Wheat straw (milled, passed through 1 mm sieve)

Dilute sulfuric acid treatment

Original liquid hydrolysate [13]

Heating up to 50°C

Addition Ca (OH)₂ until pH 7 is at room temperature

Addition Ca(OH)₂ for pH 10.0 and stirring at 50°C for 30 min

Filtration at RT

Non-detoxified liquid hydrolysate (NDLH)

Filtration and cooling down to 30°C

Neutralizing pH and filtering

Detoxified liquid Hydrolysate (DLH)

Dilutions

Ammonia pretreatment (30% v/v)

Solid Loading 10% (w/v)

Maintain for 24 hr at 50°C

Washing with distilled water until pH 7 [6]

Drying for 1 hour at 100°C

Ammonia pretreated WS (Solid loading rate 1.0, 1.5, 2.0 and 2.5g + 100ml of 50mM acetate buffer)

50m M acetate Buffer pH4.8+ 1 ml of 2% Sodium azide solution

Pre-incubation at 50°C for 10 mins.

Cellulase 80 FPU/g + β-glucosidase 220CbU/ml (24hrs’ incubation at 50°C at 150rpm)

Chilling immediately on ice centrifuge at 50,000 rpm for 10 min

Dilution of supernatant

Enzyme hydrolysis

Photofermentation

H₂ production