, researchers could feasibly identify the more metabolically active community. Similarly, by designing qPCR primers to target several specific phylogenetic groups of sulfate reducers, microbiologists could determine which are most active in a given environment. Such information could direct physiological studies of cultured representatives from the most important groups. Additionally, though not tested here, it is feasible that the oxidation of alternative electron donors, including organic contaminants such as benzene, by sulfate reducers could be monitored by measuring *dsr* expression.

When used in conjunction with an independent measurement of biomass, *dsr* expression could provide an estimate for the overall rate of sulfate reduction. Fluorescent dyes such as DAPI, Acridine Orange, or SYBR Green, for example, can stain cells prior to enumeration by microscopy. Flow cytometry can count cells suspended in the groundwater. Iron reducing bacteria and methanogenic archaea also have functional genes which have received attention in the literature (Chin et al., 2004; Steinberg and Regan, 2009). Development of similar activity probes and analysis of multiple functional genes could reveal interesting information about microbial ecology, microbiological zonation of aquifers, and carbon cycling in anaerobic environments (Bethke et al., 2008; Chapelle and Lovley, 1992; Park et al., 2006).

Perhaps the greatest unknown in applying functional gene expression to estimating rates of microbial metabolism is the diversity of sulfate reducers in natural environments. To date *dsr* expression has been tested in only two pure sulfate reducing strains, *D. vulgaris* (Strattan, 2010; Villanueva et al., 2008) and *Desulfobacterium autotrophicum* (Neretin et al., 2003), and no attempt yet has been made to determine whether a given csSRR is reflected by the same level of *dsr* expression in each. Such a comparison would not immediately confirm the widespread applicability of this approach, but could be

useful to determine its feasibility. Efforts are currently underway to repeat this experimental setup with a sulfate reducer enriched from the Mahomet Aquifer of East Central Illinois (Panno et al., 1994).

CONCLUSIONS

We measured expression of the *dsr* gene in *D. vulgaris* as a function of the rate of sulfate reduction per cell in reactors incubated under a range of geochemical conditions. We conducted experiments with formate, lactate, and ethanol as the limiting electron donor at 30 °C along with additional experiments using formate at 18 °C and 23 °C. After 96 hours of continuous electron donor input, we sampled reactors, measured biomass, and collected cells from which we extracted RNA and DNA. Reverse transcription of the *dsr* mRNA followed by qPCR amplification allowed us to determine the number of *dsr* transcripts produced per cell. *D. vulgaris* grew to larger biomass concentrations when incubated at higher temperatures and when lactate and ethanol were used as electron donors. We find in general a clear relationship between *dsr* expression and the rate of sulfate reduction normalized to mg of biomass or direct cell counts. Considering the numerous uncertainties involved in determining reaction rates in aquifers, *dsr* expression may provide a useful estimate of activity under a range of environmental conditions. Such a tool could prove useful in studying the evolution of groundwater chemistry in pristine environments and in contaminated sites where natural attenuation or biostimulation are used for remediation.

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APPENDIX

TABLES AND FIGURES

Electron donor	Reaction stoichiometry
Formate	$SO_4^{2-} + 4\ HCOO^- + H^+ \leftrightarrow HS^+ + 4\ HCO_3^-$
Lactate	$SO_4^{2-} + 2\ CH_3CH(OH)COO^- \leftrightarrow HS^- + 2\ CH_3COO^- + 2\ HCO_3^- + H^+$
Ethanol	$SO_4^{2-} + 2\ CH_3CH_2OH \leftrightarrow HS^- + 2\ CH_3COO^- + H^+ + 2\ H_2O$

Table 1 Electron donors used in experiments and stoichiometries of sulfate reduction

Experiment	Electron donor	Feed stock concentration (mM)	Bulk rate of sulfate reduction (μM/hr)	T (°C)	Sulfate reducer
1	Formate	20, 40, 60, 80	3.8, 7.5, 11.3, 15.1	18 °C	*D. vulgaris*
2	Formate	20, 40, 60, 80	3.8, 7.5, 11.3, 15.1	23 °C	*D. vulgaris*
3	Formate	20, 40, 60, 80	3.8, 7.5, 11.3, 15.1	30 °C	*D. vulgaris*
4	Lactate	10, 20, 30, 40	3.8, 7.5, 11.3, 15.1	30 °C	*D. vulgaris*
5	Ethanol	10, 20, 30, 40	3.8, 7.5, 11.3, 15.1	30 °C	*D. vulgaris*

