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Short communication

Screening of *Bacillus coagulans* strains in lignin supplemented minimal medium with high throughput turbidity measurements

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ABSTRACT

The aim of this study was to extend the options for screening and characterization of microorganism through kinetic growth parameters. In order to obtain data, automated turbidimetric measurements were accomplished to observe the response of strains of *Bacillus coagulans*. For the characterization, it was decided to examine the influence of varying concentrations of lignin with respect to bacterial growth. Different mathematical models are used for comparison: logistic, Gompertz, Baranyi and Richards and Stannard. The growth response was characterized by parameters like maximum growth rate, maximum population, and the lag time. In this short analysis we present a mathematical approach towards a comparison of different microorganisms. Furthermore, it can be demonstrated that lignin in low concentrations can have a positive influence on the growth of *B. coagulans*.

1. Introduction

Lactic acid is widely used in the food processing, cosmetics, pharmaceutical and chemical industry. Increasing prices of fossil fuels lead to increasing interests in lactic acid as a component for the production of biodegradable polymer polylactic acid [24]. There have been various attempts to produce lactic acid efficiently in bio-refineries from inexpensive feedstock such as lignocellulosic raw materials, e.g. wheat straw or hard- and soft-wood [4,16]. Lignocellulose as part of the secondary cell wall of rooted plants is one of the most abundant natural materials. It contains cellulose, hemicellulose and lignin [8]. Cellulose and hemicellulose represents polymeric carbohydrates formed from glucose, xylose, and arabinose amongst other sugars [22,16]. Therefore, lignocellulose is also the most abundant carbonate storage. After a hydrolysation process, lignocellulose can serve as a potential substrate in a biotechnological microbial fermentation for the formation of valuable products such as lactic acid [11,12,23]. Unfortunately, a non-specific chemical hydrolysis treatment, e.g. high temperature acid or alkali pre-treatment, leads to solvation of lignin and to the formation of complex sugars and inhibitory compounds such as furfural [18–21]. One way of reducing the inhibitory effect of lignin for process optimization is the reduction of the lignin concentration in the fermentation medium [7]. Another option is the use of microorganisms inhibited by lignin only to a low level, or those that can transform lignin into another compound like vanillate [10,13].

In order to improve the screening of microorganisms usable in complex and inhibitory media like lignocellulosic hydrolysates, it is necessary to characterize their growth behaviour. High throughput methods for kinetic analysis of the lignin inhibition are useful to achieve information about the lag time (λ) and the maximum growth rate (μ_m). These screening methods provide the chance to investigate the growth behaviour under different working conditions. In order to get access to lignin stable natural microorganisms (MOs) it is crucial to screen interesting bacteria in an inhibitory environment.

In this study, rapidly automated optical density (OD) measuring was applied to determine the growth response of *Bacillus coagulans* strains. The used strains are thermophilic bacteria, frequently utilized in our processes at technical scale. In studies taking place under non-sterile conditions, *B. coagulans* was shown to be the most predominant species [1]. Furthermore, the *B. coagulans* strains are known for their inhibitor tolerance [17] and their capability of utilizing pentose sugars from the hemicellulose fraction of lignocellulose [24]. These facts provide for the possibility to ferment difficult media under semi-sterile condition. Prior the fermentation in technical and pilot scale, kinetic data is

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needed to gain a basic understanding of the characteristics of the MOs for later fermentation processes and their design. Growth models are used to obtain the basic growth parameters, such as specific growth rate and duration of lag phase, in order to classify and differentiate microorganisms in respect to their behaviour towards diverse lignin concentrations. Numerous models were developed for the representation of growth curves. Widely known models are the logistic [28], Gompertz [14,25,26,28], Champbell-Richards and Stannard [28], and the model offered by József Baranyi [3]. These models have been established to fit the equations to the sigmoidal shape of a typical growth curve.

2. Materials and methods

2.1. Microbes and media

Bacillus coagulans strains were isolated from different environmental areas. They were stored in cryogenic vials (VWR, 822074ZA) at −70 °C and reactivated on MRS broth (Merck, 1.10661.0500) at 52 °C for 24 h). After reactivation the microorganisms were cultivated on slant culture tubes with MRS agar (Merck, 1.10660.0500) and stored at 4 °C for further use in inocula. The used strains were officially microbiologically characterised through the Leibniz Institute's German Collection of Microorganisms and Cell Cultures (DSMZ). Strain-1 (DSM No. 2314) was isolated from potato washing water, strain-2 (DSM ID 14-301) was isolated from chicken feed, and strain-3 (DSM ID: 14-298) was isolated from rotten foliage.

