



## The Effect of Divalent Ion Addition to Rhamnolipids Solution Through Fluid-to-Fluid Testing for Enhanced Oil Recovery: A Review

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### Abstract

Enhanced oil recovery (EOR) continues to be developed to optimize oil production from mature oil fields. One promising EOR method is the use of biosurfactant solutions, which are environmentally friendly and can reduce oil-water interfacial tension, enhancing the mobility of trapped oil in reservoir rocks. However, the effectiveness of rhamnolipid biosurfactants is strongly influenced by geochemical conditions, particularly the presence of divalent ions such as calcium ( $Ca^{2+}$ ) and magnesium ( $Mg^{2+}$ ) in formation water. This study aims to investigate fluid-to-fluid interactions between rhamnolipid solutions and divalent ions to improve oil recovery efficiency. Experiments were conducted to investigate the effects of adding calcium and magnesium ions on the physicochemical properties of rhamnolipid solutions, including changes in interfacial tension and rheological properties. Imbibition and precipitation tests were also conducted at the laboratory scale to evaluate the effectiveness of rhamnolipid biosurfactants in enhancing oil recovery. The results showed that, in terms of both interfacial tension reduction efficiency and viscosity of the emulsion formed, the presence of divalent ions can have a significant impact on biosurfactant performance. Under certain conditions, divalent ions can reduce the efficiency of oil mobilization. However, by optimizing the rhamnolipid biosurfactant formulation with the appropriate concentration of divalent ions, recovery efficiency can be improved. This study provides valuable insights into the development of biosurfactant-based EOR technologies, particularly for reservoir environments with high divalent ion content. The results are expected to serve as a foundation for developing more stable and efficient biosurfactant solutions for applications in the oil and gas industry.

**Keywords** biosurfactant, divalent ions, fluid-to-fluid interaction, interfacial tension, and oil mobility

### INTRODUCTION

In tertiary Enhanced Oil Recovery (EOR), surfactant injection is used to extract residual oil left after primary and secondary recovery, where up to 70% of the original oil in place can remain trapped (Kalita et al., 2022; Sheng, 2013). Surfactants function by reducing oil-water interfacial tension (IFT), increasing capillary number, and mobilizing trapped oil (Hayavi et al., 2022). Recently, attention has shifted toward biosurfactants, particularly rhamnolipids, a type of glycolipid produced by *Pseudomonas aeruginosa*. Rhamnolipids are biodegradable, low in toxicity, and environmentally friendly, making them suitable for green EOR applications (Kabeil et al., 2025; Sharma et al., 2017; Sorour et al., 2025). They are effective in reducing IFT, emulsifying oil, and solubilizing it, thereby enhancing displacement efficiency. A key aspect of surfactant-based EOR is the formation of microemulsions, which are stable and thermodynamically favorable oil-water mixtures that form spontaneously at specific compositions (Samak et al., 2020).

These microemulsions reduce IFT to ultra-low levels and improve oil mobility via three main mechanisms: mobilization (adhesion reduction), solubilization (oil into micelles), and emulsification (oil droplets in water) (Al-Wahaibi et al., 2014; Azad, 2021; Zhang et al., 2023). The

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rheological properties of microemulsions, particularly viscosity, also play a vital role in improving sweep efficiency and mobility control (Marhaendrajana et al., 2025).

The presence of salt and ions, especially divalent cations such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , can significantly influence surfactant performance (Derikvand et al., 2020; Marhaendrajana et al., 2018). These ions may interact with anionic head groups of rhamnolipids, creating ionic bridges that change surfactant aggregation from micelles to larger structures (e.g., wormlike micelles, lamellar phases). This can both reduce IFT further and increase viscosity, which may improve the mobility ratio and flow profile if within optimal limits. However, excessive concentrations may destabilize the system through precipitation or reduced surfactant effectiveness, underscoring the need for careful control of chemical interactions (Belhaj et al., 2020; Chowdhury et al., 2022; Yan et al., 2010). For medium to light crude oils (API > 25), rhamnolipid-based EOR is particularly suitable due to the lower oil viscosity and better responsiveness to IFT reduction and emulsification. In contrast, thermal methods remain the preferred option for heavy oils (Fardami et al., 2022). The study highlights the significance of understanding fluid-to-fluid interactions between rhamnolipids and divalent ions in formulating biosurfactants that remain stable and effective even in high-salinity reservoirs (Al-Sakkaf & Onaizi, 2023; Chen & Lee, 2022). When optimized, this approach enables efficient displacement of residual oil, enhances recovery from mature fields, and supports sustainable, environmentally friendly EOR practices.