Table 2 Details of experimental setup

Target	Primer	Primer sequence (5' – 3')	Annealing and extension temperature (°C)
D. vulgaris	DSR1F	AAG GAA CCC CGC ACC AAC	61
	DSR1R	TTA TCT CAG GTG TCT CTT GCG GT	

Table 3 qPCR primers used in this study

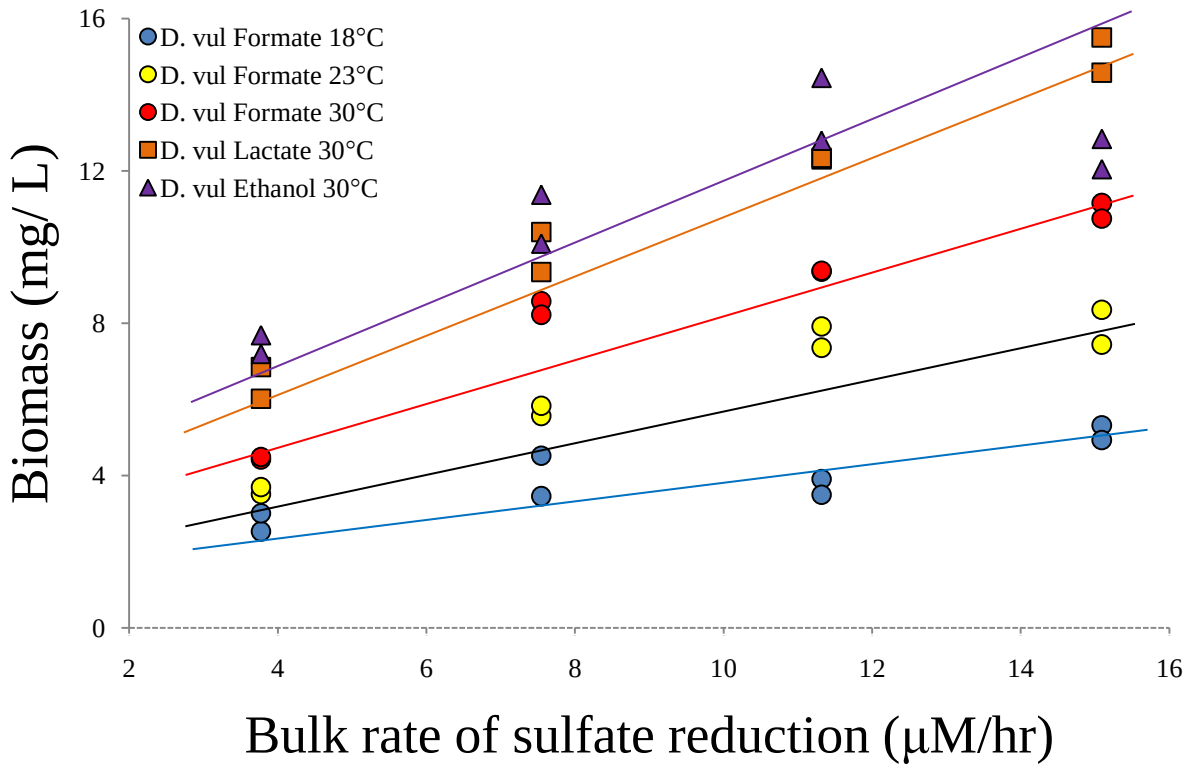

Figure 1. Accumulation of biomass in reactors after 96 hours of continuous electron donor input. Higher feed rates of electron donor resulted in more cell growth. Lactate and ethanol experiments resulted in more growth than when formate was used. Legend indicates symbols used for each experiment.

