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Kinetics study on microbial growth and surfactin production of *Bacillus subtilis* ATCC 21332 under the synergistic effect of magnetic field and Mg^{2+}

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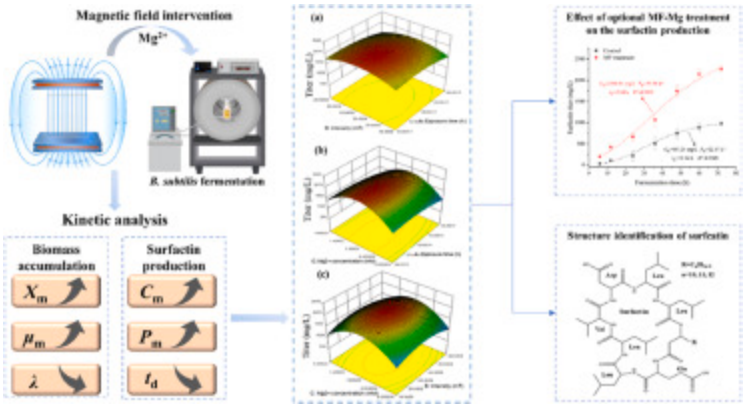
Highlights

- MF treatment promotes bacterial growth and surfactin production of *Bacillus subtilis.*
- Logistic and Gompertz models were chosen to respectively describe bacterial growth and surfactin production during *B. subtilis* fermentation.
- MF and Mg^{2+} have synergistic effects on surfactin synthesis by *B. subtilis.*
- 1.42-fold surfactin titer was achieved under optimal conditions with 58 h of MF exposure time, 24 mT MF intensity, and 1.1 mM Mg^{2+} in the medium.

Abstract

In this study, the kinetics of growth and surfactin production of *Bacillus subtilis* (*B. subtilis*) ATCC 21332 under different magnetic field (MF) exposure times, MF intensities and magnesium (Mg^{2+}) concentrations in media were investigated using Logistic and Gompertz models. Results show that a 1.42-fold increase in surfactin titer was obtained under the optimal conditions with 58 h of MF exposure time, 24 mT MF intensity, and 1.1 mM Mg^{2+} in the medium. A synergistic effect on the biological fermentation process was observed between MF and Mg^{2+} to significantly enhance surfactin yield and production efficiency by *B. subtilis*. This was accompanied by increased biomass accumulation and a higher sugar consumption rate during fermentation. Additionally, the overall volumetric surfactin productivity ($Prod_{vol}$) and the specific surfactin formation rate (qP) increased by 136% and 217%, respectively. This study provides valuable insights into the potential applications of MFs in manipulating bacterial growth and metabolism.

Graphical abstract



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Introduction

Biosurfactants have immense application potential in various industries, including food, chemical, and agriculture, owing to their excellent foaming, emulsifying, bactericidal, wetting and cleaning capabilities (Kashif et al., 2022). Compared with synthetic chemical surfactants, biosurfactants have outstanding advantages, such as natural degradability, high

selectivity, environmental compatibility, and enhanced efficiency, even under high temperatures and salinity (Ambaye et al., 2021). Biosurfactants are amphipathic secondary metabolites synthesized by microorganisms through multidomain-specific non-ribosomal peptide synthase (Das et al., 2008). Microorganisms synthesize biosurfactants through diverse molecular structures and surface activity (Campos et al., 2013). Among these microbial surfactants, lipopeptides, a subgroup produced by various *Bacillus* spp. strains, have been extensively studied. Surfactin, lichenysin, iturin, fengycin, and kurstakin are prominent examples (Ali et al., 2022).

Surfactin, a cyclic lipopeptide, comprises a hydrophilic peptide ring of 7 amino acids and a hydrophobic β-hydroxy fatty acid chain of 12–17 carbon atoms (Arima et al., 1968). The distinguished biosurfactants reportedly reduce water's surface tension from 72 to 27 mN/m at a critical micellar concentration (CMC) of 20 mg/L (Liu et al., 2015). However, widespread surfactin application is hampered by its low yield and high production cost. Numerous studies have tackled this challenge by optimizing media composition and fermentation conditions (Datta et al., 2020), utilizing cost-effective agricultural waste as a raw material (Gurjar & Sengupta, 2015), and employing genetic engineering (Jing et al., 2023; Wang et al., 2019) or heterologous expression techniques (Xia & Wen, 2022) to improve surfactin productivity. For example, Ghribi and Ellouze-Chaabouni (2011) demonstrated that a high yield of surfactin (4.92 g/L) was obtained using a glucose concentration was 40 g/L, a carbon/nitrogen ratio of seven, and dissolved oxygen saturation of the medium at 30%. Wei et al. (2007) found trace elements like Mg^{2+}, K^{+}, and Mn^{2+} particularly helpful in surfactin biosynthesis by *B. subtilis* to increase production of surfactin to 2–3.6 g/L. Considering the high energy input required and operational difficulties associated with these approaches, an alternative innovative surfactin production technology is desirable.

