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Thermokinetic responses of the metabolic activity of *Staphylococcus lentus* cultivated in a glucose limited mineral salt medium

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Abstract Biocalorimetric experiments were performed to investigate the metabolic thermal responses of the halotolerant species *Staphylococcus lentus* in glucose limited mineral salt medium. Growth factors were optimized in both shaker flask and calorimetric experiments. A limiting value of 5 g/L glucose was found to be the optimum for *S. lentus* growth. The heat flux profiles, OUR, biomass growth, and substrate depletion profiles were compared to deduce the metabolic activity of *S. lentus*. Shifts in heat flux due to the shifts in substrate uptake and three distinct phases of growth are noticeable in heat profile curves. Respirogram (OUR) and heat profiles are seen to follow the biomass growth curve. Oxycalorific coefficient is validated with the theoretical studies and those noticed in published literature.

Keywords *Staphylococcus lentus* · Metabolic activity · Heat yield · Oxycalorific coefficient · Monod kinetics · Bioreaction calorimeter

Abbreviations and symbols

Abbreviations

LPM Liters per minute

Symbols

S	Substrate concentration (g/L)
R	Correlation coefficient
k_s	Half-saturation constant for growth (g/L)
μ	Specific growth rate (h^{-1})
μ_{max}	Maximum specific growth rate (h^{-1})
$Y_{Q/S}$	Heat yield due to substrate depletion (kJ/g)
$Y_{Q/X}$	Heat yield due to biomass growth (kJ/g)
$Y_{Q/O}$	Oxycalorific coefficient (kJ/mol)
T_r	Reactor temperature (°C)
T_j	Temperature of jacket fluid (silicone oil) (°C)
Q_{acc}	Heat accumulation (Q)
Q_{est}	Heat generation rates in reaction medium (Q)

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Introduction

Heat generation is the final by-product of all metabolic processes. Heat measurement therefore, gives an overall estimation of biological activity of the system. Control of biotechnological processes requires the use of reliable, robust sensors capable of providing real time information on the main variables of the process. Sensors for monitoring pH, oxygen, carbon dioxide, and ammonia have long been available [1]. On-line measurements of biomass can also be carried out using several sophisticated biosensors, but determination of microbial activity is usually carried out after analyzing the kinetic data obtained from the bioreactor. Since microbial growth is brought about by an enthalpy change, heat dissipation measured by calorimetry represents a suitable procedure to monitor metabolic activity. [2]. Biocalorimetry has been applied to investigate metabolic activity of heterotrophic and autotrophic nitrifying bacterial populations in sludge samples from wastewater treatment plants [3]. The RC1 developed by Mettler-Toledo (Switzerland) for chemical processes was modified for bioprocess monitoring and control. [4]. Coupling of biokinetics and bioenergetics should provide better insight more about microbial growth processes and to pinpoint the unknown anomalies occurring during the biodegradation

process [5]. Especially, monitoring the biological treatment of wastewater requires knowledge of metabolic behavior of working organisms in the presence of mixed substrates. Predicting heat-yield coefficients and correlating them with stoichiometric yields in case of mixed-substrate and -microbes systems should bring biocalorimetry to an advanced phase.

The present study focuses on studying the metabolic efficiency of a halobacterial strain *S. lentus* by means of biocalorimetry. We intend to use the organism for a detailed investigation for biological treatment of tannery dye effluent. So far, there is no complete solution available for successful and efficient treatment of azo dyes employed in the tanning and textile industries. Further calorimetric investigation for the halotolerant species *S. lentus* has not yet been reported. The objective of this study is to fill that gap.

Materials and method

Materials

All the chemicals and reagents used in this study were of AR grade manufactured and supplied by SD Fine Chemicals, Bangalore (India).

Isolation and identification of halobacterial strain

The organism used for metabolism studies *S. lentus* strain a halotolerant bacterium was isolated from marine sea water and was identified by both the biochemical and 16s rRNA sequencing method. *S. lentus* was maintained at −20 °C in a 30% glycerol stock. A loop full of glycerol stock was streaked on nutrient agar plates and incubated for 24 h at 37 °C. Well grown individual pure colonies of *S. lentus* were transferred aseptically to NB media and incubated in a shaker until it reached the exponential phase and this was used as inoculum for further studies. The well grown individual pure colonies are shown in Fig. 1.

