**Application of oxygen in biofermentation engineering**

I. The effect of dissolved oxygen on fermentation

In the fermentation process， the factors affecting oxygen consumption are as follows.

(1) the composition of the medium and bacterial concentration significantly affect oxygen consumption culture medium is rich in nutrients， the bacterium grows fast， oxygen consumption; high bacterial concentration， oxygen consumption; fermentation process replenishment or replenishment of sugar， microbial uptake of oxygen then increases.

(2) the age of bacteria affect oxygen consumption respiration is high， oxygen consumption is large. Later in the fermentation period， the bacterium is in the aging state， oxygen consumption naturally weakened.

(3) fermentation conditions affect oxygen consumption in the optimal conditions of fermentation， oxygen consumption is large.

During fermentation， the elimination of toxic metabolites such as carbon dioxide， volatile organic acids and excess ammonia is also beneficial to improve the oxygen uptake of the bacterium.

At 25℃， 0.10MPa， the solubility of oxygen in air is 0.25mmol/L in water and only 0.22mmol/L in fermentation broth， while a large number of microorganisms in fermentation broth consume oxygen rapidly (oxygen consumption rate is greater than 25~100mmol/L-h). Therefore， oxygen supply is very important for aerobic microorganisms. In aerobic fermentation， microorganisms have a minimum requirement for oxygen， to meet the minimum oxygen concentration of microbial respiration is called critical valueofdissolvedoxygenconcentration， expressed in c critical. Below the c critical threshold， the respiration rate of microorganisms decreases significantly with the dissolved oxygen concentration. The general aerobic microorganism c critical is very low， about 0.003 ~ 0.05mmol / L， oxygen demand is generally 25 ~ 100mmol / (L - h). Its c critical is about 1% to 25% of the oxygen saturation solubility.

When there is no other limiting substrate， the dissolved oxygen is higher than the c critical， the specific oxygen consumption rate of the cell remains constant; if the dissolved oxygen is lower than the c critical， the specific oxygen consumption rate of the cell decreases greatly， the cell is in a semi-anaerobic state and the metabolic activity is hindered. The oxygen required to maintain microbial respiration and metabolism in the culture fluid maintains a balance between oxygen supply and oxygen consumption to meet the use of oxygen by microorganisms. Microorganisms in the liquid can only use dissolved oxygen， microorganisms at the gas-liquid interface can also use the oxygen in the gas phase， so strengthening the gas-liquid interface will also be beneficial to oxygen supply.

Dissolved oxygen is one of the most important parameters for aerobic fermentation control. Since the solubility of oxygen is very small in water and even smaller in the fermentation broth， constant adjustment of ventilation and agitation is required to meet the oxygen demand of different fermentation processes. The amount of dissolved oxygen can have different effects on the growth of the bacteria and the formation and yield of the products. Such as glutamic acid fermentation， when the supply of oxygen is insufficient， the accumulation of glutamic acid will be significantly reduced， producing a large amount of lactic and succinic acid. Another example is the production of vitamin B12 in the fermentation of Propionibacterium Xue， the components of vitamin B12 cobinamide (also known as B-factor) of the biosynthesis of the two main enzymes in the early stage of oxygen blocked， limiting the supply of oxygen in order to accumulate a large number of B-factor， B-factor and only under the conditions of oxygen supply into vitamin B12， thus using a combination of anaerobic and oxygen supply facilitates the synthesis of vitamin B12. In the fermentation of asparaginase， the first stage is aerobic culture， while the later stage is changed to anaerobic culture， the vitality of the enzyme can be greatly improved. It is quite important to master the timing of the shift. According to experimental research， when the dissolved oxygen drops to 45%， it is changed from aerobic culture to anaerobic culture， and the enzyme activity can be increased 6 times， which shows the importance of using control of dissolved oxygen to control the fermentation. For antibiotic fermentation， the supply of oxygen is even more important. As in the case of tunicamycin fermentation， stopping ventilation for a short time during the growth period may affect the sugar metabolism pathway of the bacterium during the production period， shifting from the HMP pathway to the EMP pathway and reducing tunicamycin production. Oxygen on tunicamycin C6 also comes directly from dissolved oxygen， so dissolved oxygen has an effect on bacteriophage metabolism and product synthesis.

