Bioreactor Design For Solid State Lignocellulosic Hydrolysis: A Review

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Abstract. Enzymatic hydrolysis of lignocellulose to produce reducing sugars to be used as bioethanol raw material has recently attracted many scientists’ attention to investigate. It needs to be performed on a big scale, so it is essential to select an appropriate bioreactor. This work discusses enzymatic hydrolysis in solid-state. Solid hydrolysis is suitable for the growth of filamentous fungi to produce hydrolytic enzymes. A bioreactor is the heart of the process since the chemical reactions take place in it. In biochemical reactions, bioreactor plays two complementary functions, i.e., the reaction holder and the place for microorganism to grow. Therefore, to design a good bioreactor, these two functions should be considered carefully. Besides, we also developed a bioreactor with a continuous air supply to provide oxygen for hydrolytic fungi.

1. Introduction
Lignocellulose is plant biomass consisting of three polymer components, namely cellulose, hemicellulose, and lignin. The composition of the three compounds varies widely in plants. Enzymatic hydrolysis utilizes cellulase to break down cellulose chains. It is divided into two methods, i.e., using purified enzymes or growing enzyme-producing fungi in pretreated lignocellulosic materials. Although the second method is more complicated, it is preferred since it requires lower processing costs and ensures enzymes’ availability during the hydrolysis.

Researches on the topic of enzymatic lignocellulosic hydrolysis to produce reducing sugars as a raw material of bioethanol had started to bloom since mid-1950 [1]. However, several barriers hinder further research, such as lengthy reaction time, expensive purified enzyme cost, and lack of information for designing a proper bioreactor system [2].

Hydrolysis can also be performed in limited water presence, which is called solid-state fermentation (SSF). This fermentation system has been more commonly known in the world compared to submerged fermentation. Some of the advantages of SSF include higher yields in a shorter time and a lower risk of contamination due to minimal water presence [3,4]. This method is considered the most suitable for fungi growth since fungi grow in the solid-state [4, 5, 6]. However, SSF limitations also relate to the less water in the system, including difficulty regulating the parameters such as pH, temperature, heat distribution, and nutrient conditions. In some cases, mixing also encounters problems [4].

On the subsequent progress, submerged fermentation becomes more popular, especially for the medical industries. However, due to some good advantages compared to submerged fermentation, solid-state fermentations are back to the concerns. In general, SSF is a
fermentation method with very little free water presence \cite{3,4}. In other literature, the moisture content in the SSF is from 40\% to 80\% \cite{7}, although it then becomes subjective to different substrates.

There are several advantages and disadvantages possessed by SSF, primarily because of its lower moisture content. The benefits include higher yield obtained in a shorter time, more evenly distribution of oxygen circulation, and lower contamination chance as microorganisms need sufficient water to stay alive. On the other hand, minimal water content also causes the disadvantages of SSF, such as the difficulty to set the parameters involved in the process such as pH, temperature, heat distribution, and nutrient conditions. In some cases, mixing will be quite challenging \cite{3,4}.

During hydrolysis, oxygen plays an essential role in the aerobic process. The solubility of oxygen in the water is low, about ten ppm at ambient temperature and atmospheric pressure \cite{8}. Sometimes it is necessary to provide oxygen supply into the system continuously.

2. Types of bioreactor for enzymatic lignocellulose hydrolysis

Some popular bioreactors for lignocellulose hydrolysis discussed here were tray bioreactor, stirred tank bioreactor, and rotating drum bioreactor. Besides, we also discussed a bioreactor equipped with a separating system.

2.1. Tray Bioreactor

Tray bioreactor is considered the simplest form of bioreactors and is already known for centuries. Some tray bioreactors can be used in parallel (mulitrays), each acts as a stand-alone bioreactor. Usually, they do not need complex instrumentation, but sometimes due to the properties of the material used, they may require some controls. Tray bioreactor is a considerably right choice for slow bioreaction that requires a large surface area \cite{5,7}.

