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Postprint: Synergistic Effect of Ultrasound and Surfactant on Leaky Fermentation of Vitamin K2

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Abstract

Vitamin K2 is an essential vitamin for the human body that promotes prothrombin production and osteocalcin synthesis, and also demonstrates significant effects in damaged cell repair. The microbial fermentation method for preparing vitamin K2 offers advantages such as minimal environmental impact, high biological activity, and low production costs, representing the development trend for large-scale vitamin K2 production. Utilizing ultrasound and surfactants to enhance cell membrane permeability of microbial cells during fermentation is a common approach for artificial regulation of cell metabolism. The cavitation effect of low-power ultrasound can instantaneously create micro-injuries on the cell surface, causing localized rupture of the cell membrane and thereby altering its permeability, which facilitates the release of intracellular substances or the entry of extracellular substances into cells. Surfactants help improve the solubility of nutrients, reduce the surface tension of the medium, and decrease the interfacial resistance between the cell surface and the medium, thereby promoting the transmembrane transport of nutrients and microbial metabolites. This study investigated a vitamin K2-producing Flavobacterium strain (Flavobacterium sp.) Fla-M preserved in our laboratory, subjecting it to low-power ultrasonic irradiation and surfactant treatment to examine their synergistic effects in enhancing cell leakage fermentation. First, the addition time and concentration of surfactant (polyoxyethylene oleyl ether, POE) for Fla-M were optimized in 500 mL shake flasks. The results showed that adding 1% POE at the initial fermentation stage yielded the best performance, with a final biomass of 13.4 g/L and extracellular vitamin K2 yield of 36.3 mg/L at the end of fermentation, representing increases of 83.5% and 41-fold respectively compared to the control group without POE addition (biomass 7.32 g/L, extracellular vitamin K2 0.85 mg/L). Scanning electron microscopy observation revealed that numerous surfactant micelles accumulated on the surface of cells fermented with POE addition. Due to the structural similarity between POE and cell membrane

phospholipid molecules, they may become miscible and form mixed micelles, altering the cell membrane structure and thereby improving its permeability. Secondly, the ultrasonic method, timing, power, and treatment duration for Fla-M were investigated in 500 mL shake flasks. The optimal conditions were found to be probe-type ultrasonication for 98 s (3 s per pulse with 4 s intervals) during the stationary growth phase (day 5 of fermentation) at 120 W and 20 kHz, resulting in a final biomass of 11.1 g/L and extracellular vitamin K2 yield of 50.1 mg/L at the end of fermentation, which represented increases of 51.6% and 58-fold respectively compared to the non-sonicated control group (biomass 7.32 g/L, extracellular vitamin K2 0.85 mg/L). Transmission electron microscopy observation revealed that although the cell membrane remained intact after ultrasonic treatment, the boundaries of the phospholipid bilayer became blurred, and pore-like damaged structures were present on the cell membrane surface, with suspected content leakage visible. Under the aforementioned optimal conditions, the combined application of POE and ultrasound in 500 mL shake flasks resulted in maximum biomass and extracellular vitamin K2 yield after 6 days of fermentation, reaching 11.5 g/L biomass and 59.7 mg/L extracellular vitamin K2. Compared to using POE or ultrasound alone, the fermentation cycle was shortened by 3 days, and the extracellular vitamin K2 yield increased by 64.4% and 19.1% respectively. Flow cytometry detection was performed on post-fermentation cells using the exclusion dye propidium iodide (PI). Sample 001 was designated as the negative control, representing the fluorescence signal of untreated cells without fluorescent carrier; sample 002 represented the fluorescence signal of treated cells with fluorescent carrier; sample 003 represented the fluorescence signal of untreated cells with fluorescent carrier; and sample 004 was the positive control, representing the fluorescence signal of dead cells with fluorescent carrier. The proportion of area M1 (outside the autofluorescence region of strain 001 in the negative control) to the total area was preset as 0. The results showed that the M1/total area ratio was 17.21% for sample 004 > 8.89% for sample 002 > 1.21% for sample 003, indicating that cell membrane permeability followed the order: dead cells > leakage-cultured cells > non-leakage-cultured cells. This verified that cell membrane permeability was substantially enhanced after ultrasonic and surfactant treatment. This study provides valuable insights for the industrial development of vitamin K2 preparation via fermentation.