## LITERATURE REVIEW

The literature study in this research compiled information on the latest developments in biosurfactant applications for enhanced oil recovery (EOR), with a particular focus on fluid-to-fluid interactions involving rhamnolipids and divalent ions. A summary of relevant studies is presented in Table 1).

**Table 1.** Summary of Literature Studies

No	Title	Object	Description
1	Surface active compounds from microorganisms (Cooper & Zajic, 1980)	The objects studied were microbial biosurfactants derived from hydrocarbon-utilizing bacteria.	This study identified microbial surface-active compounds, focusing on their ability to reduce interfacial tension and form stable emulsions in hydrocarbon environments.
2	Potential commercial applications of microbial surfactants (Banat et al., 2000).	The objects studied involved the application potential of microbial surfactants in diverse industries.	This reference reviewed the broad industrial use of biosurfactants, particularly in enhanced oil recovery (EOR), wastewater treatment, and pharmaceuticals, emphasizing their ecological benefits.
3	Production and Surface-Active Properties of Microbial Surfactants (Margaritis et al., 1979)	The objects studied were microbial fermentation processes for biosurfactant synthesis.	This work detailed production methodologies and examined the physicochemical properties—such as surface tension reduction—of biosurfactants applicable to oil recovery.

No	Title	Object	Description
4	Natural roles of biosurfactant (Ron & Rosenberg, 2001).	The objects studied were the environmental and physiological roles of microbial biosurfactants.	This research highlighted the roles of biosurfactants in microbial motility, adhesion, and solubilization of hydrophobic substrates, reinforcing their natural relevance to oil recovery.
5	Potential applications of microbial surfactants in biomedical sciences (Singh & Cameotra, 2004).	The objects studied were biosurfactants used in biomedical and biotechnological applications.	This paper explored how biosurfactants serve as antimicrobial agents, antiadhesives, and biofilm disruptors, suggesting use in drug delivery and medical coatings.
6	The influence of cell immobilization by biofilm forming on the biodegradation capabilities of bacterial consortia (Górna et al., 2011).	The objects studied were bacterial consortia immobilized through biofilm formation.	The study demonstrated enhanced degradation of hydrocarbons by immobilized microbial consortia, promoting efficient biosurfactant-mediated bioremediation.
7	Rhamnolipid-biosurfactant permeabilizing effects on gram-positive and gramnegative bacterial strains (Sotirova et al., 2008).	The objects studied were rhamnolipid biosurfactants and their effects on bacterial cell membranes.	Rhamnolipids were shown to permeabilize both gram-positive and gram-negative bacteria, supporting their use in oil emulsification and enhanced microbial oil release.
8	Microbial Surfactants: The Next Generation Multifunctional Biomolecules for Applications in the Petroleum Industry and Its Associated Environmental Remediation (Fenibo et al., 2019).	The objects studied were biosurfactants applied in petroleum and environmental remediation.	This study reviewed biosurfactants' multifunctionality, such as emulsification, biocompatibility, and pollutant solubilization, for eco-friendly petroleum engineering solutions.
9	Biosurfactants: Production, properties, applications, trends, and general perspectives (de Medeiros et al., 2022).	The objects studied were biosurfactant production systems and industrial trends.	This review provided extensive insights into biosurfactant synthesis technologies, commercial scalability, application versatility, and future innovation directions.
10	The Wnt/β-catenin signaling in endometriosis, the expression of total and active forms of β-catenin, total and inactive forms of glycogen synthase kinase-3β, WNT7a and	The objects studied were cellular signaling molecules in endometriosis cases.	Unrelated to petroleum or biosurfactants, this paper focused on molecular mechanisms like β-catenin and GSK-3β in reproductive health pathology.

