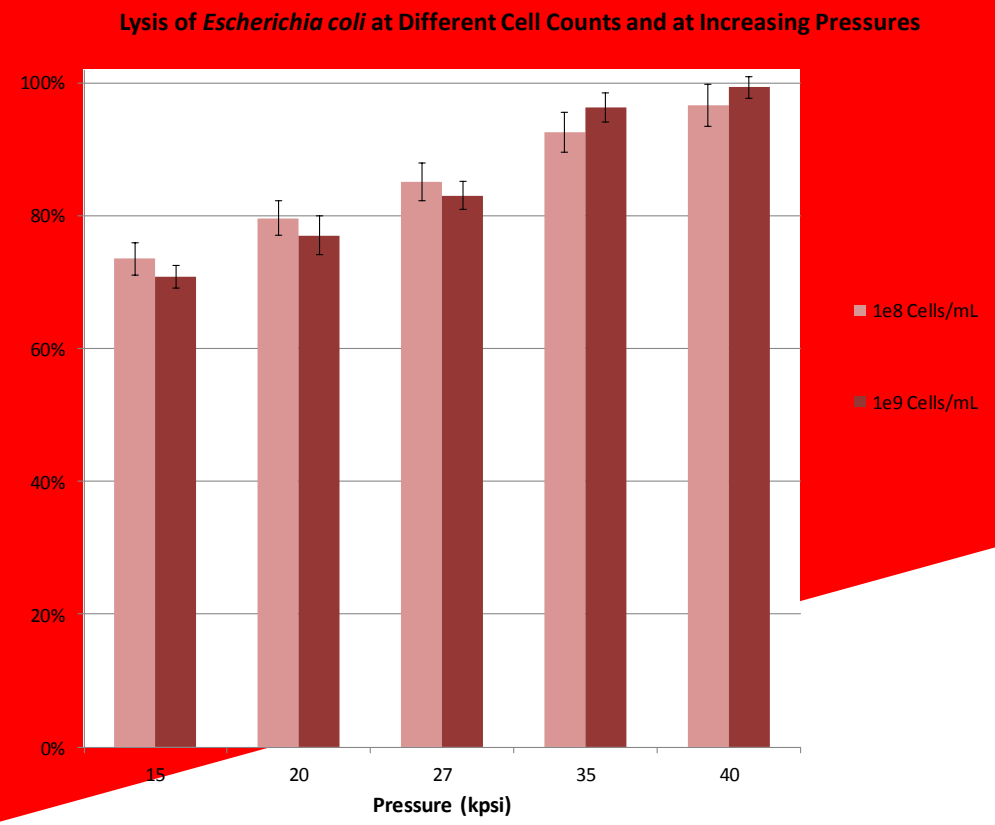
Lysis Rate of *Escherichia coli* With Varying Pressure and Cell Count