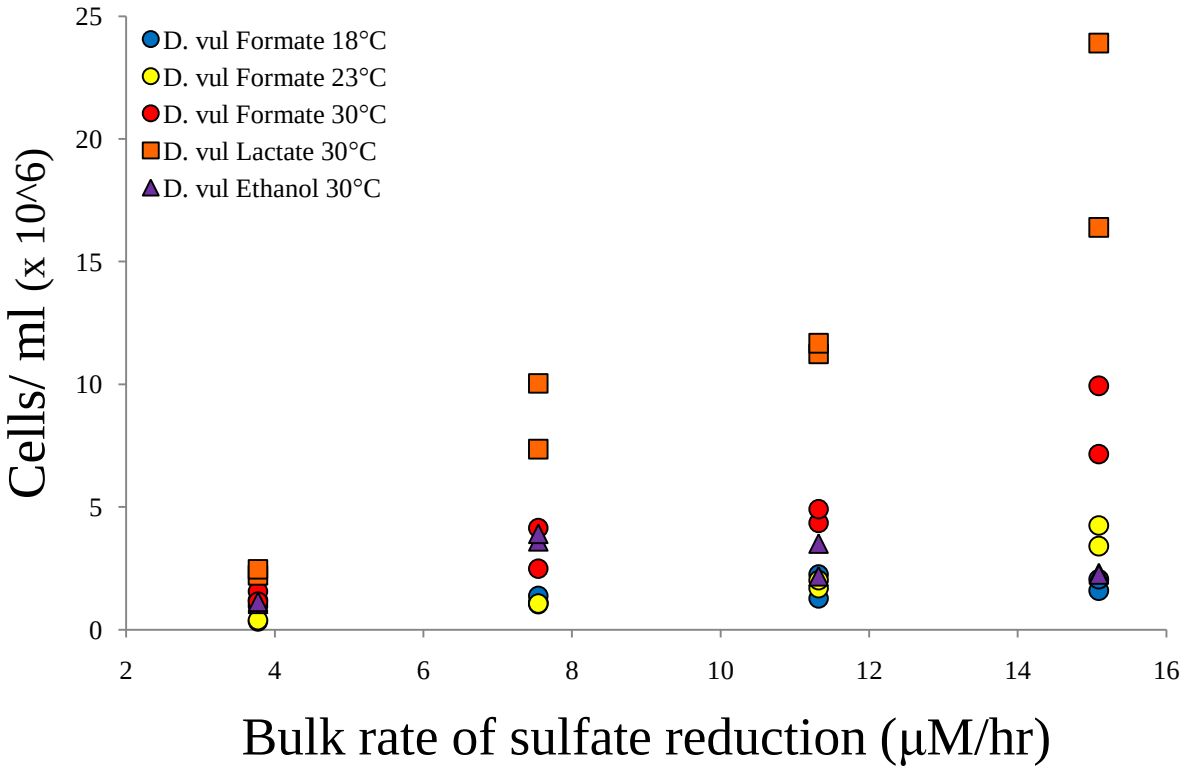

Figure 2. Final cell counts in reactors after 96 hours of continuous electron donor input.