No	Title	Object	Description
	DICKKOPF1 ( <a href="#">Pazhohan et al., 2018</a> ).		

## RESEARCH METHOD

The method in this study is designed to address identified challenges in biosurfactant application for tertiary oil recovery through a structured series of steps: problem identification, laboratory testing, data processing, and comprehensive analysis.

### Problem Identification

The primary challenge in applying biosurfactants for EOR is the unpredictable behavior of biosurfactant solutions when interacting with divalent ions ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ), which are commonly found in formation water. These ions may enhance or deteriorate surfactant performance, leading to instability in the microemulsion structure, ineffective reduction of interfacial tension (IFT), or increased viscosity beyond injectivity limits. This study aims to resolve this uncertainty by formulating a systematic investigation of fluid-to-fluid interactions and identifying the optimal conditions for biosurfactant performance under varying salinity and ion concentrations.

### Field Observation

Field observations were conducted on January 22, 2024, to collect data and information directly from the field, thereby understanding field conditions in real-time, observing factors that influence wells, identifying patterns or trends, developing more accurate prediction models, and improving strategies for determining candidate reactivation wells.

## FINDINGS AND DISCUSSION

The experimental findings indicate that the presence of divalent ions, specifically calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ), significantly influences the performance of rhamnolipid biosurfactants in Enhanced Oil Recovery (EOR) applications. At optimal concentrations, these ions enhance the formation of stable microemulsions and contribute to ultra-low interfacial tension (IFT), improving oil mobilization by promoting efficient fluid-to-fluid interactions at the oil–water interface. This is due to the ion-bridging effect, where divalent cations strengthen the molecular arrangement of rhamnolipids. However, excessive concentrations can lead to the formation of larger aggregates, such as wormlike micelles or lamellar phases, increasing viscosity and potentially reducing injectivity or causing biosurfactant precipitation. These results highlight the importance of optimizing ion concentration to balance the beneficial and adverse effects. Overall, the study confirms rhamnolipid's potential as a sustainable alternative to synthetic surfactants, capable of performing effectively in high-salinity reservoirs with complex geochemical compositions.

### Biosurfactant Composition

The *rhamnolipid* biosurfactants used in this study were obtained from Shanghai Yuchuang Chemical Technology Co., Ltd. The following are the analytical results for the composition of both biosurfactants:

**Table 2.** Biosurfactant Composition Results

Item	<i>Rhamnolipids</i>
<b>Appearance</b>	Dark Brown Liquid
<b>Solid Content</b>	29.88%

Item	Rhamnolipids
pH Value	3.95
Water Solubility	Soluble

### Rhamnolipids

Rhamnolipids are produced by *Pseudomonas aeruginosa*, in which this biosurfactant possesses two hydrophilic head groups: a carboxyl group, which imparts an anionic character to rhamnolipids, and a rhamnosyl group, which contributes to the bulkiness of the head (Eslami et al., 2020; Zhao et al., 2020). Additionally, rhamnolipids are relatively more hydrophilic compared to synthetic surfactants (Nguyen & Sabatini, 2011).

