On-line Monitoring and Estimation of the *Koji* Making Process in Japanese *Sake* Brewing

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ABSTRACT: In this report an on-line monitoring for *koji* making process in Japanese *sake* brewing on plant scale is presented. The approaches, which have been developed for laboratory scale, were applied to the on-line monitoring software for plant scale. This software could estimate mycelial content in rice *koji* and two kinds of saccharification enzyme activity using with smoothed on-line measurements. Furthermore, it could also simulate the optimal temperature trajectory.

KEYWORDS: *Koji* making process, on-line monitoring software, genetic algorithms

1 INTRODUCTION

Japanese *sake*, that is Japanese traditional beverage, is brewed from steamed rice, rice *koji* and good water. The method of brewing *sake* is peculiar fermentation, which is called “multiple parallel fermentation”. Two actions take place simultaneously in this unique characteristic fermentation. One is conversion of rice starch to glucose by rice *koji* and the other is conversion from glucose to alcohol by yeast.
Rice *koji* is solid state culture of *koji* mold on the steamed rice as a growth medium. It supplies several kinds of proteolytic and saccharification enzymes to *sake* mash. It is known that enzyme proportion of these enzyme activities in rice *koji* has effects on the *sake* mashing process, and/or flavor and taste components of product *sake*. Especially, two kinds of saccharification enzyme, α-amylase and glucoamylase related to the dissolution of steamed rice are the most important enzyme in the *sake* mashing process.
In the *koji* making process, the temperature, relative humidity, and initial moisture of steamed rice should be controlled to produce the optimal proportions of enzyme activities. However, the limited operation expert partially acquires how to control the culture conditions only from his/her experience. Preliminary culture experiments under various culture conditions should be carried out in order to look for the optimal culture conditions. After those experiments, the optimal operative conditions were determined. It requires lots of time and efforts.
We proposed the mathematical models on laboratory scale concerning the environmental temperature about the growth of *koji* mold and two saccharification enzymes [Shiba et al. 1997]. We also proposed an adaptive optimal control strategy for *koji* making process on laboratory scale [Shiba et al 1998]. In the present paper, we attempted to construct on-line monitoring strategy of the *koji* making process on plant scale. The procedure of development on laboratory scale was applied to this strategy, which include the estimation of mycelial content and enzyme activities and the simulation of temperature trajectory.

2 METHODS

2.1 MODEL DESCRIPTION

The mathematical models for *koji* making process of laboratory scale were proposed previously [Shiba et al. 1997]. The parameters in these models are modified in order to applied to plant scale and used. These models are defined as follows.

Carbon dioxide evolution rate (CER) is calculated from the partial pressure of oxygen and carbon dioxide, that is given by

$$CER = \left\{ \frac{F}{V} \cdot w_{CO_2} \cdot \left(\frac{CO_{2_{OUT}}}{100 - O_{2_{out}} - CO_{2_{out}}} - \frac{CO_{2_{in}}}{100 - O_{2_{in}} - CO_{2_{in}}} \right) \right\} \Big/ w_{rice} \tag{1}$$

The equation for estimation of the mycelial content in rice *koji*, that is related with mycelial content in rice *koji X* and growth rate d*X*/d*t*

$$CER = m_{CO_2/X} \cdot X + \frac{1}{Y_{X/CO_2}} \cdot \frac{\mathrm{d}X}{\mathrm{d}t} \tag{2}$$

By integrating above equation, *Xt* is given as

$$X_t = \exp\left(\int_{t_0}^{t} Y_{X/CO_2} \cdot m_{CO_2/X} \, \mathrm{d}t \right) \cdot \left\{ \int_{t_0}^{t} \exp\left(\int_{t_0}^{t} Y_{X/CO_2} \cdot m_{CO_2/X} \, \mathrm{d}t \right) \cdot Y_{X/CO_2} \cdot CER \, \mathrm{d}t + X_0 \right\} \tag{3}$$

where $Y_{X/CO2}$ is the yield coefficient of mycelial content in rice *koji* on carbon dioxide and $m_{CO2/X}$ is the maintenance coefficient. These parameters are expressed as a function of culture temperature based on the Arrhenius equation.
The logistic equation to simulate of the mycelial content in rice *koji* is given as follows [Okazaki, Sugama and Tanaka 1980].

