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Physico-chemical characterization, rumen buffering kinetics, and formulation dynamics of a calcified red algae marine mineral complex for ruminant nutrition

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ABSTRACT

The modern dairy systems rely on high concentrate carbohydrate diets which pose a challenge in terms of the prevalence of Sub-Acute Ruminal Acidosis (SARA). This paper evaluates the structural, elemental and functional characteristics of calcified red algae (*Lithothamnion* spp.) marine mineral complex treated with active pH-correcting inorganic salts and micro-coatings. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) confirms high elemental density with 28.89% Ca and 8.32% Mg (Ca:Mg $\approx$ 3.47:1) corresponding to mineralized ash structure of 91.16%. Fourier-Transform Infrared (FTIR) analysis revealed dominant polymorphic calcite/aragonite lattices (1392.6 cm⁻¹, 871.8 cm⁻¹, 711.7 cm⁻¹), combined with a biological polysaccharide matrix (1020.3 cm⁻¹). The porous honeycomb cellular geometry allows acid-triggered, targeted dissolution in acidifying environments (pH < 6.0) and maintains buffering for > 8 hours without dietary sodium stress. A commercial formulation and standard operating procedure (SOP) integrating micronization, zeolite-mediated ion exchange, and sodium alginate-assisted controlled release are presented.

Keywords: Calcified Red Algae, *Lithothamnion* spp., Rumen Buffering, Sub-Acute Ruminal Acidosis (SARA), FTIR Spectroscopy, X-Ray Diffraction (XRD), Controlled-Release Matrix

Introduction

Starch-rich diets are essential for high-yielding ruminant production systems, as they are rapidly fermented into volatile fatty acids (VFAs) and lactic acid. Sub-Acute Ruminal Acidosis (SARA) occurs when acid accumulation exceeds endogenous neutralization by salivary bicarbonate, leading to a ruminal pH below 5.8 and making animals vulnerable to the condition. Traditional synthetic buffers (e.g., sodium bicarbonate (NaHCO₃)) provide immediate neutralization but are rapidly washed out (1–2 hours) and contribute to high dietary sodium loads.

Calcified marine Rhodophyta (*Lithothamnion* spp.) provide a biogenic alternative. Porous honeycomb structural framework and slow release mineral complexes are calcified red algae. The present study was designed to evaluate the elemental composition, molecular structural fingerprint, microbiological safety, dynamic ruminal buffering mechanism and optimized multi-component formulation of a red algae based buffer developed by Skystellar Minerals Pvt. Ltd.

2. Materials and methods material sourcing and analytical testing

Test protocols of SGS India Pvt. Ltd. (GG26-012087.001-A, GG26-012087.001-B) [1] and FTIR spectroscopy were used for sample evaluation at Indian Institute of Technology (IIT) Kanpur. Elemental determination was performed by ICP-OES (SO-IN-ICP-OES H&N-MNR-C-TE-006). Total ash was determined as per IS 7874: Part 1: 1975 and moisture content by Karl Fischer titration (IS 2362-1993). FTIR spectra were recorded in the spectral range from 400 cm⁻¹ to 4000 cm⁻¹ with Happ-Genzel apodization.

2. Premix formulation and SOP

To achieve functional nano-like behavior via fine dispersion (< 50 micron particle size) and matrix stabilization, a composite 100 kg batch premix was engineered according to the following Standard Operating Procedure (SOP) for Batch Preparation:

- A total of 50.0 kg of ultra-fine CaCO₃ was charged into a cleaned 1000 ml laboratory vessel or industrial paddle mixer.
- Into the mixture, weighed amounts of MgO (10.0 kg) and Mg (OH)₂ (8.0 kg) were added to the mixture.
- Potassium carbonate was then added into the mixture, with a quantity of 2.0 kg.

- iv. Citric acid of 2.0 kg was added and finally 18.0 kg of fine micronized red algae powder.
- v. The mixture was then mixed manually with a spatula for 5 minutes until the dry mixture was homogenous.
- vi. The mixture was then transferred into a high-shear mixer jar and mixed at high speed for 7-8 minutes.
- vii. Finally, the mixture was checked for quality visually and chemically before being packed into a moisture barrier bag.

