Supporting Information:

Continuous Cellulosic Bioethanol Fermentation by Cyclic Fed-Batch Co-Cultivation

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Running title: Bioethanol production by a thermophilic coculture

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Optimal pH for fermentation in batch culture. Of the process parameters influencing ethanologenic fermentation, pH has been considered to be among the most important. To identify the optimal pH for semi-continuous cellulose fermentation, a series of pH was tested in batch fermentation of cellulose (10 $g \cdot L^{-1}$) with both the CT mono-cultures and the CT-X514 co-cultures. The results show that both the mono-cultures and co-cultures exhibited good cellulolytic performance at pH 6.8 or greater with the complete utilization of the cellulose substrate (Fig. S-3a). However, lowering the pH to 6.5 reduced cellulose utilization to 68.9% and 79.3% in the mono-cultures and co-cultures, respectively. A further drop in initial pH to 6.0 led to an additional 20% decrease in cellulose utilization. When initial pH dropped to 5.5, no cellulose conversion was observed in either culture.

The impact of pH on saccharolytic strain X514 was also tested in batch fermentation of glucose by theX514 mono-cultures as well as the CT-X514 co-cultures (Fig. S-3b). Similar to the pH effect on the cellulolytic strain LQRI (Fig. S3a), at pH 6.8 or greater, the impact of pH on X514 mono-cultures and the CT-X514 co-cultures was insignificant, as glucose utilization remained largely unchanged within this pH range (Fig. S3b). However, glucose utilization decreased with further drops in initial pH and eventually ceased at pH 5.0, suggesting the importance of pH for the activity of both LQR1 and X514 in ethanolic fermentation.

Since cultures of both strain LQRI and X514 were free of negative impact on substrate utilization at pH 6.8 or greater, pH 6.8 was selected as the pH target for automatic control in subsequent testing of semi-continuous ethanolic fermentation.

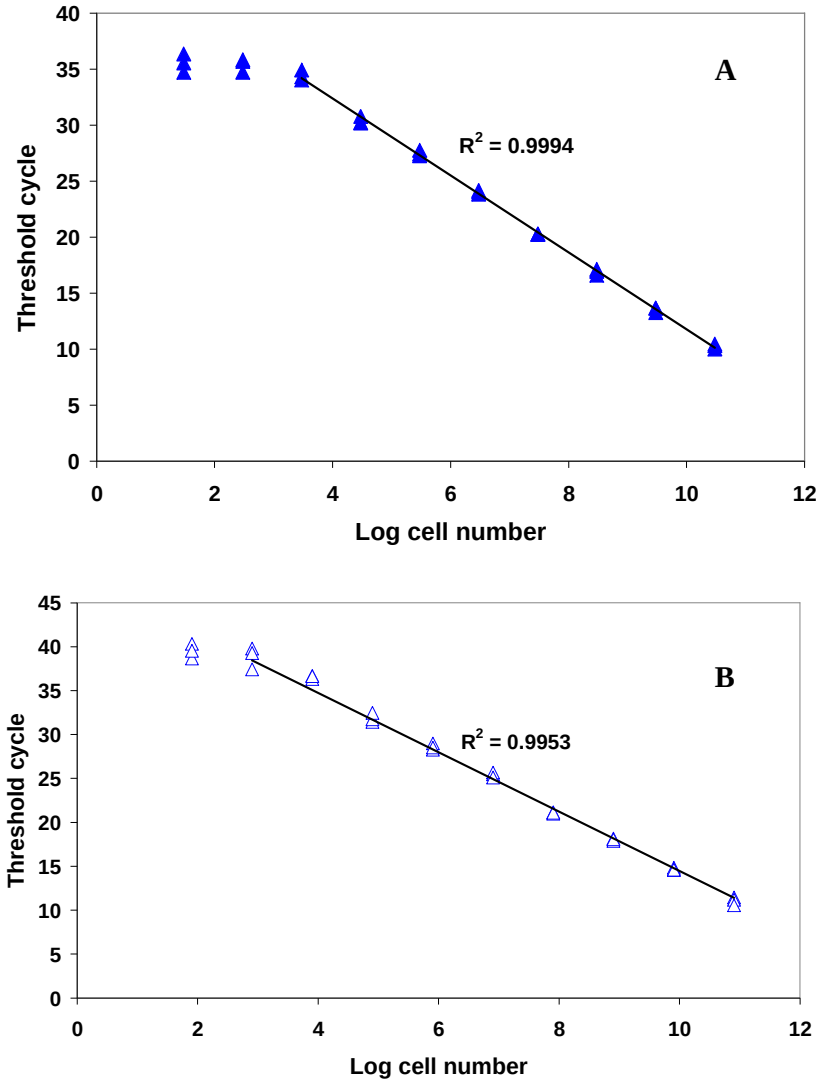

FIG. S-1 Threshold cycle measurements using real time PCR assay versus known cell concentration of strain LQR1 (A) and strain X514 (B). Real-time PCR assays were performed using triplicate samples for each cell concentration.

