Innovative Biotechnological Applications of Galdieria Sulphuraria-Red Microalgae (GS-RMA) in Water Treatment Systems

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https://doi.org/10.5109/4102483

出版情報：Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 6, pp.163-170, 2020-10-22. Interdisciplinary Graduate School of Engineering Sciences, Kyushu University
バージョン：

Innovative Biotechnological Applications of *Galdieria Sulphuraria*-Red Microalgae (*GS*-RMA) in Water Treatment Systems

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ABSTRACT: *In this study, mini review is introduced on the features of the red microalgae (RMA) and its biotechnological potential in different applications. Since it has unique extremophilic features, Galdieria Sulphuraria (GS) is considered to be a perfect microorganism candidate for various biotechnological applications in water treatment systems. Special interest has been oriented towards the applications of GS in water treatment systems, such as nutrients, biological oxygen demand (BOD) and heavy metals (HMs) removal from wastewater. Also, different future prospects have been suggested in terms of the new and innovative applications of GS in water treatment based on the gaps in the literature, including phycoremediation of HMs, bio-resin production, bio-substrate for nanoparticles, and pharmaceuticals removal from wastewater. Finally, the challenges and limitations of employing GS in biotechnological applications have been reviewed, which revealed that using GS has a great potential in largescale outdoor cultivation without becoming contaminated with other microorganisms.*

Keywords: Red Microalgae (RMA); *Galdieria Sulphuraria* (*GS*); Water treatment systems; Biotechnological applications.

1. INTRODUCTION

The red microalga (RMA) is that kind of algae which belongs to the class *Cyanidiophyceae*. The formerly mentioned class represents a group of unicellular microorganisms which goes back to around 1.3 billion years old ancestral RMA [1,2]. This unicellular RMA has three main genera classifications, *Cyanidioschyzon Merolae*, *Cyanidium Caldarium*, and *Galdieria Sulphuraria (GS)*. *Cyanidioschyzon Merolae* is well known for its size and shape characteristics as small (2 μm) and club-shaped haploid genus (Fig. 1a) [3]. The other two genera are alike some extent in the morphological features. Hence, up to 1981, *Cyanidium Caldaruim* was considered as the synonym genus for *Galdieria Sulphuraria (GS)* [4,5].

All kinds of RMA have a great adaptability to the strong acidic (pH 0.5 – 3.5) and high temperature (38 – 56ºC) conditions [6–8]. Such extremophilic features enables the RMA of growing in habitats where other microorganisms cannot tolerate. Therefore, RMA inhabits different geothermal environments, endolithic habitats around hot sulfur springs, boiling mud pools, and hot acidic waters [9]. Furthermore, RMA has an impressive metal tolerance which enables it to grow in a very toxic environments (rich with heavy metals) where other microorganisms are absent [10]. Recently, owing to the unique features of *GS* in contrast with the other RMA genera, its potential in the biotechnological applications has been intensively studied. The unicellular cells of *GS* have a spherical shape with thick walls (Fig. 1c). In addition to the previously mentioned acidophilic and thermophilic unique characteristics, *GS* has the ability to exhibit special metabolism features through growing autotrophically, heterotrophically and mixotrophically [11,12]. Hence, the cells of *GS* are considerable recognized, comparing with that of *Cyanidium Caldaruim,* owing to the heterophonic growth in the dark [13,14]. Moreover, the cell size of *GS* is larger than the other RMA genera, which is preferable for storing vacuole and energy reserve products [15–18].

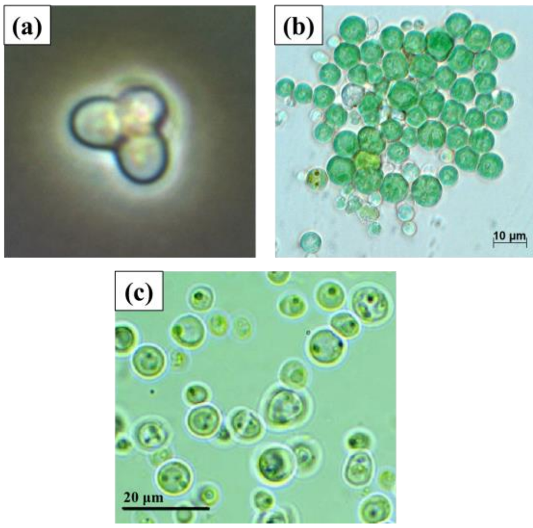

Fig. 1. Microscopic images showing the morphological characteristics of (a) *Cyanidioschyzon Merolae*, (b) *Cyanidium Caldarium*, and (c) *Galdieria Sulphuraria* [5,19,20].