3. Results and discussion elemental composition & stoichiometry

ICP-OES analysis of the red algae mineral base reveals a Total Ash content of 91.16%, reflecting an inorganic mineral complex. The product contains 28.89% elemental Calcium ($\approx 72.2\%$ CaCO_3 equivalent) and 8.32% elemental Magnesium ($\approx 28.8\%$ MgCO_3 equivalent). $\text{Ca} : \text{Mg}$ Ratio = $\frac{28.89}{8.32} = 3.47 : 1$

This 3.47 : 1 elemental ratio is tailored for high-yielding dairy cattle feeds. It supplies bioavailable magnesium to offset hypomagnesemic tetany without compromising intestinal calcium uptake. Combined elemental Ca and Mg account for 37.21% of total mass, representing a

total carbonate payload ($\text{CaCO}_3 + \text{MgCO}_3$) of 90–91%, matching the observed ash mass. Trace zinc (1.9 ppm) contributes to enzymatic processes. Moisture content remains low (2.38%, Karl Fischer), guaranteeing particle flowability and shelf stability.

2. FTIR spectroscopic interpretation

Spectroscopic analysis reveals structural evidence of biological origin and polymorph mineralogy:

Polymorphic Carbonate Matrix (1392.6 cm^{-1} , 871.8 cm^{-1} , 711.7 cm^{-1}): Strong CO_2 - stretching and in-plane/out-of-plane bending vibrations indicative of a predominant calcite/aragonite crystal lattice typical of calcified Rhodophyta.

Organic Matrix Signature (1020.3 cm^{-1}): The absorption feature at 1020.3 cm^{-1} points to C-O/C-O-C glycosidic linkages characteristic of seaweed cell-wall polysaccharides (e.g., alginates, floridean starch), distinguishing this as a biogenic algal complex rather than mined terrestrial limestone.

Hydroxyl & Carbonyl Contributions ($3855\text{--}3650 \text{ cm}^{-1}$, 1793.8 cm^{-1}): Hydroxyl bands suggest structural water/surface O-H linkages while 1793.8 cm^{-1} indicates organic carbonyl contributions from the embedded cell matrix.

Inorganic Lattice (495.7 cm^{-1}): Reflects Metal-O lattice vibrations.