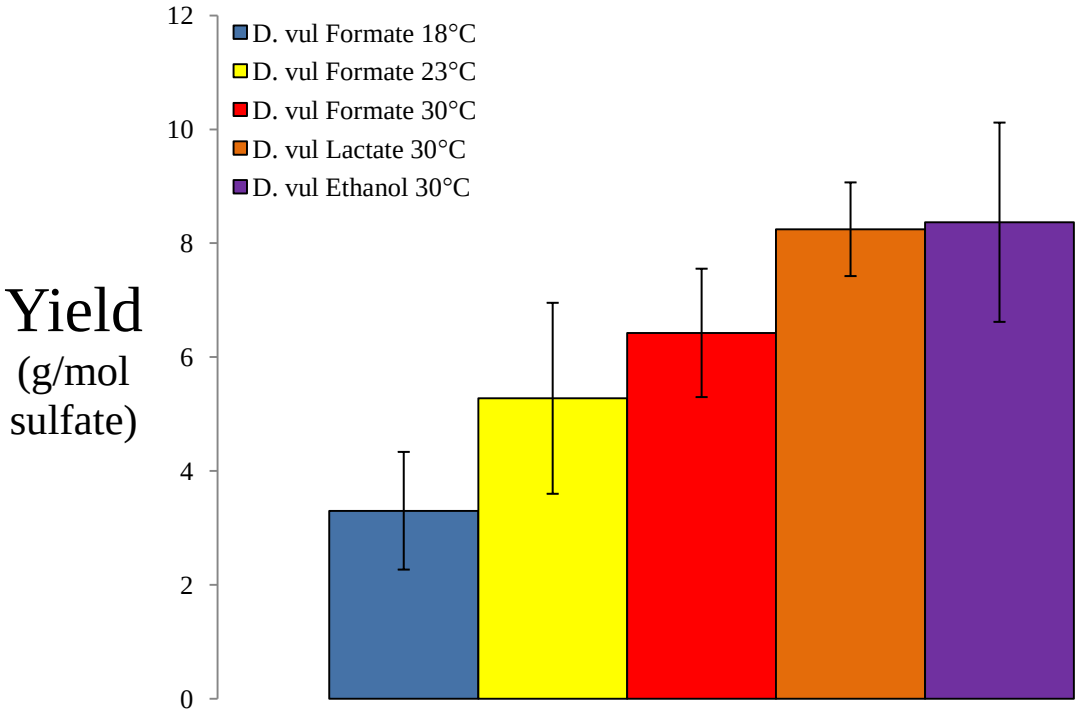

Figure 3: Calculated growth yields for *D. vulgaris* based off of fed-batch experiments, in g biomass /mole sulfate. Yields from formate at 18°C and 23°C were lower than at 30°C. All formate experiments resulted in a lower yield than from lactate and ethanol at 30°C.

