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Heat flux as an on-line indicator of metabolic activity in pilot scale bioreactor during the production of *Bacillus thuringiensis* var. *galleriae*-based biopesticides

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Abstract

The effective biotechnological process development depends on the system for monitoring the activity of the culture during cultivation. The feasibility of monitoring the metabolic flux during the cultivation of *Bacillus thuringiensis* var. *galleriae* (*Btg*) in pilot scale bioreactor was studied. All possible sources of heat generation and transfer in the system were accounted to determine the metabolic heat flux caused by the culture alone. Successful demonstration of the heat measurements has revealed the scale-up characteristics of microbial process based on the heat generation data. In addition, influence of complex media sources on heat flux, sporulation and insecticidal crystal protein (ICP) synthesis was understood. Nature of the microbial process has necessitated fed-batch cultivation with glucose solution feeding in proportions to the metabolic heat production rate and subsequent optimization of the feeding glucose solution concentration had resulted in an enhanced ICP concentration. As a significant outcome, heat flux into the cooling water for the maintenance of bioreactor temperature was found to be in definite correlation with the metabolic heat production rate. This allowed an opportunity for understanding the metabolic status of the culture with continuous on-line monitoring of the temperature profile of the cooling water in industrial scale fermentation with cost effectiveness. Continuous assessment of cultures through heat flux could be exploited by enhancing the desired metabolic process(s) in industrial scale fermentation.

Keywords: Biopesticides; *Bacillus thuringiensis* var. *galleriae*; Heat flux; Bioprocess optimization; On-line monitoring

1. Introduction

The demand of biopesticides to combat the pest population in the agricultural sector has increased tremendously in recent years. The consciousness over the environment and awareness of deleterious impact of chemical pesticides on the ecological system has led to enhanced application of biological-based pesticides. With the agricultural sector in focus, cost involved in the production of these biopesticides has to be drastically reduced to increase its application. The ICPs synthesized at the onset of sporulation by *Bacillus thuringiensis* var. *galleriae* (*Btg*) are toxic to lepidopteran pests leading to its role as a biopesticide [1].

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Biological processes are regarded as a reaction network with various interdependent biochemical reactions. The net heat released during growth represents the sum of many enzymatic reactions involved. The heat dissipation and its measurement in association with microbial metabolic activities during growth and product synthesis are well documented [2]. In particular, this has to be realized in terms of overall identifiable macroscopic phenomenon. Hence, an understanding of metabolic processes with heat generation characteristics could be employed for enhancing a desired process. Thermodynamic studies indicate that there is a strict correlation between heat production rate and oxygen requirement for aerobic growth [3]. Limiting conditions in the growing cultures could also be identified by monitoring the heat signal as reported by Marison and Stockar [4] and Ishikawa et al. [5].

With different approaches to heat measurement, heat flux calorimetry is suitable for bioprocess monitoring [4]. In this, random heat losses can be neglected and systematic contributions to heat generation or removal can be determined. Further in the heat flux calorimeter, bioreactor temperature controller needs appropriate tuning [6] for satisfactory performance. Due to its fast response characteristic, heat measurements during fermentation is important for on-line process monitoring over conventional methods of exhaust gas analysis. Further, use of exhaust gas analysis to estimate the state of the organism is not realistic in large-scale fermentations due to the gas hold up in the culture broth and in the head space caused by non-ideal fluid mixing of industrial fermentors. Besides, heat measurements in combination with mass and energy balances not only provide redundancy check on the parameters estimated, but also facilitate the determination of the stoichiometry of the growth reaction [7,8] and product formation [9]. Under well defined conditions, biocalorimetry can also be used as an tool for the estimation of biomass [10,11].

Heat flux measurements were extensively employed in bioprocess modeling, optimization and control in bench-scale calorimeter. Heat measurements were applied in the medium optimization studies [12,13]. Fed-batch cultivations were carried out in bench-scale bioreactors by several authors based on the heat production rate [14–18]. The fast response of the heat signal in comparison with other process parameters has resulted in its application in understanding the dynamic responses of microbial cultures in chemostat [15,19]. In addition, correlation of metabolic heat generation by microbial growth with other process parameters such as oxygen uptake was studied by several authors [3,20,21]. In fact, literature reports on heat measurements in large-scale fermentation are relatively less and the exploitation of the heat flux measurements in industrial application with commercial feasibility has been scarce. Hence, heat measurement was employed to monitor the metabolic status on-line towards developing cost effective processes in large-scale biopesticide production with complex insoluble medium.