Recent research has highlighted the application of magnetic field (MF) technology to enhance microbial growth and metabolism to produce nutrients, enzymes, and various high-value products (Deamici et al., 2016; Fijałkowski et al., 2017; Li et al., 2022). This method is attractive owing to its environmental friendliness and economic viability (Li et al., 2024). For instance, applying a 60 mTMF for 1 h per day resulted in an 83.2% increase in the biomass concentration of *Chlorella kessleri* and stimulated the synthesis of lipids, carotenoids, and antioxidants by 13.7%, 25.0%, and 185.7%, respectively (Bauer et al., 2017). Additionally, *Saccharomyces cerevisiae* exposed to a 10 mTMF exhibited an average increase of 33% in ethanol productivity (de Andrade et al., 2021). MFs were also effective in increasing β-carotene and astaxanthin production from *Phaffia rhodozyma* by 8.6% and 22.9%, respectively (Silva et al., 2023). Although the specific mechanisms underlying the effect of MFs on microorganisms have not been completely elucidated, MFs has been proven

to exert significant influence on key enzyme activity (Łebkowska et al., 2018), biological membrane permeability (Gebreyohannes et al., 2017; Yang et al., 2024), electron transfer processes (Yang et al., 2023), and gene expression (Sun et al., 2019), which may promote the microbial growth and improve the yields of targeted products (Chen et al., 2024).

The effects of MFs on microorganisms depend on several factors, including exposure time, magnetic induction intensity, and fermentation broth components (Łebkowska et al., 2018). For example, exposing *Monascus purpureus* in solid-state fermentation to MFs with intensities ranging from 0.4 to 0.6 mT for 24–48 h increased pigment yield by 20–40% (Zhang et al., 2015). Moreover, previous research has validated the effectiveness of optimizing Mg^{2+} concentration in culture media as a viable strategy for enhancing surfactin production in *B. subtilis* ATCC 21332 strain owing to its function as a cofactor to enhance *Sfp* phosphopantetheinyl transferase activity by forming a complex with the enzyme, thereby facilitating the surfactin synthesis (Reuter, 1999; Wei et al., 2007). Additionally, the magnetic isotope effect of Mg^{2+} acts as a mediator for MFs to modulate enzymatic reactions (Letuta & Berdinskii, 2015). Consequently, further investigation into the synergistic effects of Mg^{2+} on *B. subtilis* fermentation under MF conditions is necessary.

The application of MFs in biological fermentation remains in the preliminary stages of laboratory research, with no reported studies on their influence on surfactin production by *B. subtilis*. This study aimed to investigate the synergistic enhancement effect of MF and Mg^{2+} on the kinetics of bacterial growth and surfactin production by *B. subtilis*. Furthermore, response surface methodology (RSM) was employed to optimize the fermentation parameters for surfactin production, considering the MF exposure time, MF intensity, and Mg^{2+} concentration in the medium.

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Microorganism and culture media

The surfactin-producing strain, *B. subtilis* ATCC 21332, was obtained from the China Industrial Microbial Culture Preservation Management Center. The strain was stored in 60%

glycerin coil at −80 °C. Luria-Betrani (LB) medium containing 10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl (pH 7.4) was used as the seed medium. The mineral salt medium (MSM) used as culture media comprised glucose (40 g/L), tryptone (20 g/L), Na_2HPO_4 (40 mM), KH_2PO_4 (30 mM), $CaCl_2$ (0.007 mM), $MgSO_4$ (0.8 mM), sodium …

Effect of different treatments on *B. subtilis* biomass accumulation

The growth curves presented in Fig. 2 were fitted using the Logistic model (R^2=0.976–0.999) to elucidate the differences between the growth patterns of *B. subtilis* ATCC 21332 under different treatments, as indicated by the kinetic fitting parameters listed in Table 2. When exposed to MFs for 48 h and 72 h, the biomass of *B. subtilis* notably increased compared with MF-0 h groups (Fig. 2A and a). However, upon removal of the MFs after 24 h, the biomass began to exhibit a certain downward trend, …

Conclusion

The combination of appropriate Mg^{2+} concentration and MF exposure produced synergetic stimulatory effects on *B. subtilis*' growth and surfactin production, particularly in the middle exposure time of 48–72 h at 20–30 mT, creating a specific "window" effect. MF treatment coupled with Mg^{2+} enhanced the bioconversion efficiency of substrates into surfactin in *B. subtilis*, with overall $Prod_{vol}$ and *q*P increased by 136% and 217%, respectively. This study provides a scientific reference and theoretical …

CRediT authorship contribution statement

Sirui Chen: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Fuchao Shi:** Investigation, Formal analysis. **Fan Liu:** Writing – review & editing, Data curation. **Na Yang:** Writing – review & editing, Supervision. **Xueming Xu:** Supervision, Conceptualization. **Yamei Jin:** Writing – review & editing, Supervision, Conceptualization. …

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. …

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