

# A Novel Biological Process to Convert Renewable Biomass to Acetone and Butanol (AB)

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Butanol is an industrially important fuel and chemical that can be produced from renewable agricultural crops and residues by fermentation. Unfortunately, this fermentation suffers from butanol toxicity, resulting in accumulation of less than 20 gL<sup>-1</sup> butanol in batch reactors. This limits use of dilute sugar solutions, usually to less than 60 gL<sup>-1</sup>, and results in uneconomic recovery of butanol by distillation. Furthermore, butanol recovery by distillation is complicated by its higher boiling point (118 °C) than water.

In order to solve butanol toxicity (to the culture) and recovery problems and make butanol fermentation a commercially viable process, gas stripping, a novel technique to separate butanol was applied to this fermentation. This technique has various advantages over other techniques including adsorption, liquid-liquid extraction, perstraction (membrane assisted extraction), pervaporation, and reverse osmosis as gas stripping does not require membrane or any chemical for butanol recovery. For this application, fermentation gases (CO<sub>2</sub> & H<sub>2</sub>: produced in this fermentation) were used to remove butanol from the reactor simultaneously as AB (acetone is a byproduct of this fermentation) was produced. The objective was to keep butanol below toxic level in the bioreactor so that culture could utilize more sugar (>45 gL<sup>-1</sup>; more than in a typical batch process). In the gas stripping assisted batch reactor, a sugar concentration of 60 gL<sup>-1</sup> was used as opposed to 45 gL<sup>-1</sup> in the nonintegrated batch reactor; thus utilizing 25% more sugar and producing 33.3% more AB. In this process, AB production was more due in part to efficient utilization of acids (reaction intermediates of acetone and butanol). The reactor productivity was also improved by 110% due to reduced toxicity to the culture and to higher cell concentration.

Since gas stripping relieved butanol toxicity and the culture utilized a higher amount of sugar, another fermentation was run where the initial sugar concentration (in the reactor) was increased to over 160 gL<sup>-1</sup>. This sugar level is 356% of that used in a typical batch reactor. The fermentation was initiated in a batch mode to produce AB. Gas stripping was started after approximately 40 h of fermentation. As butanol was produced in the system, it (butanol) was recovered simultaneously. As a result of recovery all the sugar present in the reactor was used thus producing 429% more AB than in a nonintegrated batch reactor. Since it was a closed system, acids were converted to AB,

hence improving AB yield by 17.5%. An initial sugar level of higher than 160-170 gL<sup>-1</sup> could not be used due to sugar toxicity to the culture.

As a sugar concentration higher than 160-170 gL<sup>-1</sup> could not be used due to sugar inhibition, a fed-batch reactor was initiated with 100 gL<sup>-1</sup> initial glucose and product recovery was initiated after 40 h of fermentation. As the sugar level in the reactor decreased to 20 gL<sup>-1</sup>, feeding a concentrated glucose solution (500 gL<sup>-1</sup>) was started to keep glucose level in the reactor below inhibitory level (usually 80-90 gL<sup>-1</sup>). In this system 500 g sugar per L culture volume was used which is over 1100% of that used in the nonintegrated batch reactor. The culture produced 233 g AB as opposed to 17.6 g (per L culture volume) in the nonintegrated system. Examination of results revealed that fermentation possibly stopped after 201 h due to accumulation of nonvolatile components and/or decreased water activity. AB production was 1316% of that in a nonintegrated process. In the end of fermentation, it was observed that the broth became viscous and the culture started producing acids.

To overcome the problem of accumulation of nonvolatile inhibitory components, a continuous system was initiated where a continuous small bleed was withdrawn from the reactor. Fermentation was started with an initial glucose level of 100 gL<sup>-1</sup>. Continuous recovery of AB was initiated after 40 h of fermentation and depleted glucose was replaced by feeding a concentrated glucose solution containing 250-500 gL<sup>-1</sup> glucose. Under these conditions the reactor was operated for 505 h as opposed to the fed-batch system which was operated for 201 h. It should be noted that the reactor was stopped intentionally after 21 days (505 h) of continuous operation. This suggested that the fed-batch reactor stopped due to low water activity or accumulation of unknown nonvolatile inhibitory components which made the broth viscous and the culture became acidogenic. In the continuous system 460 g AB was produced (per L reaction mixture) from 1163 g glucose, which are 2600 and 2556%, respectively, as compared to the nonintegrated batch reactor data. The system showed no signs of slow fermentation during the entire operation period of 21 days. As a result of these novel developments, process scale-up is being attempted and it is anticipated that this fermentation-product recovery system could become commercial. In the above four integrated processes, concentrated product stream was obtained which would require less energy for further recovery and purification.

In conclusion, simultaneous recovery of butanol (integrated process) relieved butanol toxicity, improved reactor productivity and yield, and resulted in concentrated product stream for further separation and purification. The yield was improved due to efficient utilization of acetic and butyric acids that are reaction intermediates to acetone and butanol. Using gas stripping as a means to remove butanol from the fermentation broth, three efficient fermentation processes (batch, fed-batch, and continuous) were developed. In order to reduce overall energy requirement for butanol production, concentrated sugar solutions (250-500 gL<sup>-1</sup>) were used, as compared to 60 gL<sup>-1</sup> applied in non-integrated batch fermentations. These studies were performed employing *Clostridium beijerinckii* BA101 to produce AB.