2. Materials and methods

2.1. Microorganism

Btg was maintained as spore stocks and on nutrient broth (NB) agar plates at 4 °C. Inoculum was developed from a single colony of *Btg* maintained in NB agar. Exponentially grown culture in 10 l NB medium in a 20 l bioreactor (Bioengineering AG, Wald, Switzerland) was used as inoculum for the pilot scale bioreactor. Temperature, agitation and aeration were maintained at 30 °C, 250 rpm and 1 vvm, respectively in the 20 l bioreactor.

2.2. Media and feed solutions

NB medium: Peptone 10 (g/l); yeast extract 3; NaCl 5.0 (pH 7.2). Glucose–yeast (GY) extract medium [22]. Complex medium: soybean meal 16.0 (g/l); glucose 26.0; corn steep liquor 7.0; NaCl 5.0 [23]. Feed solutions for fed-batch cultivation were 10 l of glucose solution at two different concentrations of 104 g/l (4×) and 650 g/l (25×). Initial glucose concentration in the fed-batch cultivation was 13 g/l.

2.3. Cultivation conditions

Cultivations were carried out in pilot scale bioreactor (300 l; Bioengineering AG, Wald, Switzerland) of 200 l working volume with all available accessories

and control systems. This bioreactor has a water jacket with heat exchanger and circulation pump to maintain the temperature. This pilot scale bioreactor was modified for heat measurement studies. Bioreactor temperature was maintained at 30 °C using the water at low temperature. Aeration was carried out at controlled conditions with inlet air pressure at 2.0 bar. Foaming was controlled by the addition of polypropylene glycol by the level controller. Controllers for pH (Bioengineering AG, Wald, Switzerland) and agitation (SIEMENS SIMOREG) were used with pH controlled at 7.0 during cultivation.

Hygrometer (ROTRONIC AG) was installed in the air inlet and outlet as modification for measuring the temperature and humidity of air. Wattmeter for measuring the power consumed by stirrer, power source for temperature measurements and control unit for calibration heater were fabricated at EPFL, Lausanne, Switzerland. Further, modifications in cooling water jacket for installing temperature probes and flowmeter (Brooks Instrument, FISHER-ROSEMOUNT) were done at EPFL, Lausanne, Switzerland. Temperature measuring element (RTD, Pt100) and its transmitter (RUEGER 82000) were used in the heat measurement studies. Exhaust gas analyzer (Bioengineering AG, Wald, Switzerland) containing CO_2 analyzer (BINOS 100) was used for analyzing the CO_2 composition in exhaust gas from the fermentor. Peristaltic pump (Matson Warlow 503U) interfaced with the computer was used for feeding the external sterile solutions during fed-batch operation.

2.4. Data acquisition and control

Data acquisition of the parameters (25 in numbers) were carried out through the DAQ channel board (AT-MIO-64E-3, National Instruments) and interfacing hardware (NB-MID-32X, National Instruments) by the computer (Dell OptiPlex GX1). The entire program for data acquisition, calibration, calculation, storage and display were developed in the software, LabVIEW (National Instruments) as separate modules with the supervisory control of main (front) module. These on-line acquired values were continuously averaged (moving average) and used in the calculations involved in the program for the estimation of metabolic heat production rate.

2.5. Calibration experiments

Cascade control system with bioreactor and cooling water jacket temperature as input variables was developed in LabVIEW for the accurate control of bioreactor temperature. Total heat flux model across the pilot scale bioreactor was used for the estimation of metabolic heat signal after incorporating all the heat generation and loss terms in the model [4]. Specific heat capacity of the bioreactor and its contents (mC_P), global heat transfer coefficient (UA), heat dissipated into the medium by the stirrer in relationship with agitation and aeration (Q_{sc}) were determined by providing constant heat input (Q_h) from the calibration heater. Heat lost through the air (Q_g) was estimated by employing equations from Owens [24] with on-line measurement of temperature and humidity of the air.