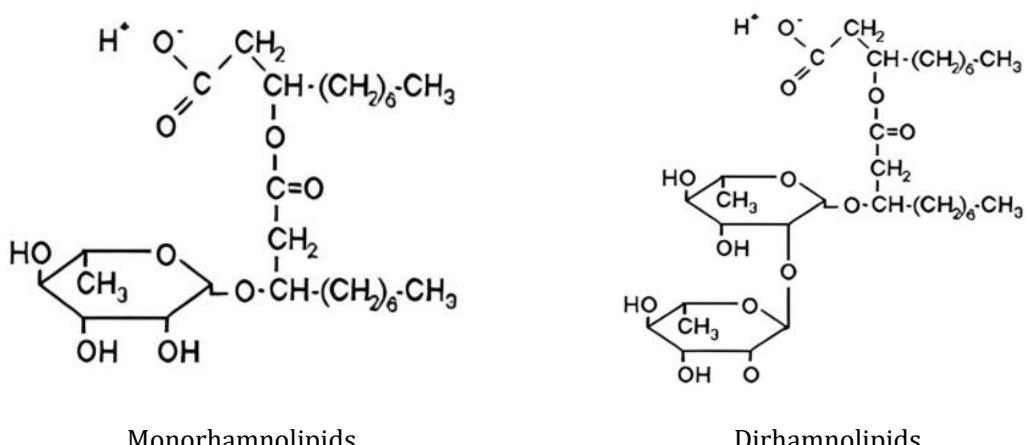


Figure 4. General Structure of Rhamnolipid Molecules

The molecular structure of rhamnolipids can vary depending on the bacterial strain that produces them and the growth conditions. However, the basic structure of rhamnolipids consists of a hydrophobic fatty acid chain and a hydrophilic rhamnose sugar.

According to a study published in the Journal of Industrial Microbiology and Biotechnology, rhamnolipids produced by *Pseudomonas aeruginosa* have a *monorhamnolipid* structure, meaning they contain one rhamnose sugar molecule. In contrast, rhamnolipids produced by *Burkholderia thailandensis* have a *dirhamnolipid* structure, which means they contain two rhamnose molecules (Arifin et al., 2025; Górná et al., 2011; Ren et al., 2023).

Another study published in the Journal of Applied Microbiology compared the molecular structures of rhamnolipids produced by various bacterial strains, including *Pseudomonas aeruginosa*, *Burkholderia thailandensis*, and *Burkholderia pseudomallei*. The study found that rhamnolipids from *P. aeruginosa* and *B. thailandensis* share similar molecular structures, while those produced by *B. pseudomallei* exhibit more complex structures (Sotirova et al., 2008).

In summary, the molecular structure of *rhamnolipids* varies depending on the bacterial strain producing them and the growth environment. The fundamental structure comprises a hydrophobic fatty acid tail and a hydrophilic rhamnose sugar. The primary structural difference among *rhamnolipids* from different strains lies in the number of rhamnose units, some possess *monorhamnolipid* structures, while others exhibit *dirhamnolipid* or more complex configurations.

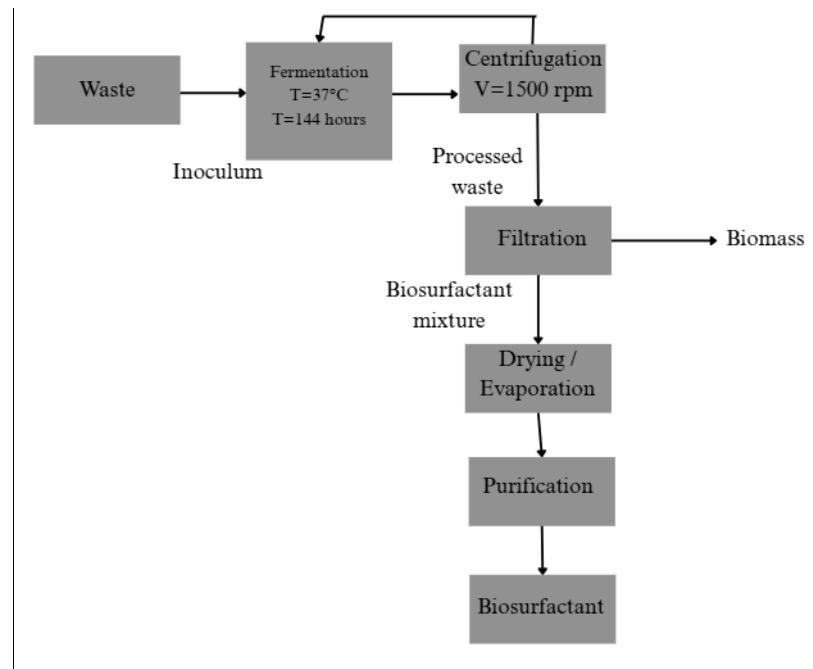
### Biosurfactant Production Process

The production process of biosurfactants can be divided into several stages as follows:

