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In this study the applicability of a "fed batch" strategy, that is sequential loading of substrate or substrate + enzymes during enzymatic hydrolysis, was evaluated for hydrolysis of steam-pretreated barley straw. The specific aims were to achieve hydrolysis of high substrate levels, low viscosity during hydrolysis, and high glucose concentrations. An enzyme system comprising Celluclast and Novozym 188, a commercial cellulase product derived from *Trichoderma reesei* and a β-glucosidase derived from *Aspergillus niger*, respectively, was used for the enzymatic hydrolysis. Among the different fed batch reactions and controls the highest final glucose concentration, 78 $g \cdot L^{-1}$, after 72 hours of reaction, was obtained with an initial, full substrate loading of 15 % total solids weight/weight (w/w). Conversely, the glucose yields, i.e. g glucose $\cdot$ g^{-1} total solids, were generally higher at lower substrate concentrations. When compared to the "conventional" reactions, loaded with the full substrate concentration from the beginning of the enzymatic hydrolysis, the reactions subjected to gradual loading of substrate, or to gradual loading of substrate + enzymes, increasing the substrate levels from 5 % - 15 % w/w DM, consistently gave lower end concentrations of glucose after 72 hours of reaction. However, because of the initial high enzyme:substrate ratios in the sequentially loaded substrate reactions (resulting from addition of the full enzyme dose required for the final substrate concentration), the initial rates of cellulose conversion were very high in all the fed batch reactions. The rapid cellulose degradation was accompanied by rapid decreases in viscosity prior to addition of extra substrate, but when extra substrate or substrate + enzymes were added, the viscosities of the slurries increased and the hydrolytic efficiencies decreased transiently. Compared to reactions to which extra substrate or extra substrate + enzyme were added, the conventional batch enzyme reactions always ended up with a higher final glucose yield and lower end point viscosity. The data signified that the higher glucose levels obtained with the conventional, batch reactions were a result of the hydrolytic reaction times on the extra added substrate being shorter when the total reaction time was kept constant at 72 hours. In addition, the introduction of a substrate slurry of 21 % w/w dry matter as opposed to addition of a 100 % dry substrate induced a dilution effect which in effect resulted in lower, final glucose levels.