2.6. Off-line analytical methods

Biomass concentration was measured from the optical density was measured using spectrophotometer (Hitachi U2000). Cell dry weight was measured by filtering 10 cm^3 of the culture broth through pre-weighed membrane (Zetapor, 0.22 μm) and the added weight was measured after drying the membrane. This cell dry weight measurement was correlated with the optical density measurement of the culture broth. The off-line analysis of residual glucose concentration was carried out in the biochemistry analyzer (YSI Inc.). The samples were centrifuged at 12 000 rpm for 10 min at 4 °C and analyzed. Spore count was estimated from the number of colonies obtained in the NB agar on incubation from the diluted heat-treated sample. The protein estimation was carried out according to Lowry et al. in 1951. The ICP concentration was estimated using enzyme linked immunosorbent assay (ELISA)-based quantification method [25].

3. Results and discussions

The extensive information from heat measurements on the metabolic activity could be effectively utilized for increasing the efficiency of metabolic processes in terms of conversion of substrate into biomass and

product(s). Metabolic heat measurement in a bench-scale calorimeter and its interpretation towards metabolic processes has already been reported [2]. The commercial feasibility of bioprocesses necessitated an efficient performance of microbial processes in large-scale fermentors. Hence, a study towards continuous understanding of metabolic activities in large-scale fermentor would facilitate in improvements of bioprocesses at production level. Measurement of heat production rate in the pilot scale bioreactor during microbial cultivation was carried out after the necessary modifications in the setup.

3.1. Batch cultivation with minimal medium

The availability of data from bench-scale bioreactor with GY medium [26] has enabled the scale-up characteristics of associated heat generation in growth and product synthesis to be determined. During the cultivation, appreciable amount of heat could be measured due to the near adiabatic operation of the pilot scale bioreactor with less surface area/volume. The metabolic heat production rate (Q_p) and CO_2 composition in the exhaust gas during the batch cultivation of *Btg* in pilot scale bioreactor with GY extract medium is shown in Fig. 1 with the maximum heat flux of 341.64 W. The correlation of heat production rate with macroscopic indication of the growth of the culture was successful. The observation of heat and CO_2 profile has lead to the inference of having constant ratio during the exponential growth indicating the culture grows under balanced condition of carbon flux through the metabolic network. The analysis of cumulative heat produced indicated the possibility of there being two different heat yields as reported in the cultivation of *E. coli* on GY extract medium by Birou et al. [3]. The first phase has a relatively high heat yield ($Y_{O/X} = 3.416$ kJ/g) due to the influence of the unconsumed substrates and metabolites from the inoculum with the biomass yield of $Y_{X/S} = 0.0383$ g/g. In the second phase, it was observed to be 1.5199 kJ/g and 2.375 g/g, respectively. This shows the pronounced effect of $Y_{X/S}$ on heat yield as it was reported that the nature of the carbon and energy substrate and its enthalpy content has a profound effect on the heat released by the microbial culture [2]. This cultivation of *Btg* on GY medium has resulted in spore concentration of 2.5×10^8 spores/cm^3 with ICP concentration of 1.0068 mg/cm^3.

The analysis of the data on maximum heat flux provided additional details regarding the relationship between the cell density and metabolic heat produced. In the pilot scale reactor at the point of maximum heat flux, heat production rate in terms of the cell density was observed to be 5.098 W/g of biomass. In the bench-scale calorimeter [26], maximum heat flux of 6.27 W was observed at 3.426 h of the cultivation with 5.421 W/g of biomass. In addition, specific growth rate of the culture was estimated to be 0.3583 in pilot scale compared with 0.2293 in bench-scale calorimeter.