In this study, mini review is introduced on the unique features of the RMA and its biotechnological potential in different applications. Special interest has been oriented towards the applications of *GS* in water treatment systems. Also, different future prospects have been suggested in terms of the new and innovative applications of *GS* in water treatment based on the gaps in the literature. Finally, the challenges and limitations of employing *GS* in biotechnological applications have been reviewed.

2. BIOTECHNOLOGICAL POTENTIAL (*GS*)

The unique features of *GS* give it a great potential in several biotechnological applications. Hence, in the following sections, different aspects of employing GS in various biotechnologies are reviewed.

2.1 Biofuel Production

There will be a serious rise in the global biofuel production by around 1.7 million barrels per day over the period between 2010 to 2040 [21]. Additionally, there is a rapid and continuous increase in the consumption of the fossil fuel all over the world within the last three decades. Hence, the need for a new and renewable energy sources has been emerged and the research on such aspect has dramatically increased. The biofuel production from microalgal biomass has attracted great attention recently as one of the possible solutions [22,23]. The microalgae biomass could be one of the most promising renewable resources for the regeneration of different types of biofuels, including bioethanol and biodiesel [24,25]. The rabid and sustainable growth of microalgae makes it a perfect biomass resource towards a viable biofuel production. The high content of carbohydrates in microalgae, which sometimes exceeds 50% of their dry weight, encouraged different researchers to conduct several studies on employing microalgal resources in bioethanol production [23,25,26].

Apparently, there is a lack of knowledge concurring the use of RMA as a renewable biomass resource for biofuel production. Despite the several advantages of the biofuel production by microalgal biomass, such technology must overcome some issues when it comes to the real competition in the fuel market. One of the major obstacles is the possible contamination of the microalgae with other microorganisms during the largescale outdoor cultivation processes, which affects the quality and the quantity of the produced biofuel through the inhibition of the algal growth as well as the decrease in the quality of the high-value products. Several studies have been focused on investigating the optimal culture and cultivating conditions for the microalgae, in addition to examining the microalgal chemical composition change by the effect of nutrients (i.e., phosphate, nitrogen and sulfur) [21,27,28]. Such studies confirmed the cruciality of the cultivation conditions of microalgae for the biofuel production viability. In this regard, RMA such as *GS* has great superiority to other microalgae in solving such cultivation problems. *GS* has a promising potential in producing high-quality biofuel with large quantities without any worries regarding becoming contaminated with other microorganisms. Moreover, the high tolerance of *GS* to the extreme conditions of pH and temperature provides an effective way to control the composition of the large-scale cultures.

2.2 Nutritional Applications

Microalgae has the ability to produce glycogen, which serves as a store of glucose, as energy and carbon reserves [29]. Glycogen has better characteristics than starch in nutritional applications owing to its solubility in cold water and the easy accessibility by enzymes. Unlike other microalgae, RMA can accumulate highly branched glycogen, such as Amylopectin, which is well-known to be used in different products including sports drinks and peritoneal dialysis solutions. *GS*-RMA could be a viable cheap source for glycogen production, as it can produce glycogen up to 50% of its dry cell weight [30,31]. Other autotrophic or heterotrophic microorganisms which act as a glycogen source and suffer from the growth limitations, exhibiting low glycogen production [32]. *GS*, as a mixotrophic cultures, can produce large amounts of glycogen 10 times greater than that of other autotrophic or heterotrophic cultures [33].

Microalgal biomass contains high content of protein which can be integrated in different food industries. However, there are different technological difficulties when it comes to introducing the microalgal-based ingredients into food industry [34]. The most concerning issue is the unattractive green-brownish color which is usually associated with the green microalgae. Moreover, green microalgae develop a fishy smell gradually during the long storing time which could be an obstacle because of the unpleasant feeling [35]. Furthermore, the bacterial contamination of the green microalgae could seriously deteriorate the commercial quality of the microalgal biomass. Hence, *GS*-RMA could easily overcome such drawbacks of the green microalgae. The biomass of *GS* is colorless, normally not contaminated with other microorganisms, and shelf-life oxidation could be negligible. Nutritional applications of *GS* were suggested because of the richness of its cell walls with protein (average of 29%) and polysaccharides (average of 66%) [35].