In summary， aerobic fermentation is not the greater the dissolved oxygen the better. Although high dissolved oxygen is beneficial to the growth of the bacterium and product synthesis， but too much dissolved oxygen sometimes inhibit the formation of products. Because， in order to avoid fermentation in oxygen-limited conditions， need to check the c critical and optimum oxygen concentration of each fermentation product (optimumoxygenconcentration)， and make the fermentation process to maintain in the optimum oxygen concentration. The magnitude of the optimum dissolved oxygen concentration is related to the characteristics of the bacterium and the product anabolism， which is determined experimentally. It is reported that the c critical for secondary metabolism of penicillin fermentation is between 5% and 10%， below this value will bring loss to penicillin synthesis， and the longer the time， the greater the loss. While the primary metabolism of amino acid fermentation， the size of oxygen requirement is closely related to the synthesis pathway of amino acid. According to the different requirements of fermentation oxygen demand can be divided into three categories (see Figure 7-4): the first category has glutamic acid， glutamine， arginine and proline and other glutamic acid family amino acids， they are under the conditions of adequate respiration of the bacteria to maximize production， if the oxygen supply is insufficient， amino acid synthesis will be strongly inhibited， a large number of lactic and succinic acid accumulation; the second category， including isoleucine， lysine， threonine and aspartic acid， namely The second group， including isoleucine， lysine， threonine and aspartic acid， which are amino acids of the aspartic acid family， can obtain the highest yield with sufficient oxygen supply， but the yield is not significantly affected when oxygen supply is restricted; the third group， including leucine， valine and phenylalanine， can obtain the largest amount of amino acids only when oxygen supply is restricted and cellular respiration is inhibited， and product formation is inhibited if oxygen supply is sufficient.

The reason for these differences in the degree of oxygen demand for amino acid synthesis is caused by their different biosynthetic pathways， which produce different amounts of NAD(P)H and， of course， different amounts of dissolved oxygen required for reoxidation. The first group of amino acids is formed by two pathways， the glyoxalate cycle and the phosphoenolpyruvate carboxylation system， which produce the largest amount of NADH. Therefore， the amount of oxygen required for the oxidative regeneration of NADH is the most， and the more oxygen is supplied， the more smoothly the amino acid synthesis will be. The second type of synthesis pathway is the glyoxalate cycle that produces NADH or the phosphoenolpyruvate carboxylation system that consumes NADH， and the amount of NADH produced is small， so the relationship with the amount of oxygen supply is not obvious. The third category， such as the synthesis of phenylalanine， does not go through the TCA cycle， with little NADH production and excessive oxygen supply， but rather acts as an inhibitor. Similar results were found for inosine fermentation. It is thus clear that the size of oxygen supply is related to the biosynthetic pathway of the product.

During the fermentation of antibiotics， there is a critical dissolved oxygen concentration for both the growth phase of the bacterium and the production phase of the product， which are c' critical and c'' critical， respectively. The relationship between the two is: ①c'critical ≈c''critical; ②c'critical>c''critical ③c'critical<c ''critical.

At present， the fermentation industry， oxygen utilization rate (oxygenutilizationrate) is still very low， only 40% to 60%， antibiotic fermentation industry is even lower， only 2% to 8%. The growth and metabolic activities of aerobic microorganisms need to consume oxygen， they can only complete the biological oxidation in the presence of oxygen molecules. Therefore， oxygen supply is essential for aerobic microorganisms.

Second， the relationship between oxygen supply and microbial respiratory metabolism

Aerobic microorganisms require oxygen for growth and metabolism， so oxygen supply must meet the needs of microorganisms in different stages. Due to the different types and quantities of oxidative enzymes (such as catalase， cytochrome oxidase， flavin dehydrogenase， polyphenol oxidase， etc.) contained in various aerobic microorganisms， the oxygen uptake or respiratory intensity of various microorganisms is different under different environmental conditions.

The oxygen uptake of microorganisms is commonly expressed by two methods: respiratory intensity and oxygen consumption rate. Respiratory intensity， also known as oxygen consumption rate， is the amount of oxygen absorbed per unit mass of dry bacteria in unit time， expressed in mmolO2/(g dry bacteria - h). Oxygen consumption rate， also known as oxygen uptake rate， is the amount of oxygen absorbed per unit volume of culture fluid per unit time， expressed as r in mmolO2/(L-h). The respiratory intensity can express the relative oxygen uptake of microorganisms， however， when there are solid components in the culture medium， it is difficult to determine， and then the oxygen consumption rate can be used to express. The oxygen consumption rate of microorganisms in the fermentation process depends on the respiration intensity of microorganisms and the concentration per unit volume of bacteria.

