FOOD COMPOSITION AND ADDITIVES

Improving Industrial Full-Scale Production of Baker's Yeast by Optimizing Aeration Control

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This work analyzes the control of optimum dissolved oxygen of an industrial fed-batch procedure in which baker's yeast (*Saccharomyces cerevisiae*) is grown under aerobic conditions. Sugar oxidative metabolism was controlled by monitoring aeration, molasses flows, and yeast concentration in the propagator along the later stage of the propagation, and keeping pH and temperature under controlled conditions. A large number of fed-batch growth experiments were performed in the tank for a period of 16 h, for each of the 3 manufactured commercial products. For optimization and control of cultivations, the growth and metabolite formation were quantified through measurement of specific growth and ethanol concentration. Data were adjusted to a model of multiple lineal regression, and correlations representing dissolved oxygen as a function of aeration, molasses, yeast concentration in the broth, temperature, and pH were obtained. The actual influence of each variable was consistent with the mathematical model, further justified by significant levels of each variable, and optimum aeration profile during the yeast propagation was found.

Baker's yeast is used extensively because of its ability to raise dough by fermenting mainly maltose and sucrose present in the dough to ethanol and carbon dioxide. It is also used in the leavening process because of its contribution to the aroma and flavor of bread (1, 2). The conditions in dough differ from those in industrial baker's yeast production, since in the latter process, the environment is aerobic and the sugar concentration is low (2, 3).

In a modern propagation plant, the question to be answered is how to control the process by ensuring optimal air supply and at the same time optimize the appropriate metabolic pathway that the yeast may encounter during its growth in the propagator. In particular, optimizing processes under pre- and poststationary phase conditions may produce a substantial economic improvement.

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The production of baker's yeast involves the multistage propagation of the selected yeast strain using sugar as a carbon source. Baker's yeast is usually produced starting from a small quantity of yeast added to a liquid solution of essential nutrients (molasses, ammonia or ammonium salts, phosphate, and vitamins) at a suitable temperature and pH (4). Once the cell population has grown enough, it is transferred into a larger bioreactor for a new growth stage; 4 or 5 stages are usually necessary to reach a satisfactory production quantity. Therefore, the bioreactor volume changes from 1 dm^3 to 100–150 m^3. The smaller bioreactors used for the initial stages operate under batch and anaerobic conditions, whereas in the larger bioreactors used for the later stages, aeration is provided and the fed-batch cultivation mode is adopted, i.e., the nutrients are fed to the culture medium at a variable rate.

The plant configuration and operative choices are the consequence of the effects that *Saccharomyces cerevisiae* metabolism produces on biomass yield and growth rate (5). During the aerobic growth of *S. cerevisiae*, both sugars and ethanol can be used as carbon and energy sources. Sugars can be metabolized via 2 different energy-producing pathways, oxidation or fermentation, depending on the sugar concentration in the medium.

Oxidative metabolism of glucose.—Theoretically, glucose is entirely oxidized and provides a high level of energy for adenosine triphosphate (ATP) synthesis:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6\,H_2O + 38\,ATP \quad (1)$$

When the yeast production yield is maximized, 0.5 g of dry matter (biomass) is produced per gram of consumed glucose.

Fermentative metabolism of glucose.—When the glucose concentration is sufficiently high, yeasts ferment glucose and ethanol is produced:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 2\,ATP \quad (2)$$

The low level of energy produced is related to low yeast growth.

Indeed, at a high sugar concentration, oxidation is suppressed and fermentation takes place [the phenomenon often referred to as the Crabtree effect (6)]; oxidation predominates when sugar concentration is below 50–100 mg/dm^3. On the other hand, under oxygen-limited growth conditions, the fermentative pathway leading to ethanol production predominates, even at a low sugar concentration.

Furthermore, an oxidative metabolism of ethanol may be produced. Without sugars, the ethanol produced during the initial fermentative metabolic pathway is reconsumed in the presence of molecular oxygen. Theoretically:

$$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O \qquad (3)$$

Biomass yields on sugars (g_{cells}/g_{sugars}) are strongly related to the prevailing metabolic pathway, being maximal only when sugar is oxidized. At high growth rates, the biomass yield of baker's yeast (*S. cerevisiae)* decreases due to the production of ethanol (4, 7). For this reason, it is standard industrial practice to use a fed-batch process (3, 8, 9) whereby the growth rate is fixed at a level very close to the point of ethanol production. Optimally, growth should be maintained at this critical level, but in practice this is difficult because the critical growth rate is dependent upon strain and culture conditions.