2.3 Recovery of Rare Metals

One of the important biotechnological applications of *GS*-RMA is the recovery or rare earth metals. The cell walls of *GS* have the ability to bi-sorb the precious metals from the metal-containing wastewaters, even at very low concentrations [36]. *GS* could selectively recover several metals from water such as lanthanides, gold (Au), palladium (Pd), and copper (Cu). Even at very low pH (1.5 – 2.5), *GS* could effectively recover around 90% of low lanthanides concentration (0.5 mg/L) from aqueous solutions via cell fractionation without any genetic manipulation of treatment of the cells [36]. Furthermore, more than 90% of Au (III) and Pd (II) were recovered from aqua-regia-based metallic wastewater, within significantly low concentration conditions which limited the use of chemical or pyro-metallurgic recycling [37].

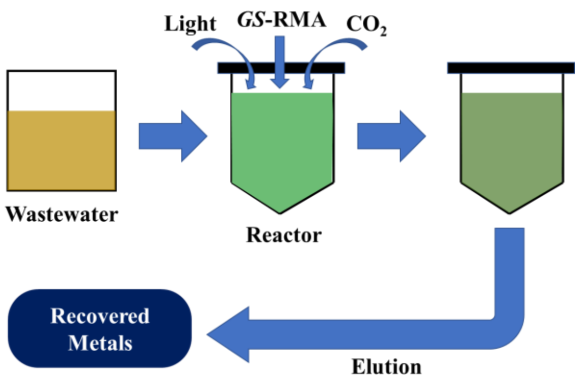

Fig. 2. Schematic of metal recovery system from wastewaters via employing *GS*-RMA.

Reviewing the literature revealed that different algae types (*Spirulina platensis*, *Chlamydomonas reinhardtii*, *Chlorella sp.*, *Chloroidium saccharophilum*…, etc.) have been considered for metals bio-sorption from wastewaters and aqueous mediums such as Cu (II), Cr(III), Zn (II), Mg (II), Cd (II) [38]. However, only few reports have been reported on the use of *GS* despite its several properties, which confirms the gap in the literature on this aspect. Hence, a simple and efficient system for the recovery of metals from wastewaters could be suggested for investigation (Fig. 2), in which it is expected that the entire process could be completed within only one hour.

2.4 Biogas Production

Employing the microalgae-based technologies in biogas production is one of the promising applications. Utilizing RMA as a biomass resource within the anaerobic digestion system could be very efficient in inducing the produced amount of biogas. The photosynthesis of RMA will be executed via the resulted CO_2 from the methanogenesis stage in addition to the light energy. That will be cost-effective as there will be no need for external CO^2 flow. Furthermore, the excess microalgal biomass in the system can be reutilized as substrate for the anaerobic digestion stage. Using *GS*-RMA in such system could be preferable cause of its auto-, hetero-, and mixotrophic ability, which could be also beneficial in terms of the required energy for the metabolism. Such combined system could be very promising within wastewater treatment plants (WWTPs) to achieve both wastewater treatment and biogas production.

3. WATER TREATMENT APPLICATIONS

Since it has unique extremophilic features, *GS*-RMA is considered to be unrivaled microorganism candidate for various biotechnological applications in water treatment systems. The possible applications of *GS* in water treatment systems with respect to its characteristics can be summarized in Table 1.

Table 1. Possible water treatment applications of *GS* considering its unique features.

No.	Feature	Designated Application
1	Enormous metabolic versatility in the utilization of carbon sources (including a large range of sugars and alcohols) for heterotrophic growth.	Perfect choice for the removal of nutrients and dissolved organic carbon from urban wastewater (UWW).
2	Spherical shape with thick-walled cells.	Higher specific surface area towards selective metal precipitation and for metal bio-sorption from wastewater.
3	Remarkable acidophilic ability.	Biological Oxygen Demand (BOD) and nutrients (phosphorus, nitrogen-forms) removal from municipal wastewater.
4	High resistance to high metal concentrations in aqueous solutions (even at low pH < 2.5).	Recovery of rare metals from industrial wastewater (even if present at low concentrations) or from solid waste materials.