2.2. Inoculum, culture conditions

Inocula were cultivated on 60 ml MRS (Merck, 1.10661.0500) broth in shaking flasks (52 °C, 100 rpm, 15 h). These were transferred into 5 ml tubes for centrifugation (5000 rpm, 15 min, 4 °C). Centrifuged bacteria were resuspended in minimal medium for the lignin test (60 g/l D-(+)-glucose, 5 g/l yeast extract, 0.025 mol/l sodium-acetate-buffer at pH 6.0). A set of five different lignin concentrations (Sigma, 471003), (0.0, 0.2, 0.4, 0.6,and 0.8 g/l) was applied.

2.3. Optical density measurement

A Bioscreen C from Oy Growth Curves Ab Ltd., was used for the optical density experiments. Measurements were taken with a wide band filter (420–580 nm).

2.3.1. Calibration curve

For the calibration curve, Bioscreen C microarray honeycomb plates were prepared as follows: all wells, except the wells of the 10th row, were filled with 250 μl of the minimal medium. The wells of the 10th row were filled with 500 μl inocula. 250 μl were removed from these wells and transferred into the next upper row. Appropriate serial 2-fold dilutions were made up to the 2nd row, mixed by repeated syringing. The 1st row was used as the medium blank. The filled plates were placed in the Bioscreen C followed by a short measurement. The OD from the non-inoculated wells was subtracted from the growth data to minimize the effect of the signal draft. The concentrations of the colony forming units (cfu) were determined by an Abbe counting chamber. On demand, additional 10-fold dilutions were prepared for counting.

2.3.2. Optical density measuring of bacterial growth

The honeycomb plates were prepared as described in Section 2.3.1. The incubation temperature was set to 52 °C with interval shaking, changing to medium and slow intensity for 30 s prior and after OD reading. Measurements were taken every 5 min for 32 h. At least two replicate wells were used in one experiment for the determination of maximum growth rate for each lignin concentration.

2.4. Models and parameter estimation

Presupposing that the cell concentration increases in sigmoidal shape, different models were used to simulate the bacterial growth curve [3,15,27]. Although these models had the same key parameters, they differed in shape and number of parameters. A logistic, the Gompertz and the Richards and Stannard model were used in a modified and reparameterised shape as it had been offered by Zwietering et al. [28]. The Baranyi equation [2] was used as a two (μ_m,λ) and three (μ_m, λ, ν) parametrical model [5,9].

- natural logarithm of the quotient of the cell concentration (N) and minimal cell concentration (N_{min})

$$y = \ln\left(\frac{N}{N_{min}}\right) \qquad \text{with} \quad [y] \equiv 1$$

- natural logarithm of the quotient of the initial cell concentration (N_0) and minimal cell concentration (N_{min})

$$y_0 = \ln\left(\frac{N_0}{N_{min}}\right) \qquad \text{with} \quad [y_0] \equiv 1$$

- natural logarithm of the quotient of the asymptotic cell concentration (N_{max}) and the initial cell concentration (N_{min})

$$y_{max} = \ln\left(\frac{N_{max}}{N_{min}}\right) \qquad \text{with} \quad [y_{max}] \equiv 1$$

- difference of logarithmic cell concentrations

$$\Delta y = y_{max} - y_o \qquad \text{with} \quad [\Delta y] \equiv 1$$

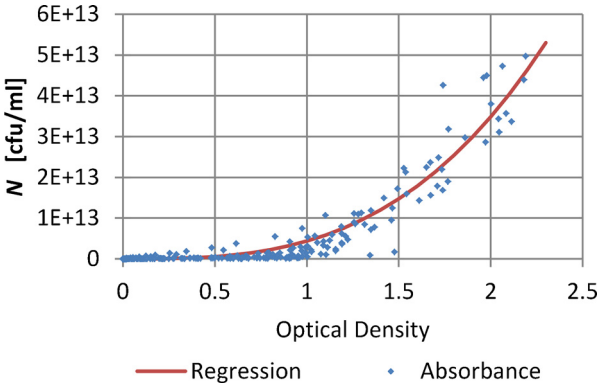

Fig. 1. Third order calibration curve between cell concentration and optical density of the different *B. coagulans* strains.