The critical growth rate may vary from batch to batch and even during the experiment. In order to avoid the risk of decreasing the yield, an alternative approach is to use the overflow metabolite as an indicator of how close or far the actual growth rate is from the critical growth rate. Thus, if ethanol production is maintained constant, it is possible to fix the growth rate at a value slightly above the critical growth rate (10, 11).

The effect of variables such as pH and temperature is well known and their optimal set-points can easily be defined. On the contrary, yield and productiveness can largely be affected by the concentration of biomass, sugar, oxygen, and ethanol formation, if any. The optimal conditions giving maximum yield and productiveness change along with time together with the biomass growth. Therefore, the feeding rate of the molasses is the most critical variable and the problem is to individuate the best feeding rate sequence (12).

Furthermore, bioprocess control runs into a number of difficulties resulting from (*1*) the nonlinear, nonsteady kinetic properties of the process dynamics as the microorganisms multiply, adapt, and change their behavior with time and with the environment (7, 13, 14); (*2*) a lack of sensors providing direct measurements of the system state variables, such as biomass, substrates, and metabolites. More often than not, sensors are not industrially available or used (9, 13, 15, 16).

The optimal process control must maximize both cell yield and productivity (13, 17). The way to overcome this productivity and yield conflict is by accurately regulating the molasses feeding to ensure that the sugar concentration is tightly maintained in such a way that only oxidation occurs and the respiratory capacity of the cells is utilized to the maximum.

The carbohydrate feedstock is an important cost factor in baker's yeast production and, consequently, biomass yield on sugar is an important optimization criterion. In order to maintain competitiveness, the fermentations must be highly consistent, with minimum variation in product quality, maximum yield on raw materials, and minimum production of undesirable side products.

Many parameters impact the metabolic activities of microorganisms and need to be controlled (14). Hence, many researchers have focused their attention on optimizing fed-batch processes for the production of baker's yeast with different aims (productivity, quality of the yeast, or energy saving). The majority of them commonly developed their research work under laboratory conditions (6, 18–30), seldom under pilot plant conditions (12, 31, 32), but never on a large industrial scale. Thus, as in our previous studies on correlation (33, 34), we report here for the first time, correlations for indirect control of dissolved oxygen through controlled aeration in an industrial full-scale production of baker's yeast.

Materials and Methods

In general, industrial fed-batch production of baker's yeast is carried out in open loop conditions, and the empirically established molasses feed profiles are kept as manufacturing secrets. Other than feed control, optimization of the entire bioprocess through the choice of optimal bioreactor size, process duration, and initial concentration of inoculum is the goal of industrial production.

Fermentation was controlled in the last phase of commercial yeast production following a fed-batch procedure. Three commercial products were manufactured, with their "recipe" characteristics (formulation used in the process for feeding the yeast) treated as confidential. The Traditional, Fast, and Acidic modes correspond to different needs and to different types of dough: Traditional produces yeast used in slow-development dough (traditional dough); Fast produces yeast used in quick-development dough (industrial dough); Acidic produces yeast used in acid-sugary dough (products which need long preservation, such as sliced bread).

With the addition of molasses to any original formulation, the medium used in this study contained glucose in the range of 5–100 g/L. During the fed-batch phase, ammonium salts and ammonia were used to provide a source of nitrogen; other minor nutrient requirements were satisfied by inorganic salts. Vitamins of the B group (factors of yeast growth) were added. Each fermentation was controlled for 16 h, 1/2 h of maturation to guarantee yeast stability.