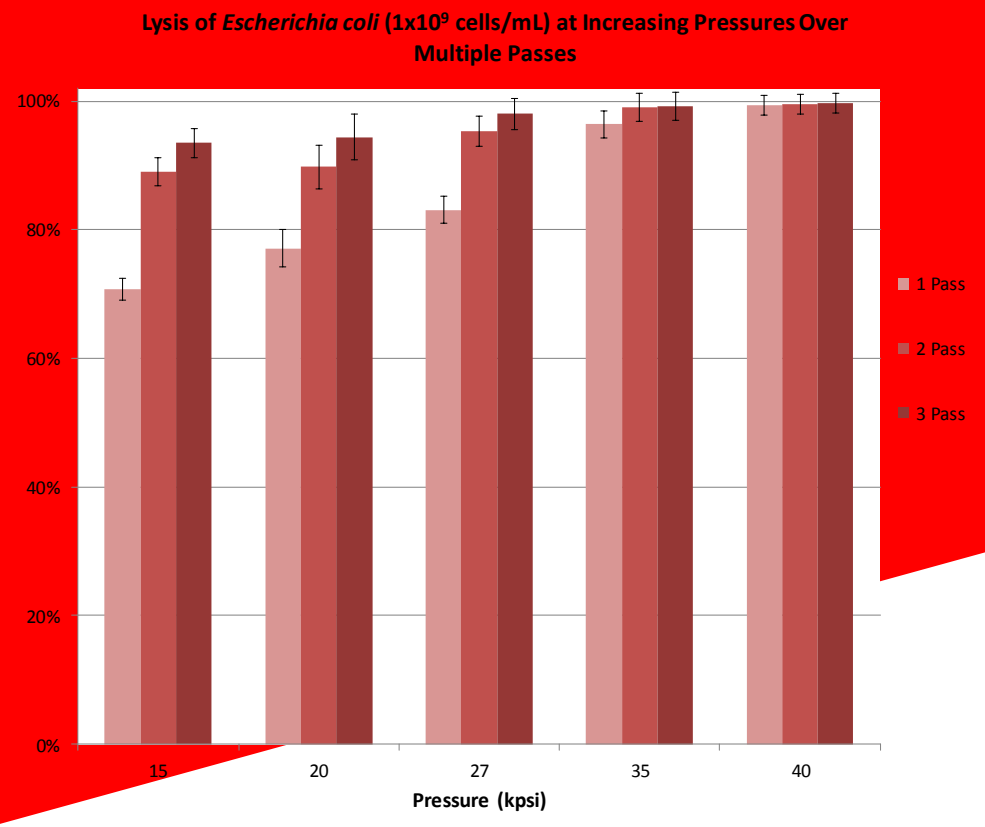
Culture: A single colony of *Escherichia coli* ATCC 8739 was used to inoculate 10 mL sterile LB (Lysogeny Broth: Tryptone 10 g/L, Sodium Chloride 10 g/L, Yeast Extract 5 g/L) and was grown overnight at 37°C with shaking at approximately 180 rpm. This starter culture was used in a ratio of 1% to inoculate 200 mL sterile LB in 1 L flasks. These were then grown under the same conditions until an Optical Density of approximately 0.6 was reached (10^8 cells/mL). At this point the culture was split; half was refrigerated and half was grown further until Optical Density of 1.2 was reached.

Disruption: The resulting culture was passed in 30 mL aliquots through a Constant Systems Ltd. 'Z+' 1.1 kW Continuous Flow Cell Disruptor at pressures indicated. The machine was rinsed with 30 mL de-ionised water between each use.

Counting: After being passed through the machines, 10 µL of lysate was mixed 1:1 with the viability stain Trypan Blue. Live cells were counted using a hemocytometer. A sample of unlysed cells from the same culture was used as a control, from which the lysis percentage was calculated.

Constant Systems Limited, Low March, Daventry, Northants, NN11 4SD, United Kingdom
Telephone: +44 (0)1327 314146
www.constantsystems.com

Lysis Rate of *Escherichia coli* with Multiple Passes and Varying Pressure



Culture: A single colony of *Escherichia coli* ATCC 8739 was used to inoculate 10 mL sterile LB (Lysogeny Broth: Tryptone 10 g/L, Sodium Chloride 10 g/L, Yeast Extract 5 g/L) and was grown overnight at 37°C with shaking at approximately 180 rpm. This starter culture was used in a ratio of 1% to inoculate 200 mL sterile LB in 1 L flasks. These were then grown under the same conditions until an Optical Density of approximately 1.2 was reached (10^9 cells/mL).

Disruption: The resulting culture was passed in 30 mL aliquots through a Constant Systems Ltd. 'Z+' 1.1 kW Continuous Flow Cell Disruptor at pressures indicated. The machine was rinsed with 30 mL de-ionised water between each use.

Counting: After being passed through the machines, 10 µL of lysate was mixed 1:1 with the viability stain Trypan Blue. Live cells were counted using a hemocytometer. A sample of unlysed cells from the same culture was used as a control, from which the lysis percentage was calculated.

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