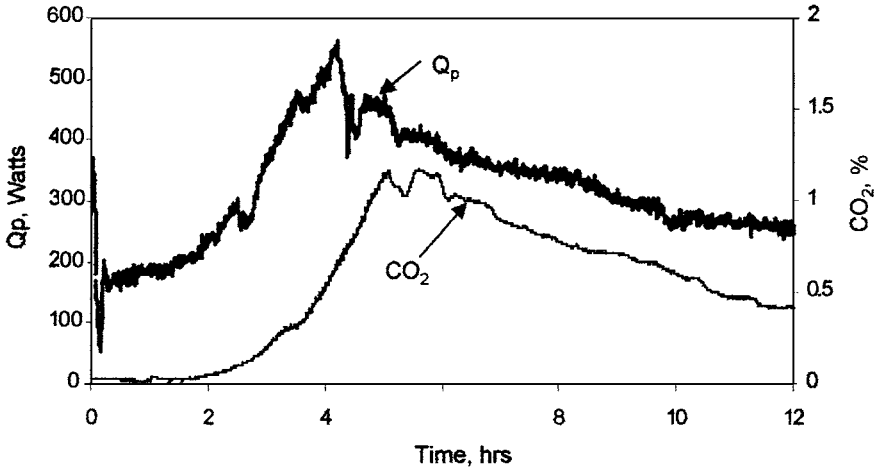

Fig. 1. Profiles of metabolic heat production rate (Q_p) and CO_2 composition in the exhaust gas during the batch cultivation of *Btg* with GY medium in pilot scale bioreactor. The maximum metabolic heat flux was observed to be 341.64 W at 4.2 h of the cultivation.

This difference in specific growth rate could be due to the dependency of the specific growth rate on the medium constituents and its concentration as 5× concentrated medium was used in the bench-scale calorimeter. In the pilot scale bioreactor, point of maximum heat flux was observed to occur later in comparison with the bench-scale calorimeter during the cultivation. Even with relatively higher growth rates in the pilot scale reactor, the observed difference in the point of maximum heat flux could be attributed to the role of residual medium unutilized in the inoculum. Also at the point of maximum heat flux, no appreciable difference in cell density was observed between the two scales of cultivation. This indicated high carbon source recovery towards biomass as growth in pilot scale bioreactor with respect to high concentration of glucose used in the bench-scale calorimeter. The net heat produced from the biological network of reactions reflecting in growth and product synthesis in macroscopic scale could depend on the nature and quantity of substrate utilized under balanced conditions as reported earlier. This indicated the feasibility of scaling up the biopesticide production process in terms of volumetric heat production and heat yield based on the nature of the substrate used for cultivation. Earlier, contribution of the heat of dilution and neutralization towards the metabolic heat flux on addition of NaOH for pH control was observed to be insignificant from the simulated experiments (data not shown).

3.2. Batch cultivation with complex industrial medium

In order to appreciate the role of on-line heat monitoring in bioprocess development, industrial complex medium containing soybean meal, etc. was employed for the cultivation of *Btg*. This on-line heat measurement could be employed as an alternative to monitor cell dry weight measurements with medium containing insolubles. The impact of medium containing soybean meal towards enhanced synthesis and toxicity of insecticidal crystal proteins has been reported [24]. Batch cultivation was carried out in the pilot scale bioreactor initially to understand the growth kinetics/characteristics of *Btg* in complex medium with reference to the heat released. The inferences obtained from these studies could be employed to optimize the metabolic process of growth and synthesis of ICP by developing appropriate feeding strategies in fed-batch cultivation of *Btg* with the above complex medium.

The heat production rate (Q_p) and CO_2 composition in the exhaust gas during the cultivation are shown in Fig. 2. The maximum heat production rate was observed to be 1327.47 W at 7.27 h of cultivation with volumetric heat yield of 6.6379 W/l of culture broth. This could correspond to the point of maximum growth rate of the culture. From the heat signal and the CO_2 profile with time, it could be inferred that the disproportionality between them was due to possible