Culture Conditions

An industrial strain (baker's yeast) of *S. cerevisiae* (property of the company) was used throughout the fermentative process. Full-scale tests were conducted in an industrial plant with a cylindrical propagator previously

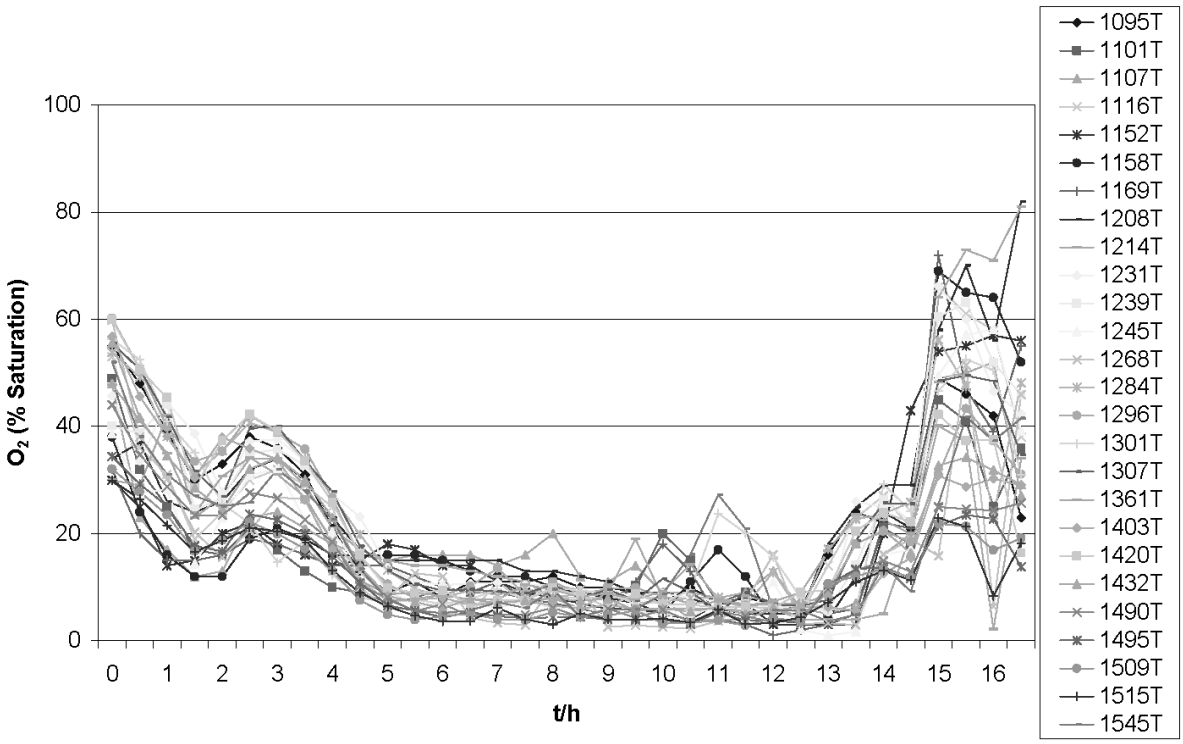


Figure 1. Online measurements of dissolved oxygen levels in fed-batch fermentations of *S. cerevisiae* in Traditional operations.

sterilized with water steam under the following conditions: 121°C, 103 kPa, 15–30 min.

The propagator tank incorporates an aeration grill through which air is introduced. The air flow, which is filtered at its entrance in order to block polluting elements, exercises 3 important functions: incorporation of oxygen for cellular metabolism, elimination of carbon dioxide accumulated in the broth, and shaking the substratum to avoid the decantation of the yeast. The air flow rate ranged from 7000 to 15 000 m^3/h.

An oxygen probe "Inpro 6800 series O2 Sensors," placed inside the tank and coupled to a microprocessor Model 4100e, was used to monitor the oxygen dissolved. The probe was calibrated before each fermentation and after replacing the electrolyte or the membrane in the probe. Saturation percentages were measured for calibration: 100% was measured before yeast spread and after vigorously airing the tank approximately 10 min with 3000 m^3/h of air; 0% saturation value remained stable.

The pH was maintained in the range of 4–7. It was started at around 4 in order to control the microbial contamination and was slowly raised, never exceeding pH 7. The temperature process was maintained close to 33°C. Foam was controlled by addition of an antifoam agent at regular intervals throughout the process.