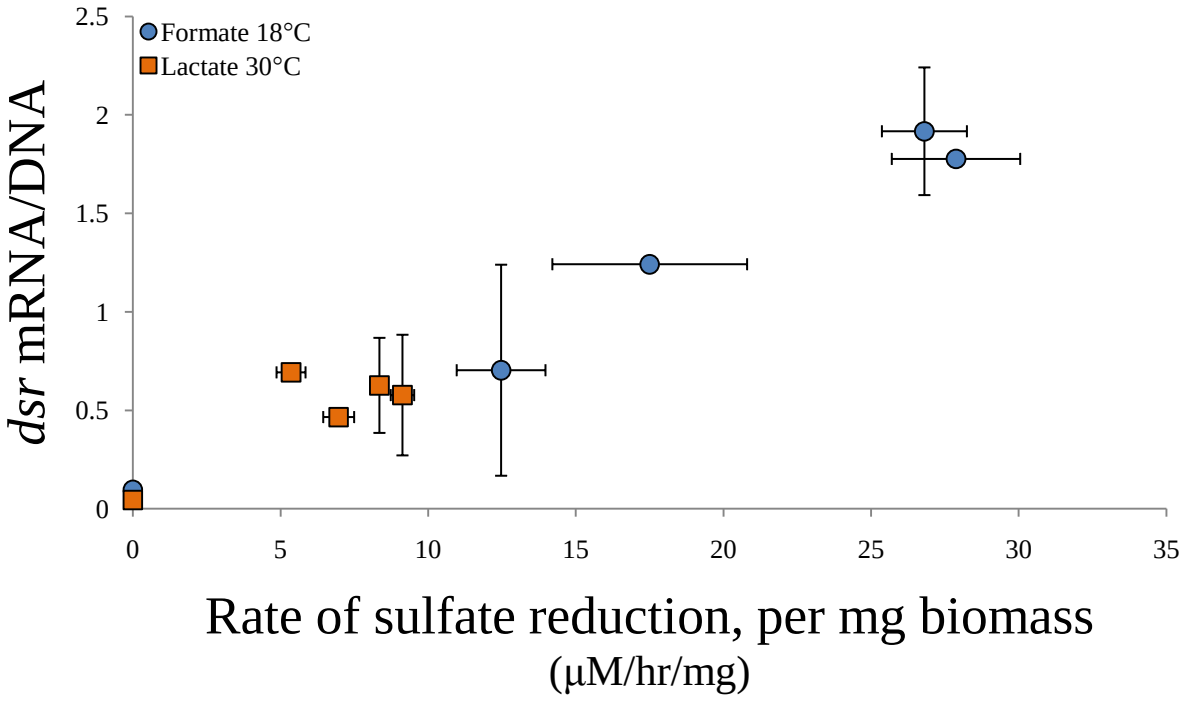

Figure 4. ***dsr*** **mRNA transcripts per cell (*dsr* cDNA /*dsr* DNA copies) vs. the csSRR for formate experiment conducted at 18°C and lactate experiment at 30°C. Error bars represent range of values for duplicate samples. Note that reactors fed lactate accumulated much more biomass than when formate was used, and the effect of growth on the csSRR.**

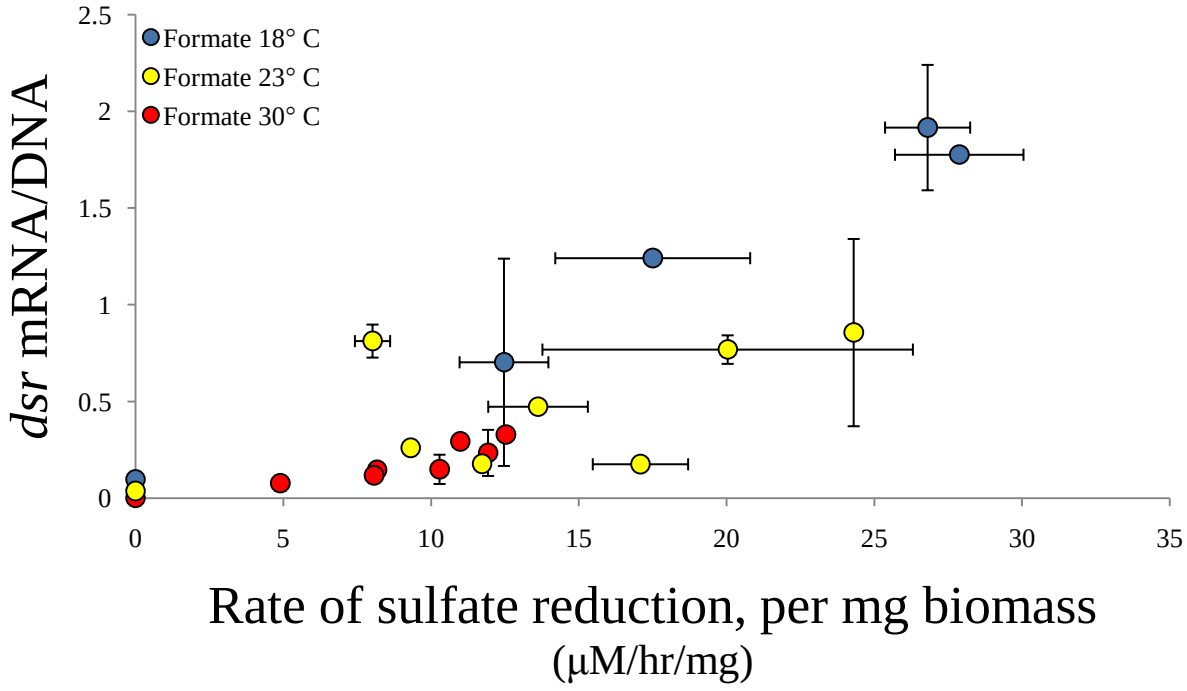

Figure 5. ***dsr*** **mRNA transcripts per cell vs. the csSRR for formate experiments conducted at 18°C, 23°C, and 30°C. A linear trend of increasing** ***dsr*** **expression with higher csSRR is evident. The slope appears to increase slightly with temperature.**