In the fermentation production， the amount of oxygen supply should be decided according to different strains， fermentation conditions and fermentation stages and other specific circumstances. For example， in glutamic acid fermentation during the bacteriophage growth period， it is desired that the maximum consumption of sugar is used for the synthesis of bacteriophage， while in the glutamic acid production period， it is desired that the maximum consumption of sugar is used for the synthesis of glutamic acid. Therefore， in the bacteriophage growth period， oxygen supply must meet the oxygen demand of bacteriophage respiration， if the oxygen demand of the bacteriophage is not met， the bacteriophage respiration is inhibited， which inhibits the growth of the bacteriophage and causes the accumulation of lactic acid and other by-products， and the bacteriophage yield is reduced. But the oxygen supply is not the greater the better， when the oxygen supply to meet the needs of the bacterium， the growth rate of the bacterium reached the maximum， if the oxygen supply is increased， not only can not promote the growth of the bacterium， resulting in energy waste， and high oxygen levels will inhibit the growth of the bacterium， and the bacterium grown under high oxygen levels can not effectively produce glutamate.

Compared with the growth period of the bacterium， the glutamic acid production period requires a large amount of oxygen. Glutamate fermentation is maximized at the maximum cellular respiration intensity for glutamate production. Therefore， adequate oxygen supply is required during the glutamate production period to meet the oxygen demand of the maximum cellular respiration intensity. When conditions are appropriate， glutamate producing bacteria convert more than 60% of the sugar into glutamate.

Third， the change of dissolved oxygen during fermentation

In the fermentation process， under the existing equipment and normal fermentation conditions， the change of dissolved oxygen for each product fermentation has its own pattern. As shown in Figure 7-5 and Figure 7-6， in the early stage of glutamate and erythromycin fermentation， the producing bacteria multiply and the oxygen demand keeps increasing. At this time， the oxygen demand exceeds the oxygen supply， so that the dissolved oxygen drops significantly and a low peak appears， and the oxygen uptake rate of the producing bacteria appears a peak at the same time. The bacterial concentration in the fermentation broth also keeps rising， and for glutamic acid fermentation， the bacteria are still growing and multiplying， and the bacterial concentration of antibiotic fermentation also appears a peak. Viscosity generally also appears a peak stage in this period. This all indicates that the producing bacteria are in the logarithmic phase. After the growth phase， oxygen demand decreases and dissolved oxygen begins to form products after a period of steady phase (such as glutamate fermentation) or a subsequent rise (such as antibiotic fermentation)， and dissolved oxygen continues to rise. The low peak of dissolved oxygen of glutamate fermentation is about 6-20h， while that of antibiotics are in 10-70h， the time of low peak appearance and low peak dissolved oxygen varies with the strain， process conditions and equipment oxygen supply capacity.

In the middle and late stage of fermentation， for wholesale fermentation， the dissolved oxygen change is relatively small. In the stabilization period， because the bacterium has multiplied to a certain concentration， the respiration intensity does not change much. However， when the outside world is replenished (including carbon sources， precursors， defoamer)， the dissolved oxygen will change， the size and duration of the change， with the replenishment of the age of the bacteria， replenishment of the type and dosage of different substances and different. Such as supplementation of sugar， the fermentation broth oxygen uptake rate will increase， causing dissolved oxygen to drop， after a period of time and gradually back up; such as continued supplementation of sugar， even down to c critical below， and become a limiting factor in production. In the late production， due to the aging of the bacterium， the respiratory strength is weakened， dissolved oxygen will also gradually rise， once the bacterium autolysis， dissolved oxygen will be more obvious rise.

In the process of fermentation， sometimes there are abnormal changes of dissolved oxygen significantly lower or significantly higher， and it is common that dissolved oxygen decreases. The reason for the abnormal change is twofold: oxygen consumption or oxygen supply has an abnormal factor or an obstacle. According to the information reported， caused by the abnormal decline in dissolved oxygen， there may be the following reasons: ① pollution aerobic miscellaneous bacteria， a large number of dissolved oxygen is consumed， may make the dissolved oxygen in a relatively short period of time down to near zero， if the miscellaneous bacteria itself is not strong oxygen consumption capacity， dissolved oxygen changes may not be obvious; ② bacterium metabolism abnormal phenomenon， oxygen requirements increase， so that the dissolved oxygen decline; ③ some equipment or process control failure or change ③ some equipment or process control failure or change， may also cause dissolved oxygen decline， such as stirring power consumption becomes smaller or stirring speed becomes slower， affecting the oxygen supply capacity， so that the dissolved oxygen decreased. Another example is the defoamer because of the automatic oiler failure or artificial add too much， will also cause a rapid decline in dissolved oxygen. Other process operations that affect the oxygen supply， such as stop stirring， boring the tank (tank exhaust valve closed)， etc.， will make abnormal changes in dissolved oxygen.