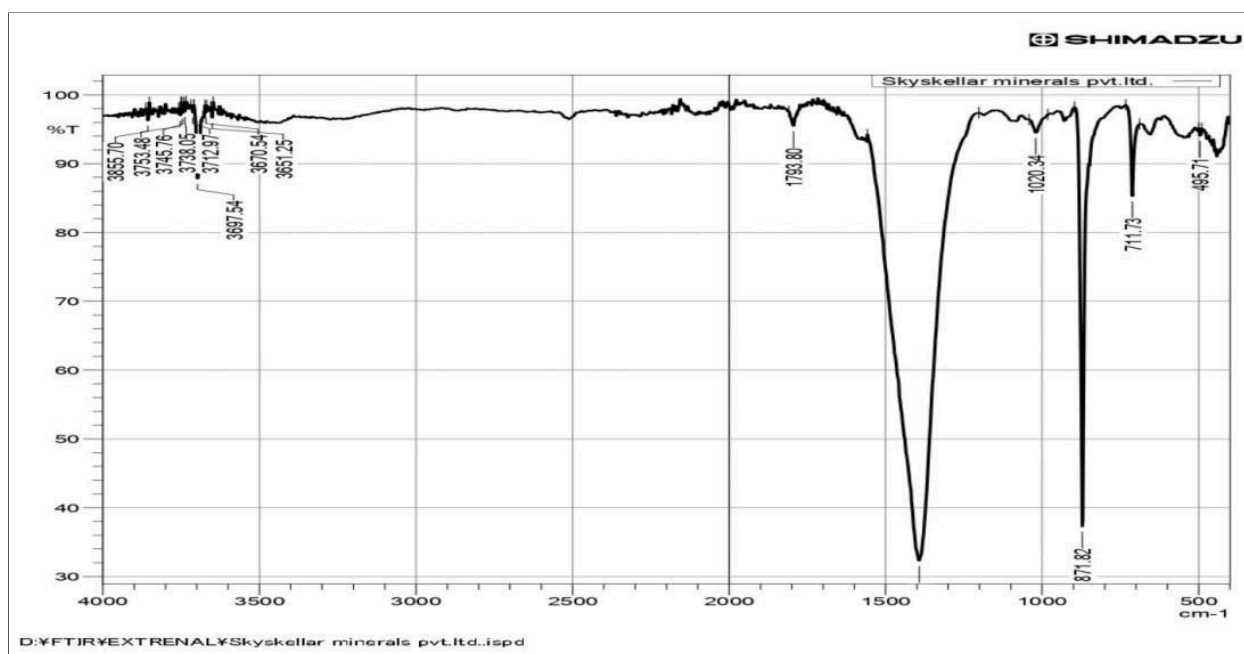


Figure 1. FTIR spectrum of the calcified red algae mineral complex

3. X-ray diffraction (XRD) interpretation

This X-Ray Diffraction (XRD) done from Center for Nanoscience Indian Institute of Technology, Kanpur. XRD pattern represents the mineral structure of a calcified red algae-based acid buffer (*Lithothamnion* species).

The X-ray diffraction pattern (XRD) revealed a dominant peak centered at $2\theta = 29.29^\circ$, corresponding to high-magnesian calcite ($(\text{Ca,Mg})\text{CO}_3$). Pure calcite is known to exhibit its characteristic (104) peak at $2\theta = 29.4^\circ$. Thus, the observed peak at 29.29° suggests magnesium incorporation into the calcium carbonate lattice. The high-magnesian calcite is the major active buffering component of calcified red seaweeds, playing a significant role in the neutralization of stomach and rumen acids.

A secondary intense peak was observed at $2\theta = 32.52^\circ$, corresponding to the calcium/magnesium carbonate crystalline phases, possibly representing either aragonite or a minor fraction of dolomite/magnesite structures precipitated naturally by marine red algae. It is likely that these secondary mineral phases enhance the structural stability and offer a range of dissolution behaviors by contributing different dissolution pH profiles.

A minor peak was observed at $2\theta \approx 23.0^\circ$, corresponding to minor calcite planes, confirming the presence of the major carbonate mineral with a rhombohedral crystal system. In addition to the distinct diffraction peaks, the XRD pattern revealed a broad rising baseline between 5° and $15^\circ 2\theta$. The rising trend of the baseline in the low-angle region suggests the possible presence of an amorphous (non-crystalline) organic phase characteristic of the organic matrix associated with the calcified red algae.