$$\frac{\mathrm{d}X}{\mathrm{d}t} = m \cdot X = m_{max} \cdot \left(1 - \frac{X}{X_{max}} \right) \cdot X \tag{4}$$

where $ì_{max}$ and X_{max} are the maximum values of $ì$ and X, respectively. The Esener model [Esener 1981] was used to express the effect of culture temperature on these parameters.
The equations to estimate and simulate of α-amylase and glucoamylase activities were expressed as follows

$$\frac{\mathrm{d}aA}{\mathrm{d}t} = d \cdot X = d_{max} \left(1 - \frac{aA}{aA_{max}} \right) \cdot X \tag{5}$$

$$\frac{\mathrm{d}gA}{\mathrm{d}t} = s \cdot X = s_{max} \left(1 - \frac{gA}{gA_{max}} \right) \cdot X \tag{6}$$

where d_{max}, s_{max}, aA_{max}, and gA_{max} are the maximum values of d, s, aA and gA, respectively. The parameters d_{max} is expressed as a function of culture temperature based on the Esener model, and the other parameters s_{max}, aA_{max} and gA_{max} are expressed based on the Arrenius equation.

2.2 KOJI MAKING SYSTEM AND CONTROL DEVICES ON PLANT SCALE

A schematic diagram of the *koji* making system for plant scale is shown in Figure 1. In this system, the master controller that run for the basically sequence program, the gas analyzer in order to monitor the partial pressure of oxygen and carbon dioxide, the personal computer for on-line monitoring and cultivator are instrumented. Inside of the cultivator consists of heater, chiller, air inlet blower, thermometer and humiditymeter. Master controller can control the culture conditions using with these devices. It displays the process variables for control of the cultivator environment and print them out.
The on-line monitoring PC communicates with master controller via RS-422 interface, and receives the data of temperature, humidity and number of the fan round. Furthermore, the PC receives the partial pressure of oxygen and carbon dioxide from gas analyzer through A/D converter. All of process variables are recorded and displayed on CRT by the PC.

2.3 ON-LINE MONITORING SOFTWARE

Figure 2 shows the flow diagram of on-line monitoring software. The software consists of simulation and estimation part. In the simulation part, optimal temperature trajectory for maximum enzyme production at the end of cultivation time is calculated by using simple genetic algorithm (GA). Calculation conditions of GA were defined as follows. The

coding to chromosomes were used the temperature values directly. The range of the temperature was separated by 0.5 C step between 34 C and 42.5 C. The number of chromosome was assumed 21, which was generated by dividing the cultivation time of 21hours by an hour. The coded chromosomes were evaluated using eqs. (4), (5) and (6).
In the estimation part, the calculation is carried out by the following procedures. The raw monitored data received from master controller were recorded by PC every minute. The extended kalman filter [Kalman 1960; Welch and Bishop 1995] is applied to these raw data in order to remove the noise. Using the smoothed data, the mycelial content and enzyme activities are estimated by eqs. (1), (3), (5) and (6) every hour.

