and considerable increase in culture volume, but the volume of the workable supernatant (approximately 7 L after biomass removal) hardly changed during the fermentation process (Figure 3). The initial carbon source was glycerol, added first as a batch, and later (after 18 h) at a constant rate of 2 g L h throughout the rest of the fermentation. The residual glycerol concentration in the culture was between 0.01 and 0.05 g L −1 , a range that does not affect the AOX1 promoter [13]. When the culture density reached approximately 150 OD at 600 nm (10% v/v), about 20 h after starting the fermentation, methanol addition was commenced and its concentration in the culture was kept at 2 g L −1 using the described methanol sensor. Although the methanol controller displayed oscillatory behavior and was sensitive to the culture temperature, the methanol level was maintained successfully at 2 (±0.5) g L −1 −1 . It was important to begin induction with methanol at a cell density between 150 and 200 OD (at 600 nm). Inducing the culture at higher cell densities resulted in significant problems with maintaining the desired temperature and dissolved oxygen level because the high amounts of oxygen, needed for methanol oxidation, generate a large amount of heat during the exponential growth phase on methanol. Higher temperatures affect the growth of Pichia, the stability of the product and the calibration of the methanol sensor. During the fermentation, 1120 g of glycerol, 1.15 L of 7 M ammonium hydroxide and 3.8 L of methanol were added. After 90 h of fermentation, of which 70 h was the duration of growth on methanol, 200 mg of angiostatin and 5 kg of biomass were produced. Since a high percentage of the culture is biomass, the appropriate way to express angiostatin production is in mg per liter supernatant instead of mg per liter culture, the average production (based on five consecutive runs) was 20 (±5) mg L supernatant. A [slightly lower concentration has been claimed in a recent patent application [15