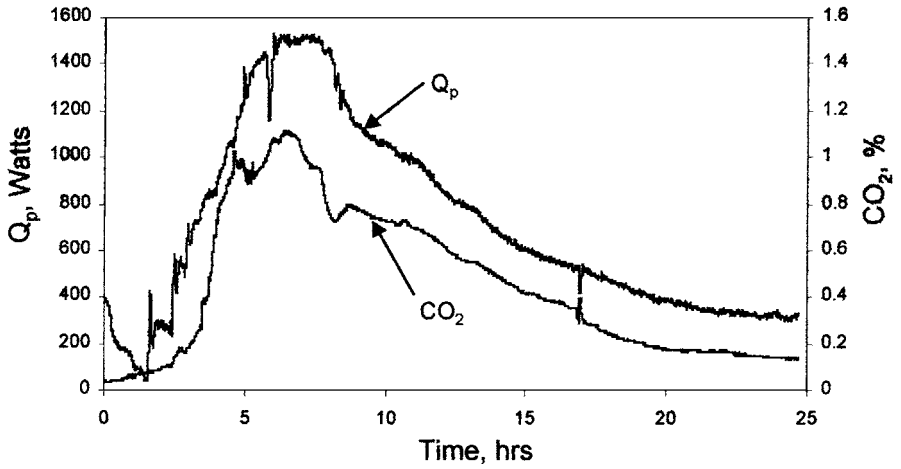

Fig. 2. Profiles of metabolic heat production rate (Q_p) and CO_2 composition in the exhaust gas during the batch cultivation of *Btg* with complex medium in pilot scale bioreactor. The maximum metabolic heat flux was observed to be 1327.47 W at 7.27 h of cultivation.

metabolic shift in utilizing the nutrients as a carbon source. During the initial period of growth excessive foaming was observed due to the presence of highly proteinaceous substances in the medium. The glucose in the medium was not consumed totally thereby indicating that the limitation of the glucose is not a contributing factor for the reduced growth. Even with the presence of residual glucose in the medium, consumption of secreted acidic intermediates/metabolites was started as reflected with rise in pH. With the results obtained during cultivation and the visual observation of the presence of insoluble soybean meal residues even at the later stage of the cultivation, the residual glucose unutilized in the medium could be due to the non-availability of the nitrogen source in the utilizable form. This indicated further scope for identification and improvement of the media components and their level by medium optimization studies.

It was observed that the concentration of the ICP remains invariably constant after 13 h of cultivation (data not shown), but sporulation was not complete as observed by the non-release of spores into the medium. This indicated that the ICP synthesis was completed within 13 h in the complex medium containing soybean meal as nitrogen source. During the sporulation process, the total heat generation from 13 to 24 h could be identified. This heat could be accounted for by the metabolic activity during the process of sporulation until spore release. The results showed the profound influence of the complex medium components on the growth and ICP synthesis. The yield of ICP was high in the complex medium in comparison with the minimal GY medium.

3.3. Fed-batch cultivation with complex industrial medium

The role of substrate inhibition and initiation of sporulation by substrate limitation has necessitated fed-batch cultivation for increasing biomass and ICP. Hence, cultivation was carried out with feeding glucose solution in proportions to the increase in metabolic heat production rate. The feeding profile using a program in LabVIEW was employed with heat production rate and weight of the feed solution from the load cell as input variables. The heat production profile (Q_p) and CO_2 composition in the exhaust gas during the cultivation are shown in Fig. 3. The high concentration (25× of 26 g/l) of glucose solution used in feeding has resulted in the elevated concentration of residual glucose during the cultivation. This elevated concentration could have disturbed the balanced flux of nutrients favoring enhanced biomass and ICP synthesis. Feeding was started after the metabolic heat production rate exceeded 250 W to ensure that the growth was established in the pilot scale bioreactor after inoculation. Flow-rate of feed solution was 47.12 g glucose/min for 1.2 h from 3.75