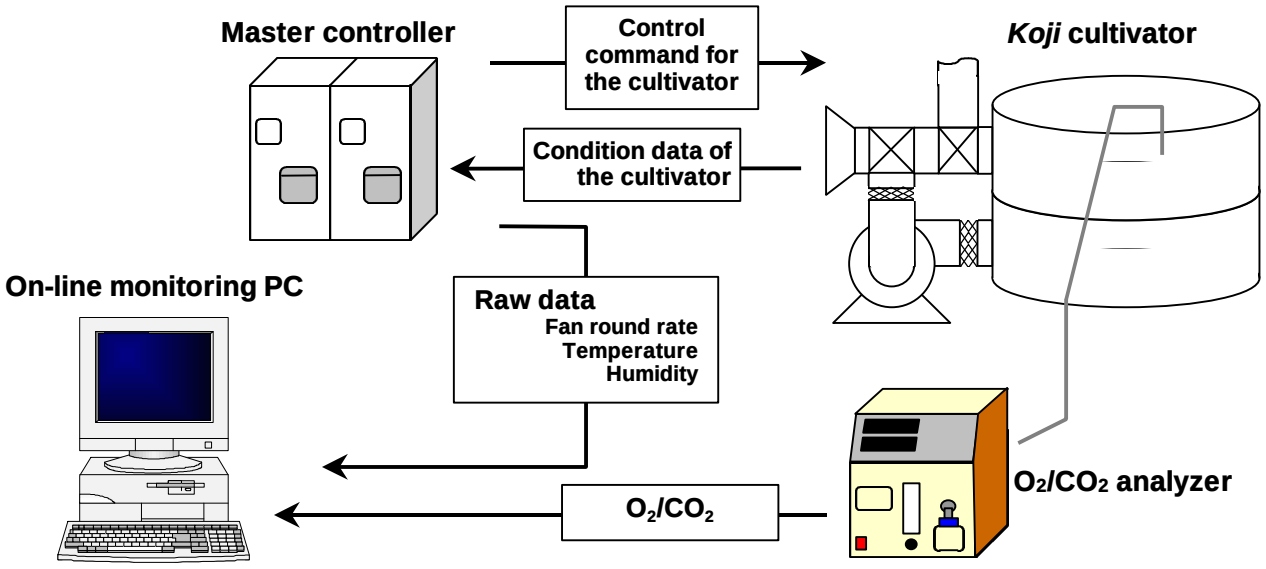

Figure 1: Schematic diagram of the *koji* making system on plant scale

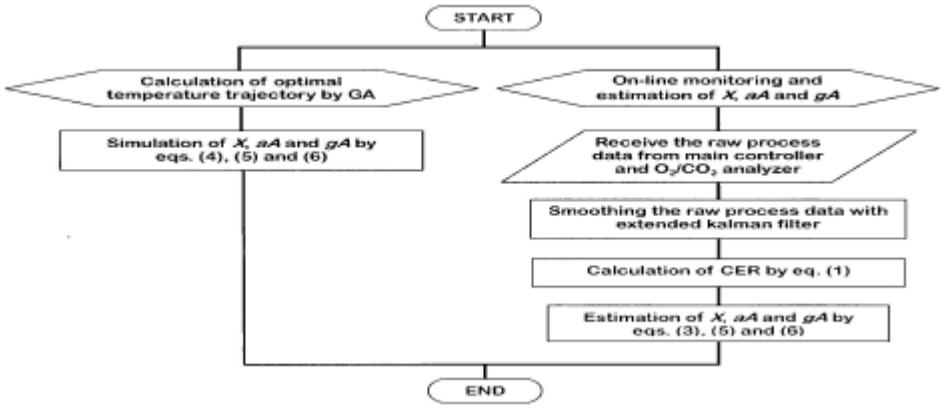

Figure 2: Flow diagram of on-line monitoring software

3 RESULTS

3.1 SIMULATION RESULTS

The simulation of the mycelial contents and two enzyme activities in rice *koji*, which was assumed that the cultivation temperature was kept constant throughout the cultivation, was carried out according to eqs. (4), (5) and (6). These simulated results were shown in Figure 3. The maximum production temperature of mycelial contents and two enzyme activities existed around 39°C. This tendency is in agreement with the result of cultivation temperature [Narahara et al. 1982].
Furthermore, several optimal temperature trajectories for maximum enzyme production simulated by GA is shown in Figure 4. The solid line indicates simulated trajectory for the maximum production of α-amylase, the dotted line is the

trajectory for maximum production of sum of α-amylase and glucoamylase and grey line is the trajectory for the maximum production of glucoamylase. Each temperature trajectories indicate gradually increase until the end of cultivation time. These tendencies are in agreement with the traditional control method of temperature and the results of gene analysis of *Aspergillus oryzae* [Ishida et al. 1998]. From these results, it is suggest that the eqs. (4), (5) and (6) can simulate the mycelial content in rice *koji* and two enzyme activities with practical accuracy.