3.1 Wastewater Treatment (WWT)-BOD & Nutrients Removal

Among the emerging wastewater treatment (WWT) technologies, algal-based systems hold promise as greener and sustainable alternatives to the current practice. Metabolic capabilities of algae enable them to grow in wastewaters, ingesting their organic- and nutrient-contents. In contrast to the current energy-intensive technologies, algal processes can be driven by energy derived either from sunlight or from the wastewater itself [39]. Depending on their metabolic choice of sources of carbon and energy, algal systems are classified as photoautotrophic, heterotrophic or mixotrophic. Photoautotrophic and chemoheterotrophic systems have been adopted in many applications including WWT. However, applications of mixotrophic systems are currently limited even though they can be seen to be the most suitable ones for WWT from a metabolic perspective [40].

Mixotrophic cultures (such as *GS*) are versatile in that, they can obtain their carbon and energy needs either from organic or inorganic chemicals. Therefore, it can be considered as a combination of both photoautotrophic and chemoheterotrophic processes as shown in Fig. 3; each process can occur independent of the other resulting in accumulation/depletion of CO_2 and O_2.

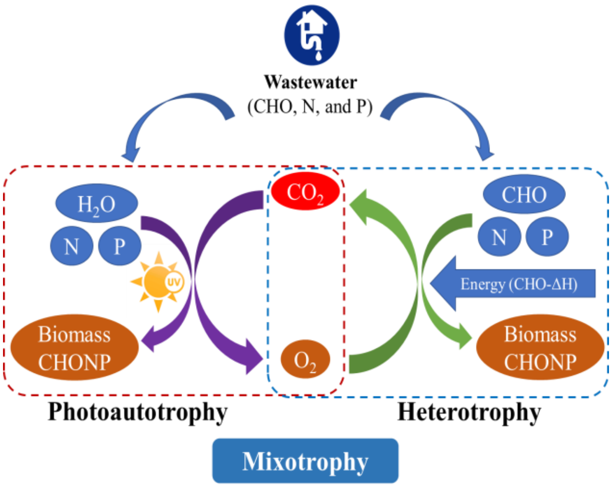

Fig. 3. Schematic of mixotrophic process for utilizing nutrient in wastewater and energy from light (in photoautotrophy) or from organics (in heterotrophy), modified from [41].

Typical wastewater treatment plants (WWTPs) equipped with secondary treatment processes are able to meet the discharge levels for organic carbon [quantified as Biochemical Oxygen Demand (BOD)], but fail to meet the discharge levels for phosphorus (P) and Nitrogen (N). To meet the discharge limits for both P and N, WWTPs are now required to add tertiary treatment systems to treat the secondary effluent. As such, *GS* is a promising strain for single-step urban wastewater treatment instead of the current two-step practices of secondary treatment followed by tertiary treatment.

Current secondary treatment by activated sludge organisms converts nearly 50% of the dissolved organic carbon (DOC) in the wastewater to carbon dioxide (CO_2). An algal system can theoretically oxidize the DOC in wastewater to CO_2 and recapture the metabolic CO_2 via photosynthesis. Thus, algal wastewater treatment would produce significantly more energy-rich biomass than current methods. Technologies are currently available to generate fuels from algal biomass. Mixotrophically derived CO_2 from respiration and O_2 from photosynthesis would also significantly reduce the total metabolic gas supply requirements relative to current secondary and tertiary processes. Based on the above, it is hypothesized that mixotrophic wastewater treatment using *GS* could be employed for energy-positive urban wastewater treatment.

In Table 2, the reported removal efficiencies in the literature of the applied *GS*-WWT systems towards BOD and nutrients removal. Peter Lammers research group in Arizona State University, USA, has been working intensively on investigating the application of *GS* in WWT from nutrient and BOD [41,42]. In their previously reported study, discharge standards (of 30 mg/L BOD; 10 mg/L N; and 1 mg/L P) were achieved for all three pollutants in less than 3 days, in a fed-batch bioreactor within in a pilot-scale system [41]. Moreover, in another study, around 99% and 98% removal efficiencies were achieved for phosphate and ammoniacal-nitrogen, respectively, within one week [42]. Furthermore, on the field application scale, Lammers' group recorded remarkable removal efficiencies of the three pollutants using *GS*.