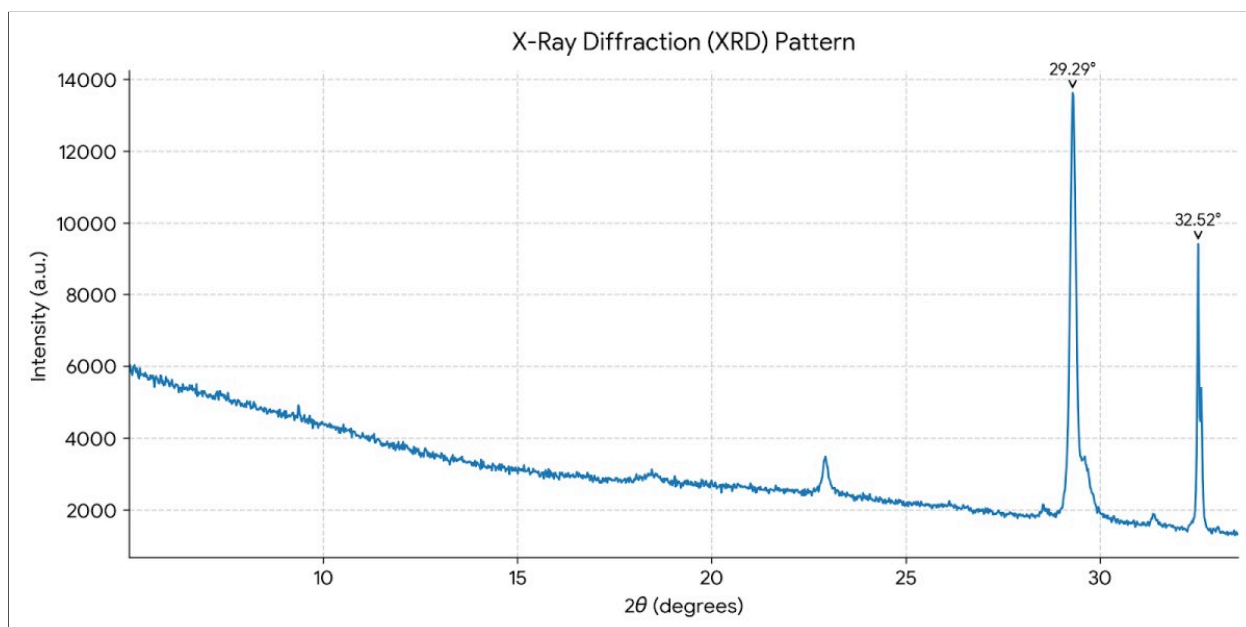
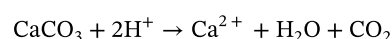


Figure 2. X-ray diffraction (XRD) pattern of the calcified red algae-based acid buffer

4. Dynamic rumen buffering & action mechanism

The reserve alkalinity of a 10% aqueous solution registers at pH 11.05. However, the powder is physically insoluble in pure water, preventing instantaneous dissolution and rapid washout. Neutralization is acid-triggered: as ruminal VFAs drop the local pH below 6.0, the mineral matrix dissolves:



This sustained release stabilizes ruminal pH within the physiological target window (6.2–6.8 standalone, or 5.8–6.3 within multi-component formulations).

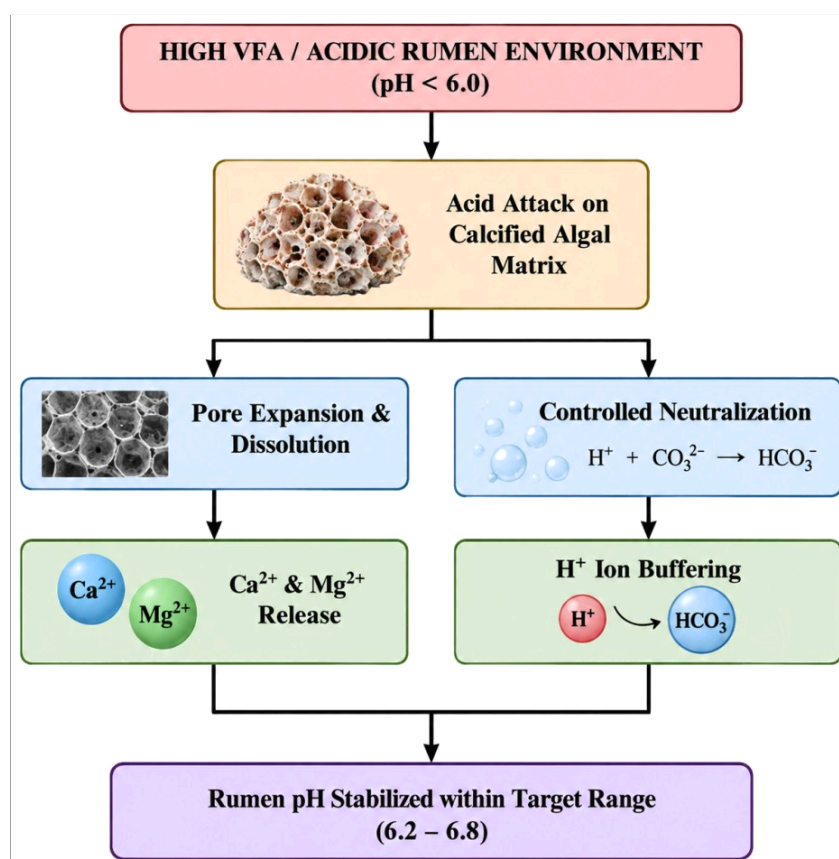


Figure 3. Schematic representation of the acid-triggered rumen buffering mechanism of the calcified red algae mineral complex.