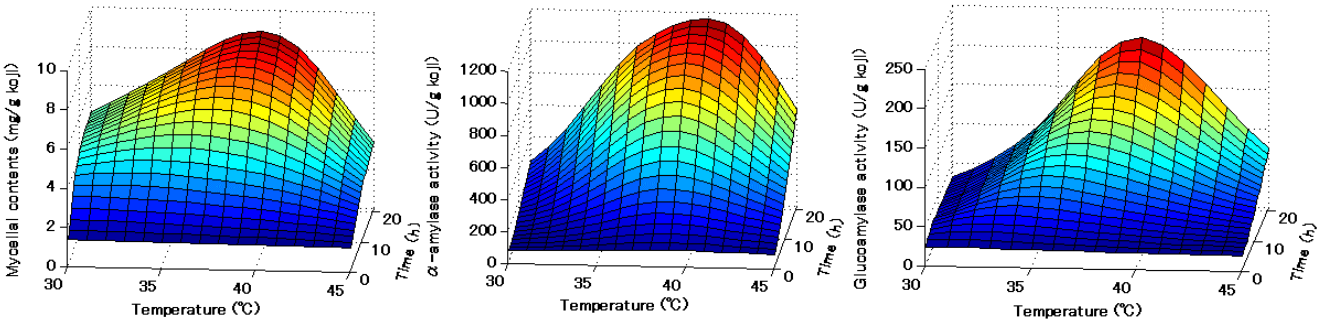

Figure 3: Simulated results of mycelial content and enzyme activities in rice *koji*

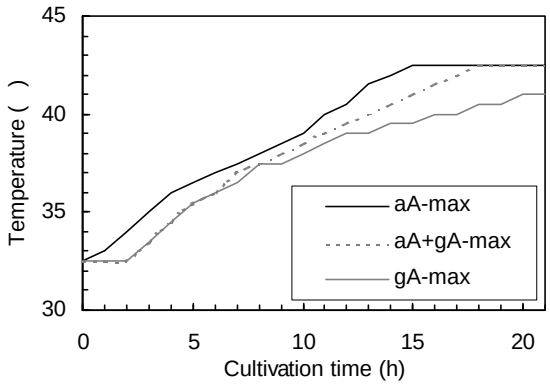

Figure 4: Several optimal temperature trajectories for maximum enzyme production simulated by GA

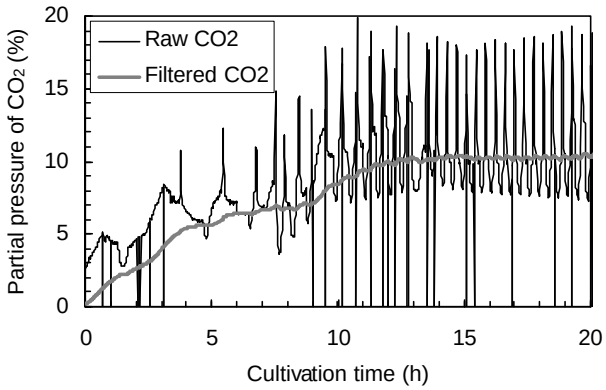

Figure 5: Comparison of partial pressure of raw CO_2 and kalman filtered CO_2 in the *koji* cultivator

3.2 ON-LINE MONITORING RESULTS

Above-mentioned the simulation tends to occur the difference between the actual cultivated results and simulated it because of the calculation parameter is only temperature. Therefore, the on-line monitoring should be estimated the components in rice *koji* by using with the temperature change and partial pressure change of oxygen and carbon dioxide during the cultivation. The following procedures were added to estimate calculation in the on-line monitoring because of slightly different from the laboratory scale in the structure of the *koji* cultivator and the conditions of the *koji* making. First, various measurement values, which were received from master controller and gas analyzer, contain the considerable noise. The extended kalman filter as the smoother was applied in order to remove the noise. Figure 5 illustrates the effect of kalman filter with the comparison between partial pressure of raw CO_2 and filtered CO_2. Next, the calculated value from fan motor round and ability of the blower substituted for the inlet air amount was assumed, because of inlet and outlet air couldn't measure in this *koji* cultivator.