Table 2. BOD and nutrients removal using *GS* in WWT applications.

Ref.	System conditions	BOD & nutrients removal efficiencies
[41]	Pilot scale system was deployed at a local wastewater treatment plant (700 L bioreactor fed by primary effluent).	• PO_4 (around 77% of initial 4.2 mg/L). • NH_3-N (around 67% of initial 23.4 mg/L). • Around 64% BOD removal (of initial 55.4 mg/L) after 2 days.
[42]	Batch tests considering primary-settled wastewater.	• PO_4 (around 99% of initial 12 mg/L) • NH_3-N (around 98% of initial 35 mg/L)
[43]	Batch tests considering synthesized wastewater simulating the concentrations of N and P in the primary effluent.	• PO_4 (around 95.5% of initial 19 mg/L) after 7 days. • NH_3-N (around 56% of initial 35 mg/L) after 10 days.
[44]	Field study considering primary-settled wastewater.	• PO_4 (around 71–95% of initial 15 mg/L) after 2 days. • NH_3-N (around 63–89% of initial 6 mg/L) after 5 days. • Around 46–72% BOD removal (of initial 32 mg/L) after 6 days.

3.2 Heavy Metals (HMs) Removal

The unique acidophilic and thermophilic abilities of GS-RMA in addition to the high metal tolerance to metals, provides several unique mechanisms for heavy metals (HMs) removal. In Table 3, several reported studies have been included related to the removal of heavy metals (HMs) via the incubation of *GS* in aqueous solutions rich with the designated metals to be removed.

Table 3. Metals removal from aqueous solutions using *GS* incubation.

Ref.	System conditions	Metal	Removal
[36]	Semi-anaerobic heterotrophic conditions (overnight incubation).	Cu (II).	Copper accumulation before recovery.
[37]	After 30 min incubation in wastewater.	Au (III) and Pd (II).	Over 90%.
[45]	90 min incubation in aqueous medium at 24ºC.	Cu (II) and Pb (II).	Selective biosorption to Copper only.

4. PROPOSED APPLICATIONS

After reviewing the up to date accomplishments as well as the gaps within the literature on the use of the RMA in water treatment applications, the following ideas are proposed:

4.1 Phycoremediation for HMs

There is a lack of knowledge on the use of *GS* in HMs removal from water as most of the reported studies only focused on the nutrients and BOD removal. However, the unique features of *GS* make it a perfect candidate for an efficient HMs removal by different possible mechanisms via several phycoremediation processes (Fig. 4).

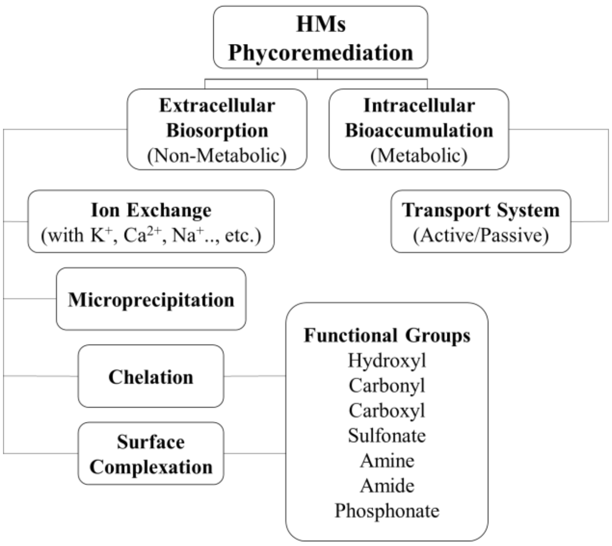

Fig. 4. Phycoremediation approaches for HMs removal by micro-algae, modified from [38].