The cause of the abnormal rise of dissolved oxygen， in the case of no change in oxygen supply conditions， mainly oxygen consumption changes， such as abnormal metabolism of the bacterium， oxygen consumption capacity decreases， so that the dissolved oxygen rises. Especially contaminated virulent phage， the most obvious impact， produce bacteria have not yet cleaved before， respiration has been inhibited， dissolved oxygen is likely to rise， until the bacterium rupture， completely lose respiratory capacity， dissolved oxygen is straight up.

As can be seen from the above， from the dissolved oxygen changes in the fermentation solution， you can understand whether the microbial growth and metabolism is normal， whether the process control is reasonable， whether the equipment is sufficient oxygen supply capacity and other issues to help us find the reasons for abnormal fermentation and control good fermentation production.

Fourth， the dissolved oxygen concentration control

(i) oxygen transfer equation

Liquid phase volume oxygen transfer coefficient KLα represents the ease of oxygen transfer from gas phase to liquid phase， which is closely related to fermentation process control， amplification and reactor design. When the dissolved oxygen concentration in the fermentation broth is kept stable， i.e.， the oxygen transfer and oxygen consumption in the fermentation process are in balance.

When the oxygen consumption rate r of microorganisms remains unchanged， while the saturated dissolved oxygen concentration c\* in liquid phase remains unchanged， the larger KLα is， the higher the actual dissolved oxygen concentration cL in liquid phase is， so the change of KLα can be used to measure the aeration efficiency of fermenter.

(ii) Dissolved oxygen concentration control

The dissolved oxygen concentration of fermentation broth is determined by both oxygen supply and oxygen demand. That is to say， when the oxygen supply of fermentation is greater than the oxygen demand， the dissolved oxygen rises until saturation; and vice versa， it falls. Therefore， to control the dissolved oxygen in the fermentation broth， we need to start from these two aspects.

In terms of oxygen supply， the main thing is to try to improve the driving force of oxygen transfer and liquid phase volume oxygen transfer coefficient KLɑ value. Combined with the actual production， under the possible conditions， take appropriate measures to improve the dissolved oxygen， such as adjusting the stirring speed or ventilation rate to control the oxygen supply. But the size of oxygen supply must also be coordinated with the oxygen demand， that is to say， there must be appropriate process conditions to control the oxygen demand， so that the growth of generating bacteria and product generation of oxygen demand does not exceed the demand of the equipment does not exceed the oxygen supply capacity of the equipment， so that the generating bacteria play the maximum production capacity. This is of great importance to the production practice.

The oxygen demand of fermentation broth is influenced by the bacteria concentration， the type and concentration of substrate and the culture conditions， among which the influence of bacteria concentration is the most obvious. The oxygen uptake rate of fermentation broth OUR is proportional to the increase in bacterial concentration， but the oxygen transfer rate OTR is logarithmically decreasing with bacterial concentration， so you can control the specific growth rate of bacteria than the critical growth rate (strains maintain a higher product synthesis enzyme activity of the "strong" cells dominate must meet a minimum specific growth rate. Below it， the older cells will gradually dominate， resulting in a decrease in product synthesis capacity. (This minimum growth rate is called the critical ratio growth rate， expressed in μ pro) a little higher level， to reach the optimum bacteria concentration [that is， c (X) pro]， the highest productivity of the bacterium. This is an important way to control the optimum dissolved oxygen concentration. The optimum bacteriophage concentration can ensure that the specific production rate of the product is maintained at the maximum， but does not make the oxygen demand greater than the oxygen supply. Controlling the optimum bacterial concentration can be achieved by controlling the concentration of the substrate. For example， in penicillin fermentation， the optimum bacterial concentration is achieved by controlling the rate of glucose supplementation. Now the sensitive type of dissolved oxygen electrode sensor has been used to control penicillin fermentation， using the change of dissolved oxygen to automatically control the rate of glucose replenishment， indirectly control the rate of oxygen supply and pH， to achieve the trinity of bacterial growth， dissolved oxygen and pH control system.

In addition to controlling the replenishment rate， in industry， process measures such as adjusting the temperature (lowering the culture temperature can increase the dissolved oxygen concentration)， liquefying the medium， intermediate replenishment， and adding surfactant can be used to improve the dissolved oxygen level.