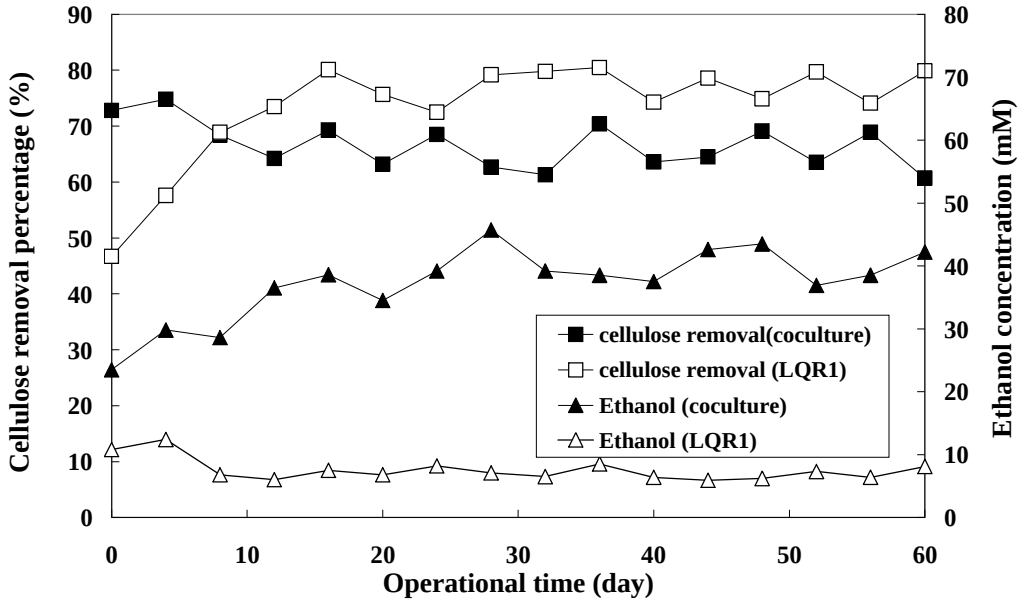

FIG. S-2 Cellulose removal percentage and produced ethanol concentration at the end of each operational cycle (four days for each cycle) in cyclic fed-batch fermentation with an initial cellulose concentration of 10 g L^{-1} and without pH control .

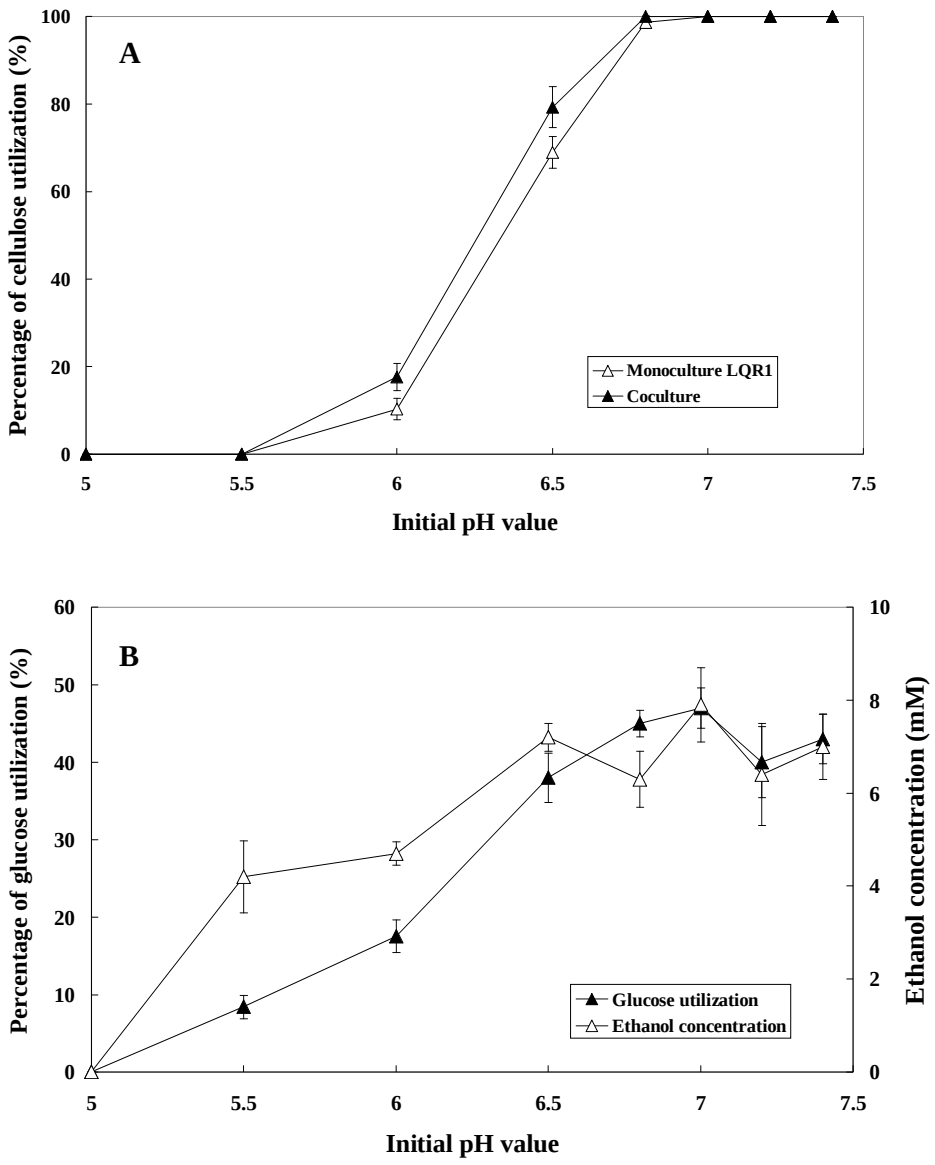

FIG. S-3 Effect of pH on cellulose utilization by monoculture LQR1 and coculture (A), and glucose fermentation by the strain X514 (B) in batch-culture incubation.