2.2. Stirred tank bioreactor

Stirred tank bioreactor is usually used for submerged fermentation with a high water content that allowing it to be mixed. However, with some modifications, it can be used in SSF as well. The reason for stirring, in this case, is the same as for submerging fermentation, i.e., to homogenize the medium. In this case, stirring must be done carefully to keep the fungal hyphae from damage. However, since most lignocellulose hydrolysis is done in a solid-state, it needs to adjust the stirrer configuration, such as a Z-blade mixer in a horizontally placed bioreactor \cite{9}. When sterilization is needed, it has to be carried out thoroughly, for sometimes additional devices such as a stirring motor and its connector can be the source of contamination.

2.3. Rotating Bioreactor

This bioreactor type is also quite popular because it provides a good mixing with a lower risk of fungal hyphae damage. Rotating bioreactor involves complex instrumentation as well as excellent power consumption. Mixing can be done either continuously or intermittently, according to the needs. In some low-speed mixing, several baffles are installed on the reactor wall \cite{7}.

2.4. Bioreactors with separation equipment

Recent innovation combines bioreactor with separating equipment, such as ultrafiltration membrane, with being separated continuously. Hence the amount of product in the reactor that hinders the enzymatic reaction can be kept low. Table 1 shows some types of bioreactors used for lignocellulosic hydrolysis. Even though almost all bioreactors perform in continuous operation, their kinetics parameters obtained from batch reaction study can be applied.
| Substrate                      | Bioreactor type                  | Source of cellulase              | Rel.       |
|-------------------------------|----------------------------------|----------------------------------|------------|
| Sugarcane bagasse             | Stirred-tank reactor (STR)       | Trichoderma viride               | [10]       |
| Sugarcane bagasse             | Rotating fibrous-bed bioreactor  | Trichoderma viride               | [10]       |
| Sugarcane bagasse             | Stirred-tank reactor (STR)       | Trichoderma harzianum            | [11]       |
| Rice straw                    | Tubular reactor coupled with UF membrane | Trichoderma reeseei             | [2]        |
| Corn cobs and brewer’s spent grain | Stirred tank bioreactor           | Fusarium oxysporum               | [12]       |
| Sugarcane bagasse             | Tray bioreactor                  | Aspergillus sp. S4B2F            | [13]       |
| Pure cellulose                | Stirred tank bioreactor          | Trichoderma  reeseei             | [14]       |
| Rice straw                    | Rotating bioreactor              | Trichoderma reeseei (extracted)  | [15]       |
| Cornstalk                     | Hollow fiber ultrafiltration      | Trichoderma reeseei (extracted)  | [16]       |

3. **Semi-continuous bioreactor**

For industrial-scale purposes, hydrolysis is carried out semi-continuously, using a fixed bed bioreactor with a continuous air supply. Traditional tray and fixed bed bioreactors are the most widely used because of their simple designs. However, upgrading the fixed bed bioreactor’s capacity may generate problems relating to the height of the bed due to the distribution of oxygen. A fixed bed bioreactor can be designed to overcome such issues by stacking up several trays to have a more effective process is worth a try with every tray acts as an individual [18].

In SSF, aeration generally has several functions, including supplying oxygen, removing CO₂, regulating temperature and humidity, and distributing volatile compounds produced during metabolism [17]. During hydrolysis, the oxygen supply is intended to maintain oxygen availability because some cellulase-producing microorganisms are known to be very aerobic fungus [3]. Also, fixed bed reactors have a good solid-liquid ratio and allow proper control of the reaction conditions [18], [20].

![Figure 1. Packed bed bioreactor with continuous air supply](image-url)
This design facilitates uniformity throughout the bioreactor. Instead of oxygen, sterile air can choose aeration. The air supply can reach remote areas within the reactor and ensure the hydrolytic fungus grows well. SSF can also minimize the formation of unwanted parameters due to the absence of mixing in the system, thus offering easy control of all parameters involved.

4. Conclusion
The selection of a bioreactor is very subjective to the nature of the hydrolysis process. The two most important criteria are contamination protection and the homogeneous conditions throughout the reactor are the keys to the success of an enzymatic hydrolysis process. Since the majority of cellulase producing-fungi are aerobic, aeration is necessary to ensure the availability of oxygen. Continuous oxygen supply can be a strong consideration to support the growth of hydrolytic fungi.

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