Temperature and kL Control Strategy for VK2 Fermentation Production by Flavobacterium sp. M1-14 (Postprint)

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Abstract

Vitamin K2 (VK2) is a series of menaquinone compounds with isoprenoid side chains, designated as MK-n according to the length of the side chain. High-activity VK2 is primarily synthesized by microorganisms and possesses physiological functions in preventing and treating diseases such as osteoporosis, hemorrhagic disorders, liver cirrhosis, and Parkinson' s disease. Flavobacterium is an important production strain that can synthesize various VK2 homologs including MK4, MK5, and MK6.

We found that by regulating fermentation temperature, the types and yields of VK2 homologs synthesized by Flavobacterium could be controlled. Within the range of 20~37°C, Flavobacterium sp. M1-14 exhibited optimal growth at 25°C, achieving a biomass of 8.8 g/L; however, the fermentation product was entirely MK6 with a yield of 13.9 mg/L and a specific yield of 1.6 mg/g DCW. When fermentation temperature exceeded 30°C, Flavobacterium could simultaneously synthesize MK4, MK5, and MK6. At 37°C, MK4 and MK5 reached their highest yields of 1.6 mg/L and 1.7 mg/L, respectively, with total VK2 yield of 12.5 mg/L, but the biomass was only 5.5 g/L, corresponding to a specific yield of 2.3 mg/g DCW. Considering the difference in optimal temperatures for cell growth and VK2 homolog synthesis in Flavobacterium, a temperature-shift fermentation strategy was adopted to enhance both biomass and VK2 production. After multi-factor optimization, we developed a two-stage temperature-shift strategy involving initial fermentation at 25°C for 48 hours followed by a shift to 37°C for an additional 96 hours, achieving a VK2 yield of 20.9 mg/L (including 2.1 mg/L MK4, 2.3 mg/L MK5, and 16.5 mg/L MK6), biomass of 8.8 g/L, and specific yield of 2.4 mg/g DCW.

Subsequently, in a 30 L fermenter, we investigated the oxygen requirements of fermentation at different temperatures by controlling aeration rate and agitation

speed. The optimal kLa values for VK2 synthesis by Flavobacterium were found to be 360 h-1 and 60 h-1 at 25°C and 37°C, respectively. In response to changing oxygen demands during temperature-shift fermentation, we developed a two-stage variable kLa control strategy. After optimization, the kLa was maintained at 360 h-1 during the initial 24 hours of temperature-shift fermentation, then reduced to 60 h-1 for the subsequent 120 hours, resulting in a VK2 yield of 28.7 mg/L (including 2.8 mg/L MK4, 3.4 mg/L MK5, and 22.5 mg/L MK6), representing a 107% increase compared to the initial condition, with biomass reaching 15.5 g/L and specific yield of 1.9 mg/g DCW.

Through the staged fermentation regulation strategy of combined temperature and kLa shifts, the types of VK2 homologs synthesized by Flavobacterium can be altered and VK2 yield significantly improved, establishing an optimized foundation for the industrialization of VK2 bioproduction.

Full Text

Preamble

Temperature and kLa Control Strategies for VK2 Production via Flavobacterium sp. M1-14 Fermentation

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Vitamin K2 (VK2) comprises a series of menaquinone compounds with isoprenoid side chains, designated as MK-n according to side chain length. Highly active VK2 is primarily synthesized by microorganisms and exhibits physiological functions in preventing and treating diseases such as osteoporosis, hemorrhage, liver cirrhosis, and Parkinson' s disease. Flavobacterium is an important production strain that can synthesize various VK2 homologs, including MK4, MK5, and MK6.

We found that regulating fermentation temperature can control both the type and yield of VK2 homologs synthesized by Flavobacterium. Within the range of 20–37°C, Flavobacterium sp. M1-14 exhibited optimal growth at 25°C, achieving a biomass of 8.8 g/L; however, the fermentation product was exclusively MK6, with a yield of 13.9 mg/L and a specific yield of 1.6 mg/g. When the fermentation temperature exceeded 30°C, Flavobacterium could simultaneously synthesize MK4, MK5, and MK6. At 37°C, MK4 and MK5 reached their highest yields of 1.6 mg/L and 1.7 mg/L, respectively, with a total VK2 yield of 12.5 mg/L, though the biomass was only 5.5 g/L, corresponding to a specific yield of 2.3 mg/g.

Given the difference in optimal temperatures for cell growth and VK2 homolog synthesis, a temperature-shift fermentation strategy was considered to enhance

both biomass and VK2 yield. After multi-factor optimization, we developed a two-stage temperature-shift strategy involving initial fermentation at 25°C for 48 h, followed by a shift to 37°C for an additional 96 h. This approach yielded 20.9 mg/L of VK2 (comprising 2.1 mg/L MK4, 2.3 mg/L MK5, and 16.5 mg/L MK6), with a biomass of 8.8 g/L and a specific yield of 2.4 mg/g.

Subsequently, in a 30-L fermenter, we investigated the oxygen requirements for fermentation at different temperatures by controlling aeration rate and agitation speed. We found that the optimal kLa values for VK2 synthesis by Flavobacterium were 360 h^{-1} at 25°C and 60 h^{-1} at 37°C. In response to changing oxygen demands during temperature-shift fermentation, we developed a two-stage variable kLa control strategy. Under optimized conditions, the kLa was maintained at 360 h^{-1} during the initial 24 h of temperature-shift fermentation and then reduced to 60 h^{-1} for the subsequent 120 h. This strategy achieved a VK2 yield of 28.7 mg/L (including 2.8 mg/L MK4, 3.4 mg/L MK5, and 22.5 mg/L MK6), representing a 107% increase over the initial yield, with a biomass of 15.5 g/L and a specific yield of 1.9 mg/g.

Through the staged fermentation regulation strategy of combined temperature and kLa shifts, the types of VK2 homologs synthesized by Flavobacterium can be altered and VK2 yield significantly improved, laying an optimized foundation for the industrialization of VK2 bioproduction.

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