Full Text

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Synergistic Effects of Ultrasound and Surfactant on Leaky Fermentation of Vitamin K2

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Abstract

Vitamin K2 is an essential nutrient that plays critical roles in prothrombin production, osteocalcin synthesis, and damage cell repair. Microbial fermentation represents a promising approach for large-scale vitamin K2 production due to its minimal environmental impact, high biological activity, and low production costs. Enhancing microbial cell membrane permeability during fermentation through ultrasound and surfactant treatment is a common method for artificial regulation of cellular metabolism. Low-power ultrasound induces cavitation that creates instantaneous micro-injuries on the cell surface, causing localized membrane disruption and altered permeability that facilitates intracellular product release or extracellular substrate uptake. Surfactants improve nutrient solubility, reduce medium surface tension, and decrease interfacial resistance between the cell surface and medium, thereby promoting transmembrane transport of nutrients and metabolites.

This study investigated the synergistic effects of low-power ultrasound irradiation and surfactant treatment on enhancing leaky fermentation of a vitamin K2-producing *Flavobacterium* sp. strain Fla-M preserved in our laboratory. First, we optimized the addition timing and concentration of the surfactant polyoxyethylene oleyl ether (POE) in 500 mL shake flasks. The optimal condition was 1% POE added at the beginning of fermentation, yielding 13.4 g/L biomass and 36.3 mg/L extracellular vitamin K2 at the end of fermentation—representing increases of 83.5% and 41-fold, respectively, compared to the control without POE (7.32 g/L biomass, 0.85 mg/L extracellular vitamin K2). Scanning electron microscopy revealed abundant surfactant micelles aggregated on the cell surface. Due to structural similarity between POE and membrane phospholipids, they likely formed mixed micelles that altered membrane structure and improved permeability.

Next, we optimized ultrasound parameters including mode, timing, power, and duration in 500 mL shake flasks. The optimal condition was probe sonication at 120 W and 20 kHz for 98 s (3 s pulses with 4 s intervals) applied during the stationary growth phase (day 5 of fermentation). This treatment yielded 11.1 g/L biomass and 50.1 mg/L extracellular vitamin K2, representing increases of 51.6% and 58-fold compared to the non-sonicated control. Transmission electron microscopy showed that although the cell membrane remained intact after ultrasound treatment, the phospholipid bilayer boundaries became indistinct with pore-like damaged structures visible on the membrane surface, suggesting possible content leakage.

Under these optimal conditions, the combined POE and ultrasound treatment in 500 mL shake flasks achieved maximum biomass (11.5 g/L) and extracellular vitamin K2 production (59.7 mg/L) after 6 days. This combined approach shortened the fermentation period by 3 days and increased extracellular vitamin

K2 yield by 64.4% and 19.1% compared to POE or ultrasound treatment alone, respectively. We validated the enhanced membrane permeability using flow cytometry with the membrane-impermeable dye propidium iodide (PI). Four samples were analyzed: 001 (negative control, untreated cells without PI), 002 (treated cells with PI), 003 (untreated cells with PI), and 004 (positive control, dead cells with PI). The proportion of area M1 (outside the autofluorescence region) was set to 0 for the negative control (001). Results showed M1 area proportions of 17.21% for 004 > 8.89% for 002 > 1.21% for 003, demonstrating that permeability followed the order: dead cells > leaky fermentation cells > non-leaky fermentation cells. This confirms that ultrasound and surfactant treatment substantially increased cell membrane permeability. These findings provide valuable insights for the industrial development of vitamin K2 fermentation processes.

Keywords: Ultrasound, Surfactant, Vitamin K2, Leaky Fermentation

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Note: Figure translations are in progress. See original paper for figures.

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