Analytical Methods

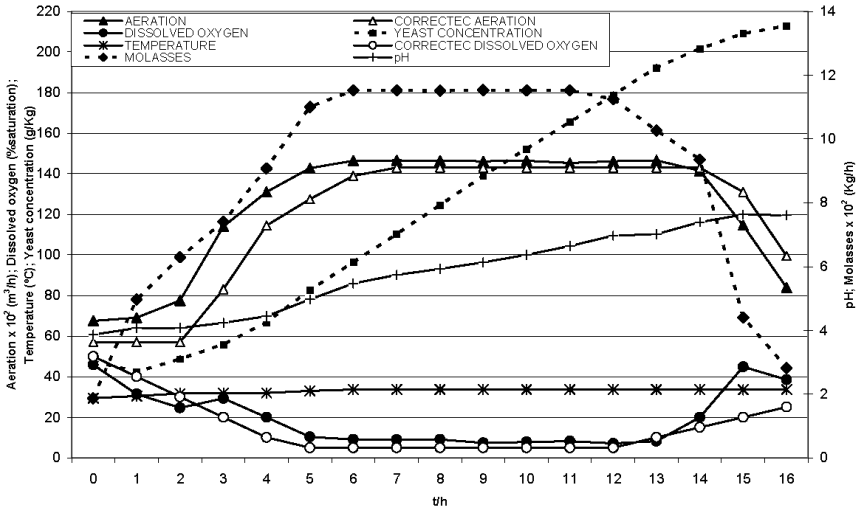
In the control room, online measurements of dissolved oxygen (percentage), aeration (m^3/h), molasses (kg/h), temperature (°C), and pH were recorded. Simultaneously, ethanol (mL/L), yeast concentration in the fermentation broth (g/kg), and specific growth were determined offline. Specific growths were calculated from yeast concentration data and Equation 4 (35).

$$ln\, x_t = ln\, x_o + \mu t \qquad (4)$$

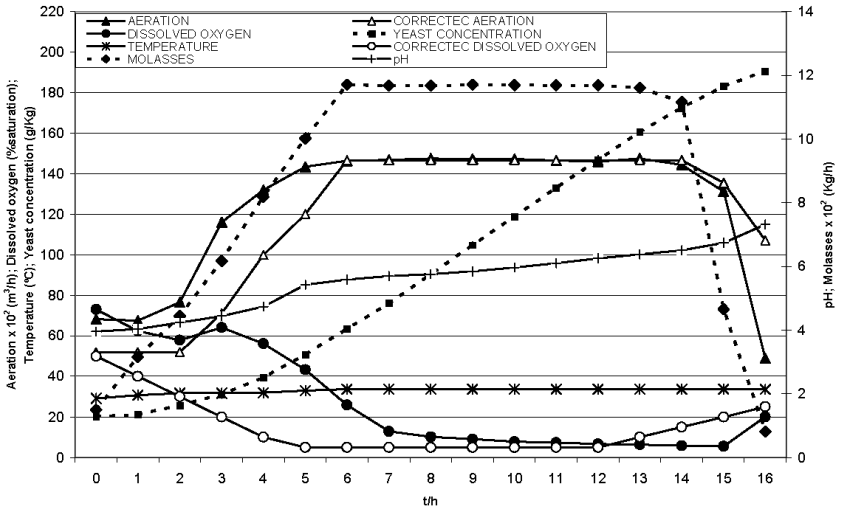
where x_t and x_o are the yeast concentrations in the broth (mg/cm^3) at time t, and at the beginning of the fermentation, t is the time (h), and μ is the specific growth (h^{-1}).

Yeast concentration in the fermentation broth was determined by filtering 10 mL of fermentation broth through preweighed glass fiber filters, washed and dried in a microwave oven for 7 h at 100°C, and kept in a desiccator for 1 h before reweighing. The molasses feed profile was controlled with a feedback loop based on previously collected data. Samples of the fermentation broth were distilled to determine ethanol concentration. The distilled product was collected in a flask containing potassium dichromate and sulfuric acid and neutralized against Mohr salt.