4.2 Pharmaceuticals Removal

Environmental pollution due to the presence of pharmaceuticals in wastewater is one of the major threats to aquatic environment globally and it attracts great attention nowadays as one of the hot topics around the world. Huge amounts of pharmaceuticals enter the surface water bodies after the partial removal by the conventional WWTP [46–49]. Different studies have been focused on integrating different kinds of algae for this process [50]. However, there are almost no other studies concerned with integrating *GS* in such applications. Therefore, investigating the removal of different pharmaceuticals (such as antibiotics and personal care products) by *GS*-based treatment system could be a promising technology and worth to be investigated

4.3 Bio-resin Production

Working on this aspect will represent a great novelty as it will be the first time to produce a bio-resin by immobilizing *GS* on silica gel or proprietary polymer into adsorption columns for biotechnological cleanup of contaminated sites as well as the removal of HMs. Silica gel is an inorganic material which has several crucial features, such as the stability in acid conditions, no swelling, high mass selectivity, high porosity, and high specific surface area. Such properties make silica gel an ideal supporting material for environmental remediation applications. The main idea is modifying the silica gel surface via immobilizing the organic functional groups (i.e., carboxyl, hydroxyl, and carbonyl), which are derived from the *GS*-RMA. Such functional groups will enhance the ability of the composite for the bio-sorption of HMs as well as ion exchanging. Utilizing the bare microalgal powder could be ineffective to be used as column filling material because of its low density. Hence, immobilizing the *GS* biomass on the supporting matrix of silica gel will increase the quality of the adsorbent to be used as column filling material for the continuous sorption process and to increase the chemical stability of the composite in general. The bond was proven previously in the literature by Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy-energy dispersive spectrum (SEM-EDS) analyses. It was confirmed by the loss of the Si-O stretching by the reduction in the silanol group (Si-OH) from the biomass condensation on the surface, in addition to the presence of NH and CH groups. Figure 5 shows the proposed production steps of the *GS*/silica-gel bio-resin.

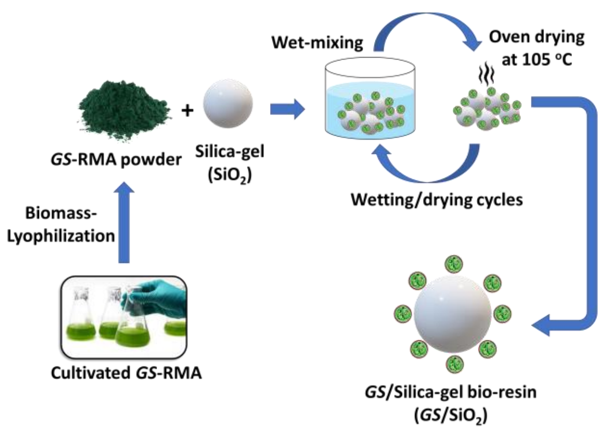

Fig. 5. Schematic of the production steps of *Galdieria Sulphuraria*/Silica-gel bio-resin (*GS*/SiO_2).

4.4 Bio-substrate for Nanoparticles

As one of the pioneering technologies in the world, nanotechnology still attracts great attention in the field of water treatment applications [51–56]. Nanoscale zero-valent iron (Fe^0) has great advantages in the removal of most of the soluble contaminants in aqueous solutions [57–59]. Combining the unique features of Fe^0, such as the core shell structure and the high redox potential, with the special characteristics of *GS*-RMA could yield a novel composite with extraordinary remediation abilities [60–62]. Supporting the nanoparticles on the microalgal surface will provide various removal pathways for different contaminants via either the reactive surface of Fe^0 or the organic functional groups of *GS* [63–67].

5. CHALLENGES & LIMITATIONS

The main challenges for the application of microalgae in wastewater treatment are:

- The harvesting of the algae, due to the settling characteristics and operational conditions: can be overcome by the unconventional harvesting systems such as the paddlewheel system developed by Lammers' group [42].
- The control of biomass composition is complicated by the selection of the desired species: using *GS*-RMA has a great potential in largescale outdoor cultivation without becoming contaminated with other microorganisms, under both mixotrophic and heterotrophic conditions, owing to its tolerance with extreme pH and temperature conditions.
- The determination of an optimal ratio of algae and bacteria biomass: considering different operating conditions in the batch-scale before scaling up to the pilot level could be very beneficial in such aspect.
- The possible need for external CO_2 present additional obstacles: such drawback will be overcome by the use of mixotrophic cultures such as *GS*-RMA which has the ability to accumulate CO_2 within the system.

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