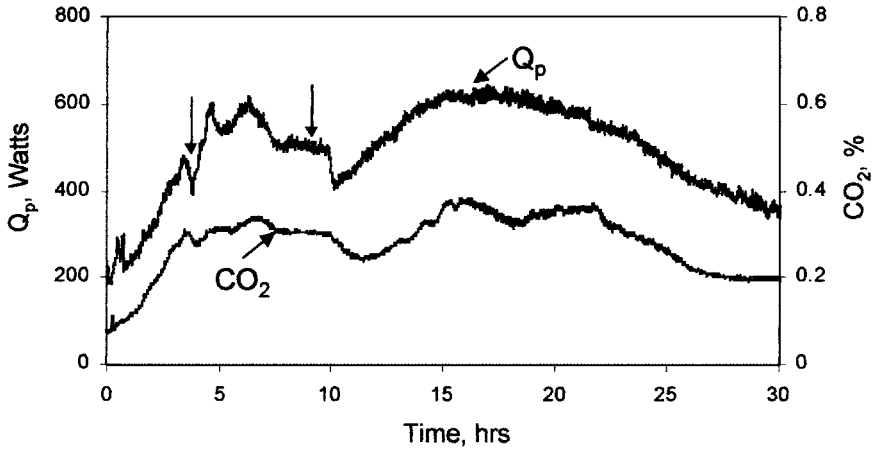

Fig. 3. Profiles of metabolic heat production rate (Q_p) and CO_2 composition in the exhaust gas during the fed-batch cultivation of *Btg* with complex medium in pilot scale bioreactor by feeding glucose solution at 25× of 26 g/l. The maximum metabolic heat flux was observed to be 445.49 W at 17.03 h of cultivation. The arrows indicate the point at which feeding of glucose solution was started.

to 4.95 h of cultivation. The feeding was stopped as the rate of increase in the heat production rate was observed to be insignificant. Also during the later growth phase, increase in both heat production rate and dissolved oxygen concentration has resulted in feeding with 11.53 g glucose/min for 0.7 h from 9.45 to 10.16 h of cultivation.

Since the utilization of the glucose was relatively slower in comparison with the glucose feed rate, accumulation of glucose could result in the medium. This indicated the possibility of change in the internal metabolic network in response to changes in the environmental conditions provided. Hence, this could have resulted in the enhanced accumulation of intermediates/metabolites which was consumed in the later phase resulting in the diauxic growth pattern with maximum heat flux observed during this phase. Diauxic growth was observed in the heat profile as reported by Alexander and Jeffries [27]. In addition, insoluble soybean meal would not be available in a utilizable form for culture as the enzymes, especially proteases, needed for its degradation were not available due to the disturbances happened in the internal metabolic network. Further, it was observed that the amount of glucose consumed during cultivation was less and its metabolic flux was diverted away from biomass formation. Hence, it could be concluded that the adopted feeding concentration would not result in enhanced biomass and ICP formation. The microscopic observation of the culture indicated the presence of heterogeneous population of vegetative cells, sporulating cells and released spores from 22 h of cultivation. This observation also indicated the stress to which the culture was subjected due to the disturbance in the flux of glucose for metabolic activities. Hence, disturbance produced due to the addition of concentrated glucose solution could have altered the optimum ratio of carbon/nitrogen from that needed for balanced flux.

From the insight provided from the impact of feeding glucose at high concentration over the metabolism of *Btg*, feeding glucose at low concentration, 4× was selected so that the condition of glucose limitation could be created during cultivation. Feeding was carried out at a flow-rate of 6.6873 g glucose/min for 2.47 h from 2.589 to 5.059 h of cultivation. The metabolic heat production rate (Q_p) and CO_2 composition in the exhaust gas during the cultivation are shown in the Fig. 4. During this fed-batch cultivation, maximum heat flux of 1189.61 W was observed at 5.893 h of cultivation with ICP concentration of 6.969 g/l and spore count of 37.67×10^8 spores/cm^3. The profile of CO_2 concentration in the exhaust gas was observed to correlate with the metabolic heat production rate. But during the exponential growth phase, increase in CO_2 concentration in the exhaust gas could not be matched with the rise in the heat production rate. This could be due to the redirection of carbon flux away from the respiratory pathway of