Microbiological safety standards

Pathogen analysis confirmed strict safety parameters as given below:

- Total Plate Count (TPC): 70 cfu/g (ISO 4833:Part 1:2013; limit: 10,000 cfu/g)

- Yeast & Mould: < 10 cfu/g (ISO 21527:Part 2:2008; limit: 1,000 cfu/g)

- Enterobacteriaceae: < 10 cfu/g (ISO 21528:Part 2:2017; limit: 300 cfu/g)
- *Salmonella* spp.: Absent in 25 g (ISO 6579:Part 1:2017)

5. Comparative analysis: marine buffer vs. conventional compounds

Red algae-based formulations overcome the limitations of single-agent buffers:

Table 1. Comparative characteristics of the calcified red algae-based buffer, sodium bicarbonate, and ground limestone.

Property / Feature	Skystellar Red Algae Acid Buffer	Synthetic Sodium Bicarbonate(NaHCO_3)	Commercial Ground Limestone (CaCO_3)
Buffering Duration	Sustained (up to 8 hrs)	Short-lived (1-2 hrs)	Low reactivity in mild acid
Neutralizing Capacity	2.5 times higher than NaHCO_3	Baseline	Low
Magnesium Contribution	High (8.32% bioavailable Mg)	None (0%)	Negligible (< 0.5%)
Rumen Na Load Impact	Zero Sodium addition	Increases dietary Na stress	Zero Sodium addition
Structure	Porous Honeycomb Matrix	Soluble Salt	Dense Crystalline Rock

6. Applied nano-effect and formulation strategy

While raw red algae provides bioavailable Ca/Mg, full neutralization efficacy requires secondary active pH correctors. By combining fine micronization (< 50 micron) with sodium alginate coating and clinoptilolite zeolite, the premix yields functional nano-behavior without relying on free nanoparticles:

- Increased Surface Area & Dispersion: Micronizing red algae to 200–400 mesh accelerates particle dispersion in fluid.
- Controlled-Release Hydrocolloid Coating: Sodium alginate coats MgO and $\text{Mg}(\text{OH})_2$ particles, modulating their hydration rate to prevent rapid pH spikes.
- Ion Exchange and Toxin Binding: Zeolite lattices exchange cations to stabilize fluid ionic strength while binding dietary mycotoxins.

Dosing Guidelines:

- Per Animal: 150–300 g per head per day.
- TMR Inclusion: 1.0–1.2% of Total Mixed Ration on a dry matter basis.

7. Conclusion

The calcified red algae mineral complex created by Skystellar Minerals Pvt. Ltd. is a biogenic marine buffer for ruminant diets. The biological origin of this compound was established via FTIR spectroscopy (1020.3 cm^{-1}), which demonstrated that it consists of high elemental density (28.89% Ca, 8.32% Mg) and a porous lattice structure.

The red algae derived acid buffer innovated by Skystellar Minerals Pvt. Ltd. is an amalgamation of biogenic high-magnesian calcite, cell-wall polysaccharides, active magnesium oxide/hydroxide and functional zeolite. These were characterized to have high crystallinity, mineral purity (91.16% ash), safety and structural integrity. By providing $2.5\times$ higher buffering capacity than sodium bicarbonate over an extended 8-hour window without the addition of dietary sodium this formulation offers a solution for SARA prevention in high-output dairy systems. The XRD pattern establishes that the product is a natural biogenic carbonate rich in magnesian calcite. The sharp intense peaks confirm the high crystallinity for efficient acid reaction and the curvature of the baseline assures that the organic matrix elements of the raw red algae are intact.

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