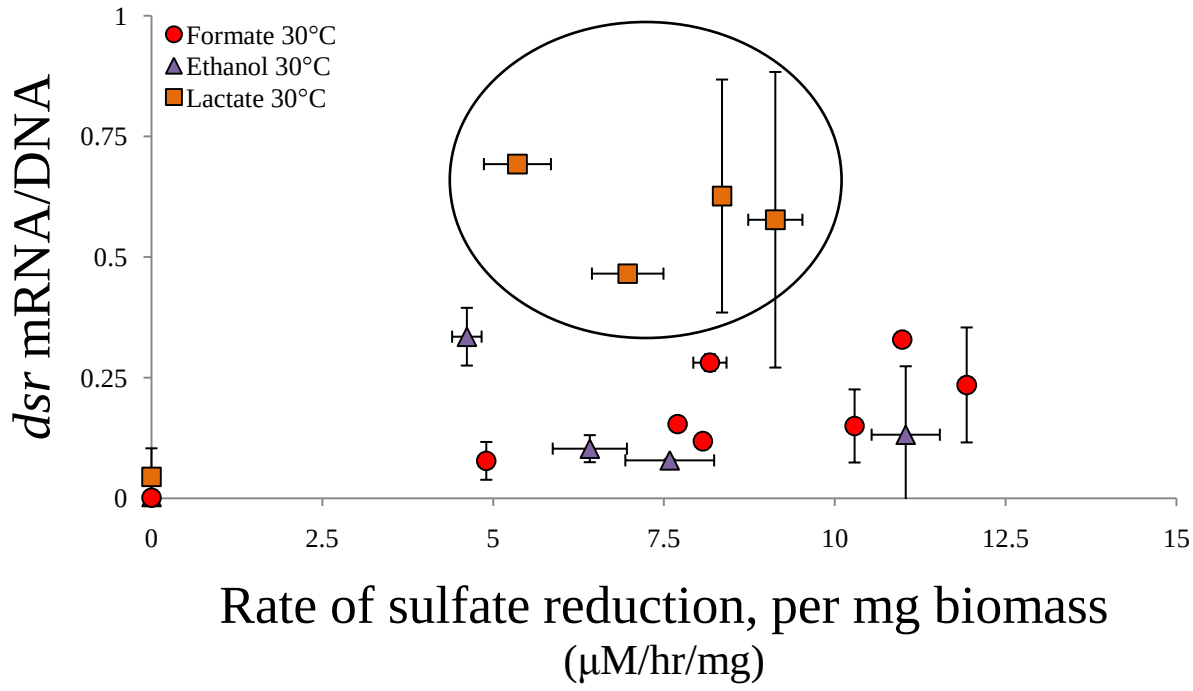

Figure 6. *dsr* expression vs. the csSRR for different electron donors at 30°C. Formate data show the best linear, increasing trend. Lactate data, circled, plot above the those for formate and ethanol.