Fig. 1 *Staphylococcus lentus*

Table 1 Composition of mineral salt medium

Chemical	Formula	Amount (g/L)
Dipotassium hydrogen phosphate	K_2HPO_4	1.73
Potassium dihydrogen phosphate	KH_2PO_4	0.68
Magnesium sulfate heptahydrate	$MgSO_4{\cdot}7H_2O$	0.1
Sodium chloride	NaCl	4
Ferrous sulfate heptahydrate	$FeSO_4{\cdot}7H_2O$	0.03
Ammonium nitrate	NH_4NO_3	1
Calcium chloride	$CaCl_2{\cdot}2H_2O$	0.02
Glucose	$C_6H_{12}O_6$	5

Media for growth studies

The composition of mineral salt medium used in the study is given in Table 1. The pH of the medium was adjusted to 7.0 by means of 0.1 N HCl solution. The medium without glucose was sterilized at 121 °C for 20 min. Glucose was sterilized separately and added to the medium.

Inoculum preparation

Preculturing of bacterial strain was done by plating the glycerol stock suspension on nutrient agar. The agar plates were incubated at 37 °C for 24 h. A single colony isolated from the plates was further streaked over agar plate to check contamination. Finally, pure culture was used to inoculate 15 mL of complex medium. After incubation in a shaker at 37 °C for 24 h, well-grown colonies with absorbance (≈ 1) were used as preculture. Bio-RC1 was inoculated with a pre-optimized seed concentration of 4% throughout this study.

Analytical methods

Bacterial growth studies

Growth of S. *lentus* was monitored spectrophotometrically and by gravimetric method. Samples withdrawn at regular time intervals from calorimeter were analyzed for growth of *S. lentus* by recording the absorbance in a UV–Spectrophotometer (Shimadzu, Kyoto, Japan UV-210 PC) at 600 nm. By gravimetric method, samples taken from the calorimeter were centrifuged at 10,000 rpm for 5 min. The supernatant was decanted and cells were separated. The harvested cells were freed from soluble salts, nutrients, and waste products by washing thrice with sterile water, and their dry weights were recorded after 24 h of drying at 80 °C.

Colony count

The serial dilution technique and plating was adopted to count the number of colonies. Various dilutions were

plated on pre-sterilized petri dishes containing nutrient agar and the plates were incubated at 37 °C for 24 h.

Glucose analysis

Glucose analysis was performed by di-nitro-salicylic (DNS) method [6]. The sample was taken from the calorimeter (BioRC1e) at periodic intervals and the cells were separated by centrifugation. The supernatant was used for the glucose analysis.

Growth studies

Shaker flask experiments

Shaker level experiments were carried out at 37 °C in an orbital shaker (Scigenic Model LE) at 100 rpm in 250-mL conical flasks (12 nos.) containing 100 mL of the medium. Samples were withdrawn for analysis at regular time intervals.

Biocalorimeter

All the calorimetric experiments were carried out in an isothermal real time reaction calorimeter (Bio RC1e-RTcal, Mettler-Toledo AG, Switzerland) composed of 2 L jacketed reactor. The system was well equipped with pH probe, turbidity probe, and inbuilt DO probe (Mettler-Toledo). The inbuilt DO probe was used to monitor the variations in dissolved oxygen (DO) value. Heat generated from the biological reaction was monitored and controlled by icontrol RC1e 4.0 software. In the isothermal mode, the reactant temperature (T_r) was maintained constant by controlling the jacket temperature (T_j). This was done by circulating at high rate (2 L s^{-1}) low-viscosity silicone oil through the reactor jacket. The jacket temperature was carefully controlled by blending oils from "hot" and "cold" circuits, via an electronically controlled metering valve. Agitation was achieved through a 4-blade Rushton turbine impeller 100 rpm for effective mixing. Aeration was performed by supplying pure oxygen from an oxygen cylinder and the flow was controlled by a Rotameter. The inlet air was sterilized through a membrane filter (0.2 μm) and sparged through the bottom of the reactor. The computer attached to the RC1e acquired the temperatures of both reactor (T_r) and jacket (T_j) and evaluated the heat production rate (heat flow). The measured heat production rate (qf in J s^{-1}) is given by Eq. 1 as

$$Q_{RTC} = A \cdot q_s 0. \quad (1)$$

where Q_{RTC} is heat flow through the part of the reaction vessel wall that is wetted by its contents (W), A is the effective heat exchange area determined by the sensors of the vertical band (m^2), and $q_s 0$ is the specific heat flow through the horizontal sensor band (W/m^2)