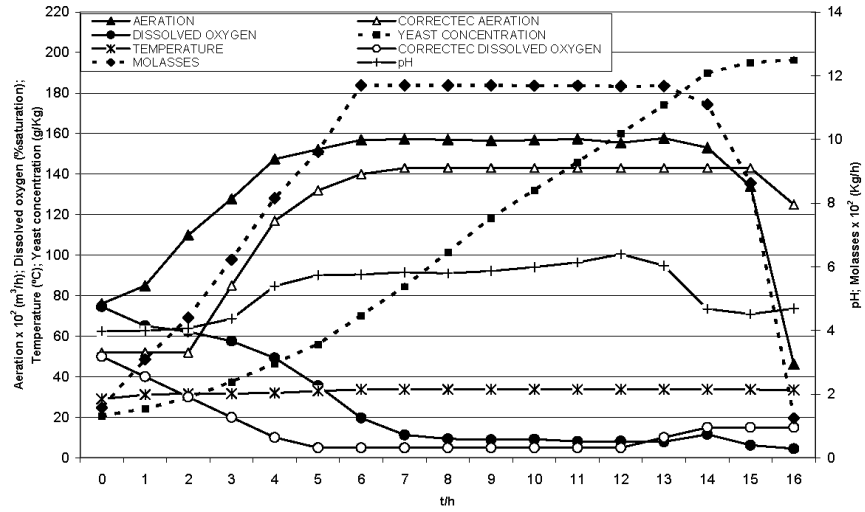
A large number of experiments were performed and the information acquired was stored in the system database in order to achieve a full optimization. Statistica 5.0 was the program chosen to illustrate the model dependence of the dissolved oxygen on the operating variables.



(a) Traditional



(b) Fast



(c) Acidic

Figure 2. Plot of the parameters' values measured in fed-batch cultures operated under Normal, Fast, and Acidic recipes. Closed symbols and asterisks correspond to experimental values. Open symbols correspond to model-created values.

Table 1. *P*-values associated with aeration, yeast concentration, molasses, pH, and temperature for Traditional, Fast, and Acidic recipes, obtained with Statistica 5.0 program

	P-value		
Parameter	Traditional	Fast	Acidic
Intercept	0.108863	0.207013	0.170375
Aeration, m^3/h	0.000000	0.020878	0.002563
Yeast, g/kg	0.000000	0.000254	0.687794
Molasses, kg/h	0.000002	0.049202	0.009337
pH	0.000030	0.080400	0.577680
Temperature, °C	0.285457	0.957062	0.459558
Time, h	0.169738	0.001405	0.022355
Time squared, h^2	0.018405	0.000132	0.073013

Results and Discussion

Aeration, sugar, and yeast concentration in the broth have been considered as fundamental variables influencing the concentration of dissolved oxygen. Air incorporates oxygen, and yeast and sucrose influence its consumption. However, the influence of temperature and pH on dissolved oxygen must also be kept in mind. Thus, air flow rate (A), concentration of molasses (M), yeast concentration (Y), pH, temperature (T), and time (t) were considered independent variables which had an effect on fed-batch aerobic yeast production, and dissolved oxygen (O) was selected as the dependent variable.

In order to optimize conditions for biomass growth in the fed-batch feeding of the industrial reactor, a large number of experiments for each type of fermentation were performed. In particular, a series of 26 fed-batch fermentations (16 h from inoculation) were performed under controlled aerated conditions for Traditional recipes.

Figure 1 shows dissolved oxygen per experiment performed along a timeline. An analysis of variance of Figure 1 data by means of Statistica 5.0 was performed. Average values and prediction limits of the oxygen dissolved were obtained with a 95% confidence level. Figure 2a shows average values. A similar procedure was used for the other variables under consideration: air flow rate, concentration of molasses, yeast concentration, pH, and temperature. Results are also included in Figure 2a. Figure 2b and c show the results of similar procedures for Fast and Acidic fermentations.

In all the different fermentations, an upward trend in aeration is observed early in the process while a downward trend occurs in the later stages, associated with the feeding of molasses according to each of the recipes. In general, an excess of dissolved oxygen was observed in all the types of fermentations, in both the early and later stages of the process, as a negative factor in the overall cost of the full process.

These results are in agreement with previous results, whereby, at the initial stages of the process, the maximum feasible growth rate is dictated by the threshold-specific growth rate at which respirofermentative metabolism sets in (3). In later stages, the specific growth rate is decreased to avoid problems with the limited oxygen transfer (2, 3, 8, 36, 37).