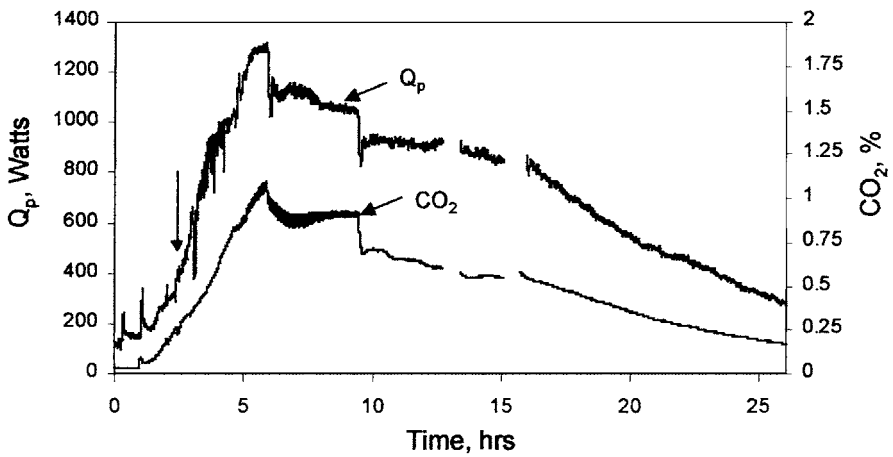

Fig. 4. Profiles of metabolic heat production rate (Q_p) and CO_2 composition in the exhaust gas during the fed-batch cultivation of *Btg* with complex medium in pilot scale bioreactor by feeding glucose solution at 4× of 26 g/l. The maximum metabolic heat flux was observed to be 1189.61 W at 5.89 h of the cultivation. The arrow indicates the point at which feeding of glucose solution was started.

producing CO_2 towards the formation of biomass. This indicated that the present strategy of feeding glucose at low concentration results in a balanced flux of nutrients towards the formation of biomass.

Enhanced uptake of glucose was observed during growth thereby leading to its depletion after 12.5 h. This condition simulated the culture undergoing sporulation from the vegetative phase. It was also observed that the ICP concentration increased from 16 to 26 h of cultivation with the spores released at 26 h (data not shown). Also, it was reported earlier that the major portion of amino acids for ICP synthesis came from the protein turnover of the vegetative proteins [28]. In addition, microscopic observation showed the presence of uniform population of sporulating cells during the sporulation phase of the cultivation. In this cultivation, it was observed that the fed-batch phase with the glucose feeding at low concentration followed by the glucose limited batch cultivation has resulted in the enhanced yield of ICPs in comparison with the batch cultivation. In addition, the total quantity of glucose consumed during this fed-batch cultivation was less in reference to the batch cultivation. The enhanced synthesis of ICP which resulted with the present feeding strategy could be due to the balanced utilization of glucose preventing its accumulation in the medium. Further, effective initiation of sporulation process on the depletion of glucose could lead to high ICP synthesis rate. In particular, the maximum heat production rate was less in comparison with the batch cultivation indicating the efficiency in the metabolic conversion process.

3.4. Impact of medium and process conditions on metabolic heat flux

The significance of the heat measurements can be substantiated by a detailed understanding of the complex relationship between the heat production rate and the influence of substrate(s) and process conditions on the metabolic process towards biomass and ICP synthesis. The observed maximum heat flux and cumulative heat produced at different cultivation periods for different media are summarized in Table 1. Silman [29] reported a cumulative heat output of 17.24 and 39.25 kJ/l was reported during the cultivation of *Zymomonas mobilis* with initial glucose concentrations at 40 and 80 g/l, respectively. Several literature reports indicate the influence of carbon and nitrogen sources in the medium on heat production, which was also valid for the complex media sources [4,17,30].

The data from Table 1 indicated that the total metabolic heat produced to be 26.37 kJ/l during the fed-batch cultivation of *Btg* at the time of harvest (26 h) in complex medium with feeding glucose at 4× concentration of 26 g/l. In particular, heat produced during the initial stages was less with relatively high total heat produced at the time of harvest resulting in high concentration of ICP at 6.969 g/l. This could be interpreted in terms of the correlation of heat production rate with the balanced flux of nutrients favoring enhanced biomass and ICP synthesis. In comparison, the total heat produced in GY medium was considerably less with respect to the complex medium indicating the predominant role of the complex media sources in heat production. This characteristic of the complex medium could be exploited in large-scale fermentation as an on-line metabolic status indicator. Also, the process of ICP synthesis could be monitored with the total heat produced during the cultivation providing an indirect measurements of the secondary metabolites during cultivation.