Q_{RTC} is real the time measurement of Q_{flow}, $Q_{accu_{est}}$ estimates Q_{accu} online based on C_p estimates

$$Q_{r_{est}} = Q_{accu} + Q_{rtc} + Q_{dos} + Q_{loss} \quad (2)$$

Inflow = Accumulation + out flow of the heat

Q_{RTC} heat flow through reactor wall (rtc: real time calorimetry), Q_{accu} is the heat storage (accumulation) by the reaction mass and through the inserts, Q_{dos}, the heat input due to dosing; power that is needed to bring the inflow from T_{dos} to T_r, Q_{loss} is the heat flow through the reactor head assembly, and $Q_{r_{est}}$ is the estimated heat generation rate in the reaction medium that are caused by all biochemical reaction running simultaneously and through phase changes such as mixing.

The UA factor was determined using $Q_{RTC,}$ which is real time measurement of Q_{flow} by the inbuilt sensors bands, i.e., (i) horizontal sensor band placed below the level of the reactor contents and (ii) vertical sensor band placed well above the level of the reactor contents. The sensors bands are thermocouples which are integrated in polymer matrix. Heat flow across the matrix leads to the voltage signal, and the measured voltage signal is converted into heat flow (W).

Oxygen uptake rate

Dissolved oxygen (DO) sensor in built in the biocalorimeter was initially calibrated in the autoclaved growth medium by purging the medium with pure oxygen and nitrogen. A minimum DO value of 2 ppm was always maintained to ensure aerobic conditions throughout the reaction. Oxygen uptake rate (OUR) was calculated by the dynamic method [7].

Bioenergetics studies

Though power-time profiling depicts the metabolic shifts occurring during a growth process, quantitative information on relative consumption of substrates $Y_{Q/S}$ (kJ heat evolved per gram of glucose consumed), energy changes associated with biomass growth $Y_{Q/X}$ (kJ heat evolved per gram cell dry weight formed) and oxycalorific coefficient $Y_{Q/O}$(heat generated in kJ/mol of oxygen consumed) are evaluated by integrating cumulative heat production values by integrating the power-time curve [8].

Growth kinetics

Growth kinetics, the relationship between specific growth rate and the concentration of the substrate, is one of the basic information needed in biological system. It is a

mathematical description of the metabolic process of microorganisms. Currently, the concepts in microbial kinetics were dominated by the relatively simple empirical model proposed by Monod. Monod model relates the growth rate to the concentration of a single growth-controlling substrate [$\mu = f(S)$] via two parameters, the maximum specific growth rate and substrate affinity constant

$$\mu = \mu_{\max}\frac{S}{S + k_S}. \quad (3)$$

Substrate depletion kinetics

Single substrate driven growth of the biomass is governed by the following equations:

$$\frac{dX}{dt} = \mu(S)X, \quad X_0 = X\,@\,t = 0 \quad (4)$$

$$\frac{dS}{dt} = -\frac{\mu(S)}{Y_{x/s}}X, \quad s_0 = s\,@\,t = 0 \quad (5)$$

$$\mu = \mu_{\max}\frac{s}{s + k_S}. \quad (6)$$

Where X is the biomass concentration, S is the substrate concentration, X_0 is the initial biomass concentration, S_0 is the initial substrate concentration, μ is the microbial growth rate, and $Y_{x/s}$ is the yield coefficient. Various mathematical models are available to correlate single substrate dependency of microbial growth rate μ on substrate concentrations, [9], among which the most commonly used model is the Monod kinetics given by Eq. 6.