In order to describe the relationship between the dissolved oxygen (O_2) and the independent variables referred to along the process, the data were adjusted to a model of multiple lineal regression by means of the program Statistica 5.0. Equations 5–7 show the relationships obtained for each type of fermentation. Also, the *P*-values associated with the variables are represented in Table 1. When *P*-values are <0.05, they correspond to a significant level of the variable, becoming more significant as it approaches zero.

Traditional:

$$O = 64.9694 + 0.0037A - 0.0508M - 0.32767Y + 6.8178\,pH - 1.4078T - 2.8073t + 0.2674t^2; \quad R^2 = 77.29 \qquad (5)$$

Fast:

$$O = 69.2612 + 0.014A - 0.0139M - 0.4057Y + 6.0945\,pH - 0.098T - 8.30957t + 0.5163t^2; \quad R^2 = 88.3301 \qquad (6)$$

Acidic:

$$O = 109.863 + 0.0042A - 0.0387M + 0.046Y - 1.5038\,\text{pH} - 1.8892T - 8.7873t + 0.3184t^2; \quad R^2 = 94.3035 \qquad (7)$$

Equations 5–7 show that aeration is always a positive factor, since air flowing through the broth contributes to an increased amount of dissolved oxygen. The addition of sugar is a negative factor, because when substratum is fed, it is metabolized through the corresponding consumption of oxygen, thereby diminishing the oxygen concentration in the tank. Yeast concentration in fermentation broth is also a negative factor, since the yeast needs oxygen for its in-tank metabolizing processes. It is necessary to point out an inconsistent positive factor in the case of the Acidic fermentations, which is explained in the statistics, since the associated *P*-value of 0.6877 eliminates any effect of this variable and therefore can be neglected.

Temperature ought to remain constant, with no significant fluctuations, since growth should remain at its optimum level; for our strain, this corresponds to temperatures close to 33°C. The oxygen solubility in aqueous phase should decrease as temperature increases, as seen in Equations 5–7. However, the high *P*-values obtained correspond to a nonsignificant level of temperature. These high *P*-values can be explained by considering factors such as aeration and concentration levels of yeast or of molasses, which highly influence the percentage of dissolved oxygen affecting yeast growth, favoring a nonsteady equilibrium throughout each operation.

The pH should be maintained within certain limits. In order to avoid microorganism contamination, the process begins at approximately pH 4; the fed-batch process including ammonia decreases acidity along the process, although pH should never exceed pH 7. For Traditional and Fast operations, the oxygenation increases when pH increases; however, in the later stages of Acidic operations the tendency is reversed. This may be explained in terms of substratum composition (sulfuric acid concentration is 4 times that of Traditional and Fast operations) since this yeast is applied to acidic dough. On the other hand, the *P*-value equal to 0.57 disagrees with the sign and the meaning of the corresponding coefficient.

In Figure 2, the temporary dependence of the dissolved oxygen is consistent with the quadratic dependence appreciated in the mentioned equations. The corrected aeration was calculated taking into account the obtained relationships (Equations 5–7). Optimized values of dissolved oxygen were considered as reference followed by a recalculation of the optimized aeration through each equation. Optimized values of dissolved oxygen were determined considering both process parameters, such as residual concentration of ethanol and maximum produced ethanol time, and manufactured product parameters such as ferment capacity in preservation and consistency in fermentation. The procedure ensured keeping the ethanol concentration at a very small nonzero level, thereby forcing the culture to grow very close to the critical growth rate and with a near-optimal biomass yield. Figure 2 shows the corrected values as open symbols for each of the fermentations.

Although in an aerobic culture of *S. cerevisiae* (DS 28911), limited in glucose, the metabolism was totally respiratory below a specific growth of 0.28 h^{-1} (3), in our research, based on an industrial productive process, in the 3 types of fermentative processes, ethanol was produced when growth was higher than 0.20 h^{-1}, and only below these values was growth completely aerobic. The maximum of ethanol produced also coincided with the maximum growth.

Since controlling a fed-batch fermentation process is a complex business and requires sophisticated methodologies, the expert knowledge of this process can be used in conjunction with statistical treatment and predictive relationships. By making a little readjustment in aeration, important energy was saved without altering the quality parameters of the final product, which contributes, in a general way, to an effective control of production processes in the yeast industry.

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