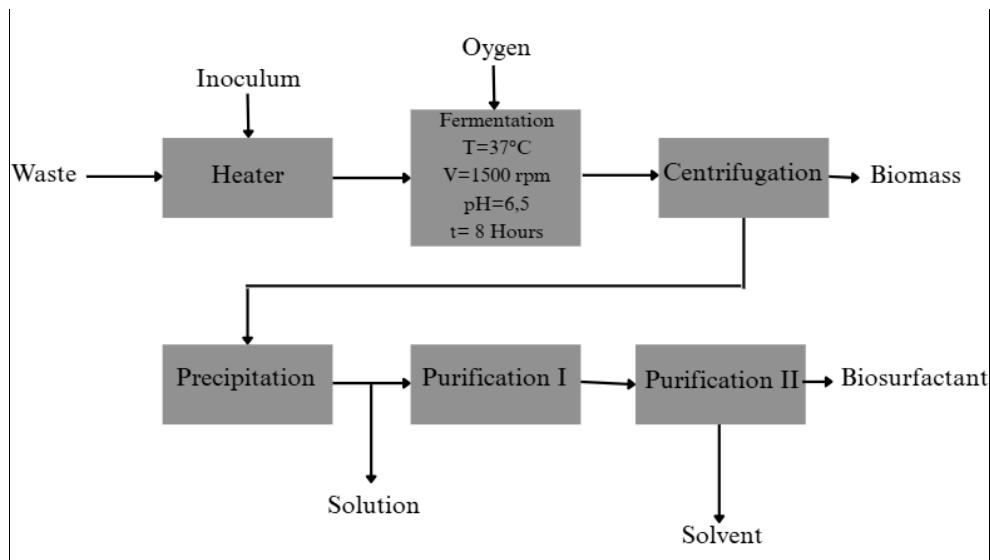
1. Product Storage Stage

2. Product Formation Stage
3. Product Separation and Purification Stage

Various technologies are used in the production of biosurfactants, which can generally be categorized into two methods: batch (staged) systems and continuous systems. The diagrams below illustrate these two production methods.



**Figure 5.** Biosurfactant production from bacteria using a batch (staged) system



**Figure 6.** Biosurfactant production from bacteria using a continuous system

## Biosurfactant Production Systems

### Batch System Production Workflow:

- The batch system is the simplest method for producing biosurfactants using bacteria.
- In this method, bacterial cultures are allowed to grow in a closed container or fermenter under controlled conditions.
- Nutrients and substrates required for bacterial growth and biosurfactant production are added at the beginning of the process.
- Bacterial growth and biosurfactant production occur over a defined time period (a batch).
- After this period, the production is stopped, the tank is emptied, and a new cycle begins by introducing fresh bacterial cultures and nutrients into a clean tank.
- The advantage of the batch system is its simplicity and ease of operation; however, the drawback is that production must pause during tank emptying and restarting the new cycle.

### Continuous System Production Workflow:

- The continuous system is a more efficient and sustainable method for biosurfactant production.
- In this approach, bacterial cultures grow and produce biosurfactants continuously without halting the production process.
- The production tank in a continuous system is typically equipped with a steady feed system for nutrients and substrates.
- Nutrients and substrates are supplied periodically in accordance with the optimal growth and production rates of the bacteria.
- The produced biosurfactant is continuously harvested from the production tank using a controlled output system.
- The main advantages of the continuous system are uninterrupted production, higher yields compared to batch systems, and more efficient resource utilization.

The choice between batch and continuous systems depends on production needs, cost, efficiency, and other factors related to product specifications and production scale. However, in industrial-scale biosurfactant production, the continuous process using *Pseudomonas aeruginosa* is often preferred because:

- Biosurfactant production using bacteria is more biodegradable and applicable to many fields.
- The resulting product includes biomass that can be removed or further processed.
- The fermentation process using *Pseudomonas aeruginosa* does not require high temperatures.

## Interfacial Tension

Interfacial tension, commonly referred to as IFT, is a measurement of the force required to expand the interface between two immiscible liquids over a certain area (Han et al., 2022; Zhang et al., 2023). Surface tension refers to the forces occurring along the surface of a single liquid, whereas interfacial tension refers to the energy acting along the interface between two different liquids. This force arises from the contact between two fluids of different phases.