Here, $\mu_{\max}$ is the maximum specific growth rate, and k_s is substrate affinity constant for growth.

Results and discussions

The thermodynamic responses for the cultivation of *S. lentus* were carried out in a bioreaction calorimeter. Before the biocalorimetric studies, optimization of process variables such as temperature, pH, inoculum concentration, and carbon source were carried out in shaker flask experiments. On optimizing the above parameters, attention is focused in biocalorimetric experimentation where in few more parameters such as aeration rate and agitation rate were optimized. Finally, biocalorimetric experimentation on the cultivation of *S. lentus* was carried out with optimized parameters of shaker flask and biocalorimeter. The results are discussed in the same fashion below

Optimization of process parameters at shaker level

The bacteria *S. lentus* is a gram −ve species belonging to the halotolerant group. During active growth phase the organism developed coccus with white pigmentation as shown in Fig. 1. Before thermokinetic studies, shaker lever experiments were performed to determine the suitable substrate for growth. Several experiments were performed by changing the concentrations of inoculum, pH, temperature, and carbon source. The optimized conditions for maximum growth are arrived from the shaker level experiments and results are summarized in Table 2. In this manuscript, only the effect of carbon source on cultivation is discussed in detail.

The effect of carbon sources on growth of *S. lentus* are presented in Figs. 2, 3, and 4. The different carbon sources studied were glucose, fructose, sucrose, and lactose, respectively, and their concentrations were varied from 0.5 to 6 g/L. From the results shown in Fig. 2, 3, and 4, it can be inferred that increase in carbon source concentration, increased the growth level considerably. The peak growth rates were seen at 30 h of inoculum addition. A maximum growth rate is observed at 5 g/L of glucose concentration. On further increasing the concentrations (results not shown), the growth rates were found to decrease. Therefore, for further studies a limiting glucose concentration of 5 g/L was chosen.

Table 2 Optimized parameters of metabolic studies of glucose-limited (5%) mineral salt medium

No.	Optimized parameters	Conditions
1	pH	7
2	Temperature	37 °C
3	Inoculum	4%
4	Aeration	1 lpm
5	Agitation	100 rpm

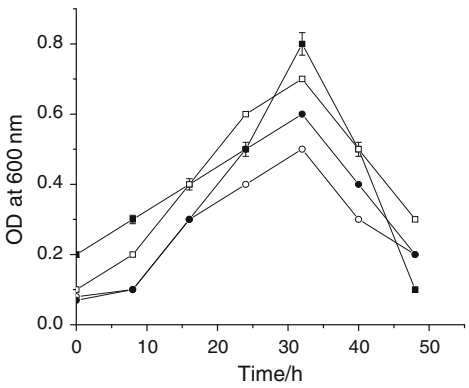

Fig. 2 Growth pattern of *S. lentus* in the presence of various carbon sources at a concentration of 0.5 g/L (glucose (*filled square*), fructose (*empty square*), sucrose (*filled circle*), and lactose (*empty circle*))

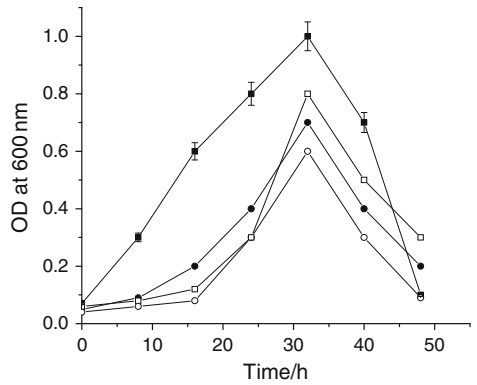

Fig. 3 Growth pattern of *S. lentus* in the presence of various carbon sources at a concentration of 1.0 g/L (glucose (*filled square*), fructose (*empty square*), sucrose (*filled circle*), and lactose (*empty circle*))