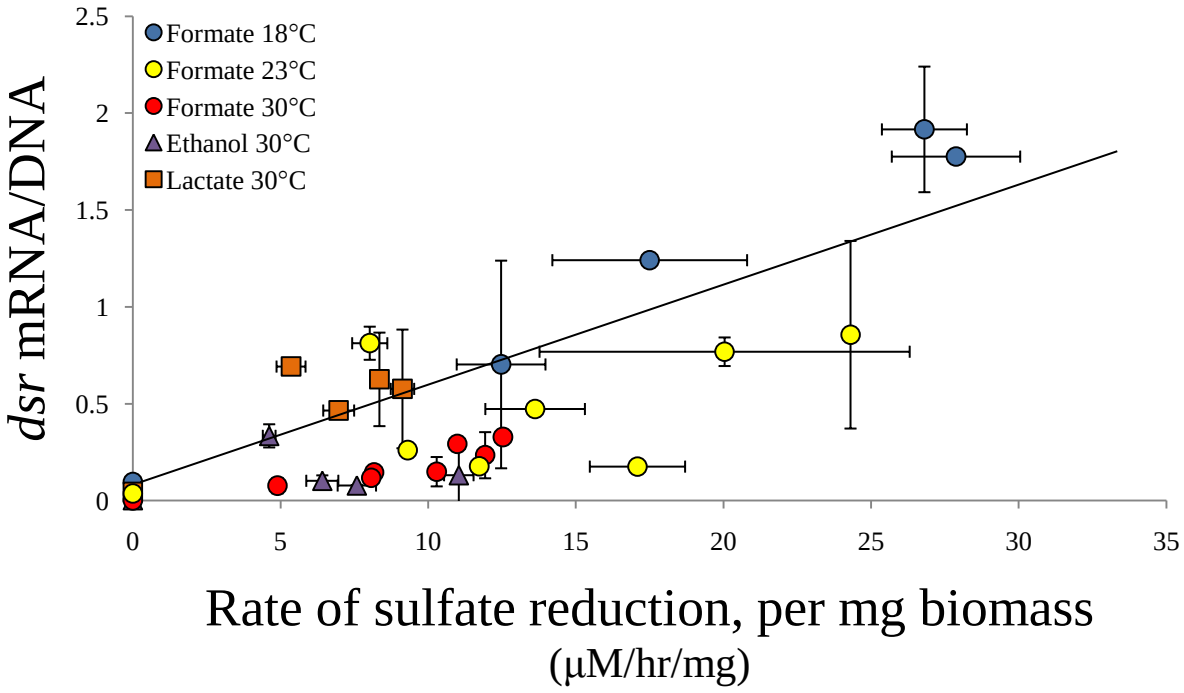

Figure 7. ***dsr*** **expression vs. the csSRR under all experimental conditions, normalized to mg of biomass**

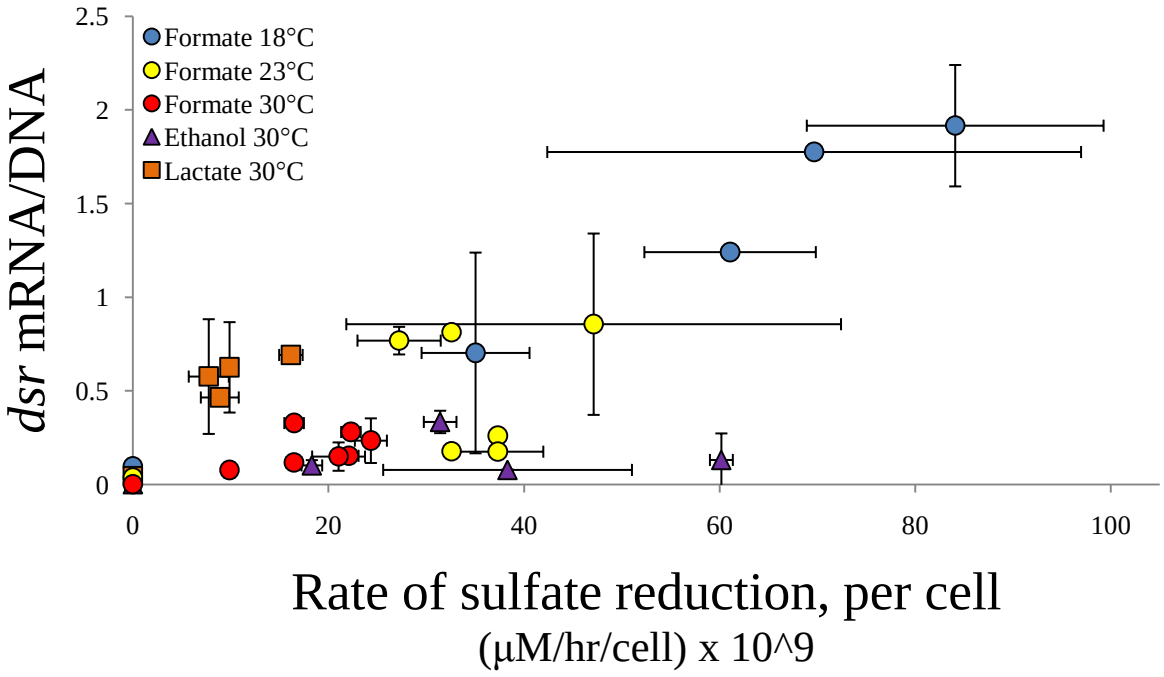

Figure 8. ***dsr*** **expression vs. the csSRR under all experimental conditions, normalized to cell counts**