In the International System of Units (SI), interfacial tension is expressed in mN/m or dyne/cm. A reduction in interfacial tension decreases cohesive forces and conversely increases adhesive forces. Cohesive forces are the forces between molecules of the same kind, while adhesive forces are the forces between different kinds of molecules. Interfacial tension is calculated using the Vonnegut equation, which is based on the width of an oil droplet. The equation is as follows:

## Microemulsion Viscosity

Microemulsion viscosity plays a crucial role in controlling the mobility ratio and sweep efficiency in Enhanced Oil Recovery (EOR) applications. The addition of biosurfactants such as rhamnolipids can form microemulsion structures that reduce interfacial tension and alter fluid viscosity. The presence of divalent ions like  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  enhances molecular interactions through ionic bridging, leading to the formation of aggregates such as wormlike micelles or lamellar phases, which increase viscosity. This viscosity enhancement improves oil displacement efficiency; however, at excessive ion concentrations, the system may become unstable and lead to precipitation. Therefore, careful control of biosurfactant formulation and brine salinity is essential to maintain microemulsion stability and achieve optimal performance in complex reservoir conditions.

## Phase Behavior

In the phase behavior test, it was observed that Rhamnolipids can form stable emulsions, but only in small volumes. The testing was conducted on 90 samples with varying concentrations of Rhamnolipids (0%, 0.5%, 1%, 1.5%, and 2%) using three types of synthetic brine (5000 ppm, 10000 ppm, and 15000 ppm), considering the effect of added  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions, and with two kinds of oil characteristics (medium and light oil). The results of the tests across various scenarios indicated that the formed emulsions fell under Winsor Type III. However, the volume of stable microemulsion after a week of observation was relatively small. Overall, the phase behavior results showed similar trends; one example of the phase behavior test result with 10000 ppm (NaCl) brine and 2% Rhamnolipids is attached.



**Figure 7.** Phase Behavior at 10,000 ppm NaCl and 2% Rhamnolipids

## CONCLUSIONS

Based on the results of the analysis and design that have been made from the modelling *decision tree* in determining reactivation candidates for the *idle well*, conclusions can be drawn as follows:

1. Interfacial tension (IFT) measurements revealed that the addition of divalent ions ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) to rhamnolipid solutions significantly affects IFT reduction. Under optimal ion concentrations, IFT values were reduced to ultra-low levels, which are essential for mobilizing trapped oil. The mechanism is attributed to ion-bridging, which enhances surfactant packing at the oil–water interface.
2. Microemulsion viscosity tests demonstrated that increasing biosurfactant concentrations resulted in a decrease in viscosity, thereby improving flow characteristics. Additionally, the viscosity was shown to be more stable at higher temperatures, a critical factor for application in real reservoir conditions.
3. Data from laboratory tests also indicated that biosurfactant performance is sensitive to the concentration of divalent ions. Low to moderate concentrations improved emulsification and interfacial behavior, whereas excessive levels led to micelle aggregation or precipitation, thereby reducing the effectiveness of the biosurfactant.
4. The batch and continuous production systems were compared, with continuous production using *Pseudomonas aeruginosa* identified as more efficient for large-scale application due to its lower temperature requirements, steady productivity, and biodegradable output.
5. The overall analysis revealed that rhamnolipid-based biosurfactants, when formulated adequately with suitable salinity and ion levels, can significantly enhance oil recovery efficiency in medium- and light-oil reservoirs, underscoring their potential as a sustainable alternative to synthetic surfactants in tertiary recovery processes.

Based on this research, this study contributes to Enhanced Oil Recovery (EOR) by demonstrating the ion-bridging mechanism, which is crucial for the effectiveness of rhamnolipids, and establishing continuous production as the most efficient method for industrial scalability. This provides a path for more practical and sustainable EOR technology.

## LIMITATIONS & FURTHER RESEARCH

This review is limited to laboratory-scale analyses and literature-based evaluations of rhamnolipid–divalent ion interactions, without integrating molecular-level simulations or core-scale displacement experiments that could provide deeper mechanistic insights and field applicability. Future research should incorporate molecular dynamics or spectroscopic studies to elucidate ion-binding behavior and micelle structural changes under varying salinity and temperature conditions. Additionally, pilot-scale coreflooding and long-term stability assessments in actual reservoir brines are recommended to validate the optimized formulations and ensure scalability for field deployment in diverse geological settings.

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