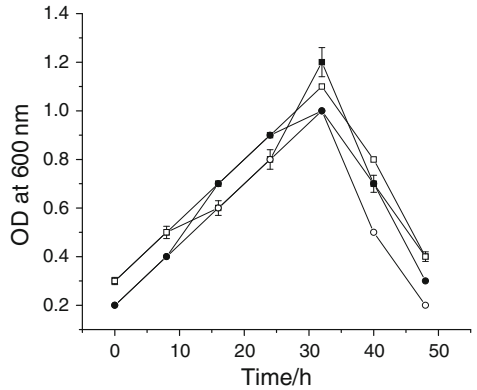

Fig. 4 Growth pattern of *S. lentus* in the presence of various carbons sources at a concentration of 5.0 g/L (glucose (*filled square*), fructose (*empty square*), sucrose (*filled circle*), and lactose (*empty circle*))

Kinetics of biomass growth and substrate depletion based on Monod model

For the bacteria under study, the glucose concentration is found to be growth limiting at a concentration of 5 g/L. Analysis of the experimental data showed that biomass growth is exponentially dependent on growth concentration. Hence Monod model was taken into consideration in this study to determine μ_{max} and k_s. It may be noted that the governing equation (Eq. 6) for biological process are applicable only for growth phase of biomass and do not take into account either lag or death phase that occurs in real system.

The typical Monod model curve obtained for the biomass growth of *S. lentus* is shown in Fig. 5 and the biokinetic constants are summarized in Table 3. For each glucose concentration μ_{max} is determined by numerically evaluating dx/dt from the experimental data and finding the maximum of (dx/dt)/x, since $\mu_{max} = \max((dx/dt)/x)$ according to Eq. 2. Once μ_{max} is determined, k is found out. Table 3 lists the biokinetic parameters for five

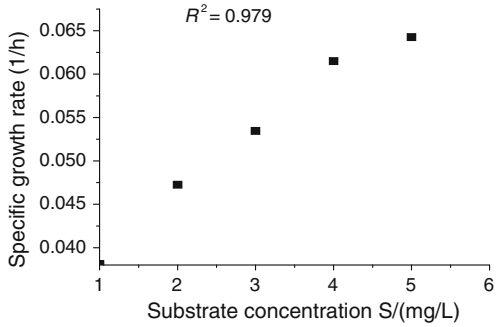

Fig. 5 Monod kinetics for *S. lentus* cultivated in glucose-limited (5%) mineral salt medium

Table 3 Monad kinetic constants for the growth of *S. lentus* at different glucose concentrations

S, g/L	k_s (g/L)	μ_{max} (h^{-1})	μ (h^{-1})	R^2
1	3.54	0.1732	0.038	0.9705
2	1.55	0.084	0.047	0.9785
3	5.92	0.174	0.058	0.9836
4	2.65	0.101	0.061	0.9798
5	2.94	0.102	0.064	0.9686

different glucose concentrations. It may be noted that the parameters are within the range reported in the literature [10].

Biocalorimetric studies: measurement of metabolic heat

Biocalorimetric studies for the growth of *S. lentus* were carried out in batch mode (1 L). Batch runs were performed in mineral salt medium under optimized conditions determined from the shaker level experiments (Table 2). Aeration and agitation rates were optimized at the biocalorimetry. Turbine type impeller was employed for effective mixing and oxygen transfer. The agitation rates studied were 50, 100, and 150 rpm. The increase in agitation rate resulted in increased growth up to 100 rpm. The heat added due to stirring power was systematically eliminated using baseline heat values. In our experiments, increase in stirring rate above 100 rpm resulted in excessive foaming and fall in growth of *S. lentus*. Therefore, the stirring rate was fixed at 100 rpm for the biocalorimetric experiment for the cultivation of the organism under study. The power-time curve for the metabolic activity of *S. lentus* is shown in Fig. 6.

Similarly, aeration studies were conducted with pure oxygen stored in oxygen cylinder (120 kg capacity, normally used in medical emergencies). Aeration rate was varied through the three different ranges (0.5, 1.0, and 1.5 lpm). It was observed that increase in aeration rate