Figure 6 shows the on-line monitoring results of mycelial content and enzyme activities in rice *koji*. These on-line monitoring experiments were performed by 24 batches of *koji* making, which were used with the actually operated and fixed temperature trajectory in our factory. It seems from this figure that the on-line monitoring results of mycelial content and enzyme activities in rice *koji* was different from each batches in spite of using the same culture condition of all *koji* making. Furthermore, figure 7 shows the comparison of estimated results by on-line monitoring with experimental data at the end of cultivation. Although the observed and estimated results had a distribution and some errors, it seems from this figure that the estimated results correspond relatively well with experimental results with the practical accuracy.

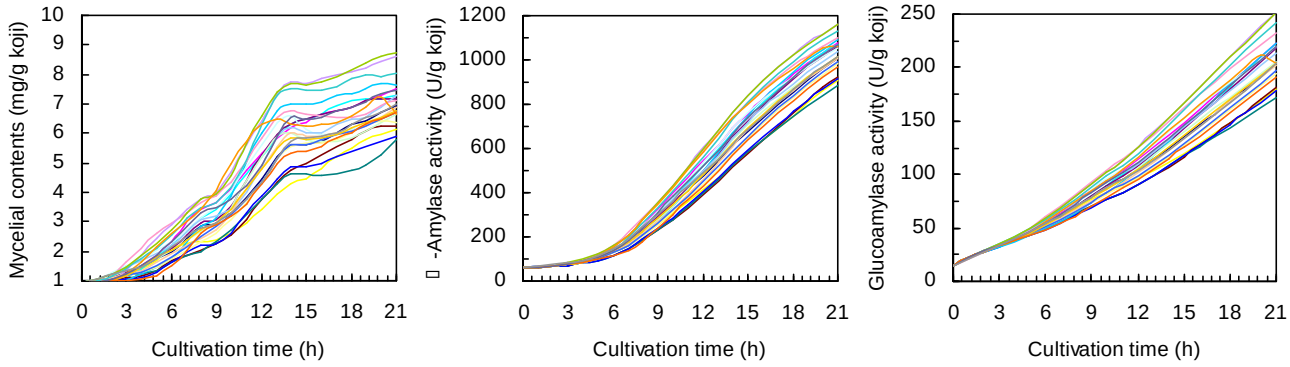

Figure 6: 24 batches of on-line monitoring results of mycelial content and enzyme activities in rice *koji*

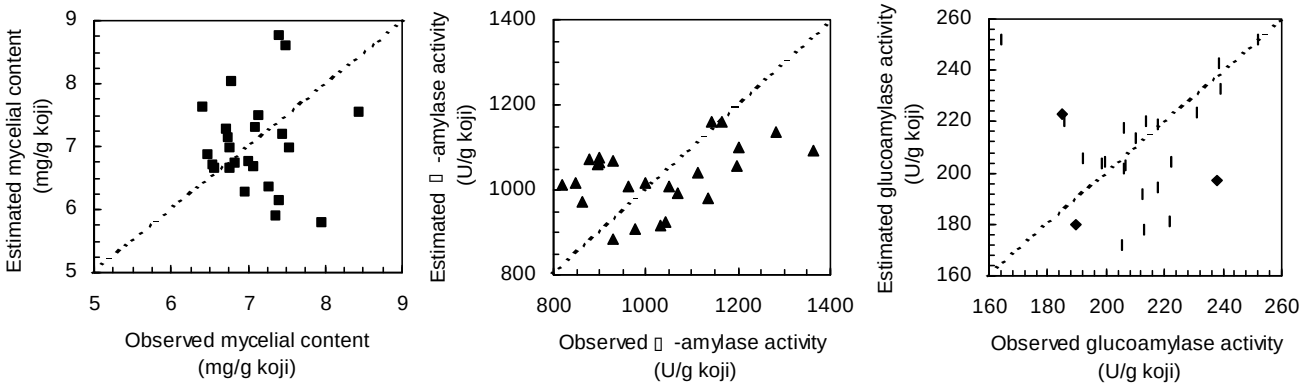

Figure 7: Comparison of estimated results by on-line monitoring with experimental data at the end of cultivation

4 CONCLUSION

In this paper, the strategy of on-line monitoring and estimation of the *koji* making process is illustrated. We improved the laboratory scale strategy by the addition of some assumptions and filtering procedure. Therefore, we could simulate several optimal temperature trajectories and estimate the mycelial content and enzyme activities in rice *koji* with the practical accuracy.

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