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Ethanol Evaporation from Fermenter – Often Overlooked?

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Ethanol is a widely used biofuel. Currently, ethanol is blended with gasoline at a level of 10% (in US) to 85% (in Brazil), so that consumption of non-renewable gasoline can be reduced. Ethanol provided 2.2% of the world's fuels for road transport in 2010. Among all the biofuels, ethanol has the highest level of production. In 2010 worldwide biofuel production reached 28 billion gallons, of which ethanol accounted for 23 billion gallons. According to the International Energy Agency, biofuels have the potential to meet more than a quarter of world demand for transportation fuels by 2050. Most of ethanol produced today is obtained from corn starch or from sucrose contained in sugarcane and sugar beet. Demonstration plants have been built for producing ethanol from lignocellulose, the most abundant organic resource on earth. Extensive research is being undertaken for commercialization of cellulosic ethanol. Besides developing efficient enzymes or thermochemical processes for breaking down lignocelluloses to sugar, a major portion of the research also focuses on metabolic engineering of microbes so that sugars present in lignocellulosic hydrolysates can be fermented to ethanol with high yield and productivity. Typically, characterization of these recombinant microbes is carried out in fermenters up to 10 liter volume, under anaerobic conditions in a batch process. Comparison of ethanol productivity and yield data is used to establish the effectiveness of the recombinant microbes.

In a fermenter, anaerobic conditions are maintained by sparging an inert gas such as N_2 [1] or Ar [2] through the fermenter that displaces the O_2 from the culture media and positive pressure of exiting gas prevents any inflow of atmospheric oxygen into the fermenter. However, the exiting N_2 or Ar gas also carries ethanol vapors with it. Besides this, ethanol evaporation also occurs. The exiting gas flows through a condenser so that ethanol vapors condense and fall back into the fermenter. In spite of this, ethanol vapors still escape from the fermenter. If the ethanol escaped is not taken into account, the calculated ethanol yield and productivity values will be inaccurate. Rate of ethanol evaporation from a sterile fermenter containing culture media fitted well to a first order kinetics with a decay factor of 0.0044 h^{-1} [3] and 0.006 h^{-1} [4]. This translates into a loss of as much as 5% of produced ethanol for fermentation lasting more than 1 day.

Several researchers have developed methods to account for this ethanol loss. Some have used a GC-MS to measure the amount of ethanol escaping from the fermenter [5]. However, a continuous online GC-MS measurement has to be done to find out the total amount of ethanol loss. Some researchers have modeled the rate of ethanol evaporation from the fermenter and applied this correction to their results [3]. However, the model is usually developed under sterile conditions and may not accurately reflect the ethanol loss from a live culture. Still others have measured the total amount of CO_2 produced and used this value to calculate the total amount of ethanol produced using reaction stoichiometry [4]. The downside of this approach is that assumptions have to be made for chemical reactions involving CO_2 and ethanol. Some of the chemical reactions may not be known as well. However, even after accounting for the ethanol loss, an important factor still gets overlooked. Due to the loss of ethanol, which is the product, there is mitigation of product inhibition and to get the right measure of the biocatalytic efficiency, this also needs to be accounted for. To get a true picture of biocatalytic efficiency, all the produced ethanol should be present in the culture media.

A simple and effective method for resolving this problem has been described by Agrawal et al. [6,7]. Here, the commonly used set up that involves sparging by an inert gas was modified. In the modified set up, a tube was attached to the exhaust, the other end of which was immersed at the bottom of a water column. This set up prevented the diffusion of atmospheric oxygen as it now has to partition into the water phase, diffuse through the water column, again partition into the gas phase and then move through the tube to reach the fermenter vessel. This provides an excellent barrier to O_2 diffusion and thus anaerobic conditions are maintained even without sparging an inert gas. As purging is not done, ethanol loss does not occur due to carry over by the inert gas. Any ethanol evaporation can be recovered in the water column as well. However, during the experiments, it was seen that negligible amount of ethanol was recovered in the water column suggesting that this set up prevents any loss of ethanol. Though this setup helps to report accurate biocataytic efficiency, the setup may not be scaled to industrial scale. In industry, it will be desirable to reduce product inhibition by flowing inert gas so that fermentation can occur at a faster rate.

Thus, for a fair comparison of ethanol producing microbes, use of a fermenter set up with negligible ethanol evaporation is desirable. If significant ethanol evaporation occurs, then data on ethanol evaporation or corrections applied to yield and productivity data must be mentioned.

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