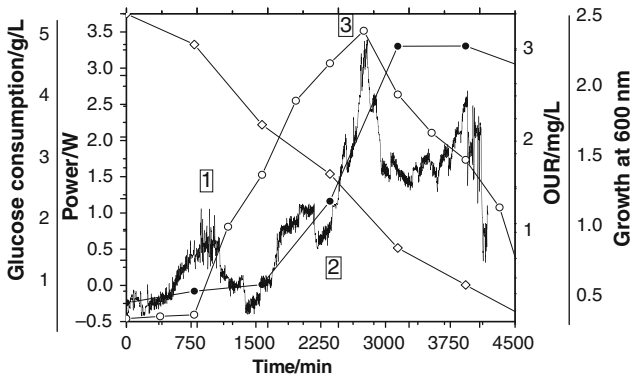

Fig. 6 Thermodynamic responses of *S. lentus* cultivated in glucose limited mineral salt medium: comparative plot of heat production rate (*solid line*), oxygen uptake rate (*empty circle*), bacterial growth (*filled circle*), and glucose consumption (*empty diamond*)

above 1 lpm resulted in excessive foaming and caused severe turbulence, which disturbed the measurement of metabolic heat production. Therefore, the aeration rate was fixed at 1 lpm for biocalorimetric studies. A comparative plot of heat flux, biomass, glucose uptake, and OUR for the growth of *S. lentus* under optimized conditions in bio reaction calorimeter on glucose limited (5 g/L) mineral salt medium is projected in Fig. 6. Figure 6 shows three distinct phases of growth. Power-time curve indicated the current activity of the cell culture, changes in slopes of the curve showed the effects of limiting factors on metabolic activity of *S. lentus*. On the basis of the power-time curve the behavior of the culture was deduced. Phase I in Fig. 6 (0–1600 min) comprises both lag and earlier exponential phase, where the culture adapted to MS media and effectively utilized the glucose. This indicates that *S. lentus* adapted to MS media slowly and rapidly metabolizes glucose (about 40%) present in the media. It can be seen that there is no marked increase in the growth of *S. lentus* in Phase I. Maximum heat flux (0.5–0.8 W) observed in Phase I further suggests that glucose consumption by *S. lentus* contributes the major heat release. This clearly suggests that the bacterium is more active in depleting the energy rich glucose, rather than in cell multiplication. The drop in heat flux before a rise after phase I may be related to the relaxation process of the bacterium.

A sudden rise in heat profile in phase II (Fig. 6) corresponds to the initiation of exponential growth of *S. lentus*. In phase II (1600–3220 min) the organism continues to use the sole carbon source glucose for cell maturity and multiplication. During Phase II, a maximum of 3.5 W energy release is recorded. It is also clearly seen that biomass concentration increases exponentially until the glucose concentration depletes to 0.2 g/L. In phase III, although a small quantity of the glucose is available, a declining trend in heat release, biomass, and OUR confirms that the organism is reaching the endogenous death phase. A total metabolic heat of production of 3.9 kJ g^{-1} was observed during the cultivation of *S. lentus* in a biocalorimeter.

The OUR profiles in Fig. 6, very closely follows the power-time curve and corroborated to the previously reported finding that both power time and OUR profiles yielded the same information during the bacterial cell cultivation [11–14]. Our study further substantiated the application of calorimetry for inline monitoring of the bacterial systems.

In order to determine the energy efficient process in a bacterial metabolism (anabolic or catabolic), to observe the substrate shifts, and to validate stoichiometry, it is necessary to estimate the yield coefficients. Heat yield due to growth (anabolic) of *S. lentus* ($Y_{Q/X}$) was calculated by finding the average value of slope from a plot between cumulative heat and cell dry weight of *S. lentus.* Similarly, heat yield (catabolic) due to glucose consumption ($Y_{Q/S}$) values was calculated by finding the value of slope of a plot between cumulative heat and glucose uptake. Heat yield due to growth ($Y_{Q/X}$) was found to be 14.42 kJ/g, and heat yield value due to glucose uptake was ($Y_{Q/S}$) 9.6 kJ/g. Both the heat yield values were found to be much lower than the standard values of single C-substrates for pure aerobic processes [15]. Determination of heat yield values for the growth of *S. lentus* in glucose limited MS medium has revealed that the organism in addition to degrade the energy rich source glucose may also be involved in degrading some of the salts like ammonium nitrate present in the MS medium. Further studies are required to confirm the diauxic behavior which is currently in progress in our laboratory.

Oxycalorific coefficient

The oxycalorific coefficient (heat generated in kJ/mol of oxygen consumed) is determined from the slopes of the heat generated and cumulative oxygen uptake (shown in Table 4). $Y_{Q/O}$ for *S. lentus* grown at glucose limited MS media is found to be 427 ± 10 kJ/mol and correlates well within the values reported in the literature [16]. *S. lentus* thus confirmed to be an obligate aerobic bacteria and the resultant oxycalorific value confirms its aerobic efficiency.

Table 4 Heat yields due to biomass growth, substrate consumption and oxygen uptake by *Staphylococcus lentus*

S.no	Activity	Heat yields
1	Biomass growth, $Y_{Q/X}$ (kJ/g)	14.42
2	Substrate consumption, $Y_{Q/S}$ (kJ/g)	9.6
3	Oxygen uptake, $Y_{Q/O}$ (kJ/mol)	427

Conclusions

Staphylococcus lentus was successfully cultivated in a glucose limited MS medium in a bioreaction calorimeter under aerobic conditions. From the power-time curve, three phases of the growth can be distinctly seen. Heat time profiles found to follow the growth profiles. Heat yield coefficients were estimated for the growth of *S. lentus* and the values were found to be low as compared with the standard yield values for single substrate systems reported in the literature.

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References

1. Senthilkumar S, Surianarayanan M, Sushleela R. Biocalorimetric studies of the metabolic activity of *Pseudomonas aeroginasa* aerobically grown in glucose limited complex growth medium. Biosci Biotechnol Biochem. 2008;72:936–42.
2. Higuera-Guisset J, Rodriguez-Viejo J. Calorimetry of microbial growth using a thermopile based microreactor. Thermochimica Acta. 2005;427:187–91.
3. Ligthart J, Daverio E. Application of calorimetric measurements for biokinetic characterization of nitrifying population in activated sludge. Water Res. 2003;37:2723–31.
4. Vonstockar U, Duboc P, Menoud L, Marison IW. On-line calorimetry as a technique for process monitoring and control in biotechnology. Thermochimica Acta. 1997;300:225–36.
5. Liu JS, Marison IW, Von Stockar U. Microbial growth by a net heat up-take: a calorimetric and thermodynamic study on acetotrophic methanogenesis by Methanosarcina bakeri. Biotechnol Bioeng. 2001;75:170–80.
6. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426–8.
7. Singh V. On-line measurement of oxygen uptake in cell culture using the dynamic method. Biotechnol Bioeng. 1996;52:443–8.
8. Surianarayanan M, Balaji D, Senthilkumar S, Asit Baran M. Batch kinetics on growth of salt tolerant *Pseudomonas aeroginasa* secreting in a biocalorimeter. Biotechnol Bioprocess Eng. 2010;15:670–5.
9. Bailey J, Ollis F. Biochemical engineering fundamentals. New York: McGraw-Hill; 1986. p. 383–6.
10. Robinson JA, Tiedj JM. Nonlinear estimation of Monod growth kinetic parameters from a single substrate depletion curve. Appl Environ Microbial. 1983;45:1453–8.
11. Senthilkumar S, Surianarayanan M, Swaminathan G. Biocalorimetric and respirometric studies on biological treatment of tannery saline wastewater. Appl Microb Biotechnol. 2008;78:249–55.
12. Senthilkumar S, Surianaryanan M, Madhu B. Biocalorimetric and respirometric studies on metabolic activity of aerobically grown batch culture of *Pseudomonas aeruginosa*. Biotechnol Bioprocess Eng. 2007;12:340–7.
13. Birou B, Marison IW, Von stockar U. Calorimetric investigation of aerobic fermentations. Biotechnol Bioeng. 1987;30:650–60.
14. Surianarayanan M, Senthilkumar S. Bioenergetics studies on aerobic growth of *Pseudomonas aeroginasa* in a single substrate media. J Chem Technol Biotechnol. 2009;84:1234–9.
15. Von Stockar U, Marison IW. The use of calorimetry in biotechnology. Adv Biochem Eng Biotechnol. 1989;40:93–136.
16. Cooney CL, Wang D, Mateles RI. Biotechnol Bioeng. 1968;11:269.