In all cultivations performed with *Btg* in pilot scale reactors, the heat production rate profile was observed to be an exact correlation with cooling water inlet and outlet temperature profiles. With constant cell density, total heat produced will be in exact proportion to the scale of the bioreactor used for cultivation. This will result in a higher metabolic heat flux in pilot scale reactors in comparison to bench-scale calorimeter. As observed in our experiments, this flux resulted in an increased temperature of water in the jacket. This increase could be observed in the jacket water inlet and outlet temperature. Higher the metabolic heat flux, higher the difference in the jacket water inlet and outlet temperature with constant cooling water circulation in the jacket. During the cultivation of *Btg* in GY medium, the jacket inlet and outlet water temperature profiles has a definite correlation with the heat production rate as shown in Fig. 5. This indicated that the heat transfer across the reactor into the cooling water present in the jacket was significant and directly proportional to the metabolic heat as shown in Fig. 6. It was observed that the heat transfer across the jacket (Q_j) and heat carried away by the cooling water (Q_w) during cultivation were similar and there was

Table 1
The observed maximum metabolic heat flux and the estimated cumulative heat produced at different cultivation periods with different media under varying process conditions[a]

Medium	Mode of operation	Net maximum heat flux (W)	Baseline heat signal (W)	ICP concentration (mg/ml)	Cultivation time for maximum heat flux (h)	Cumulative heat production (kJ/l)				
						Maximum heat flux	12 h	24 h	26 h	30 h
Glucose–yeast extract	Batch	341.64	221.55	1.01	4.2	2.07	5.72	–	–	–
Complex medium	Batch	1327.47	223.85	6.15	7.27	8.79	16.27	24.61	–	–
Complex medium with feeding glucose at 25×	Fed-batch	445.49	197.4	1.77	17.03	11.95	7.85	17.53	18.82	20.98
Complex medium with feeding glucose at 4×	Fed-batch	1189.6	123.88	6.97	5.89	5.35	14.01	25.48	26.37	–

[a] Baseline heat signal values and ICP concentrations obtained in each cultivations are also shown.

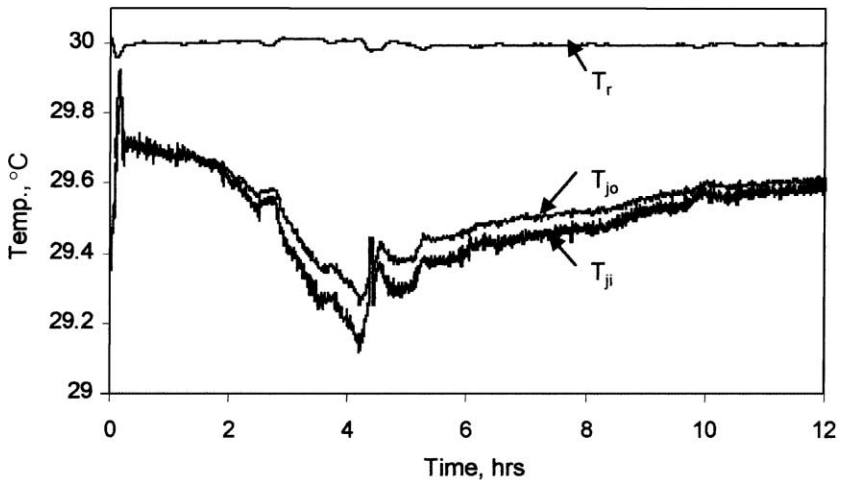

Fig. 5. Profiles of the cooling water temperature at jacket inlet (T_{ji}) and outlet (T_{jo}) during the batch cultivation of *Btg* with GY medium in pilot scale bioreactor and T_r indicates the temperature in the bioreactor.

a constant difference as indicated in Fig. 7. This difference in the estimated heat transfer rates resulted in a shift in the metabolic heat production rate profile from the baseline. In addition, Q_w could not be used in the heat flux calculations due to its noisy characteristics as observed in Fig. 7. Due to the significant heat transfer into the cooling water, the resulting increase in the difference in the jacket water inlet and outlet temperature as shown in Fig. 7 could be used as the indicator of the on-line metabolic status of the culture. No significant difference was observed in the baseline heat signal with GY and complex medium. In contrast, slight difference was observed in the baseline heat signal during fed-batch cultivation due to the variation in the non-estimated heat fluxes constituting the baseline.

Since industrial scale fermentations will normally be performed at constant aeration and agitation, measurement of jacket water inlet and outlet temperature could be used for calculating the heat transfer across the jacket for maintaining the temperature during cultivation. This calculated heat flux could indicate

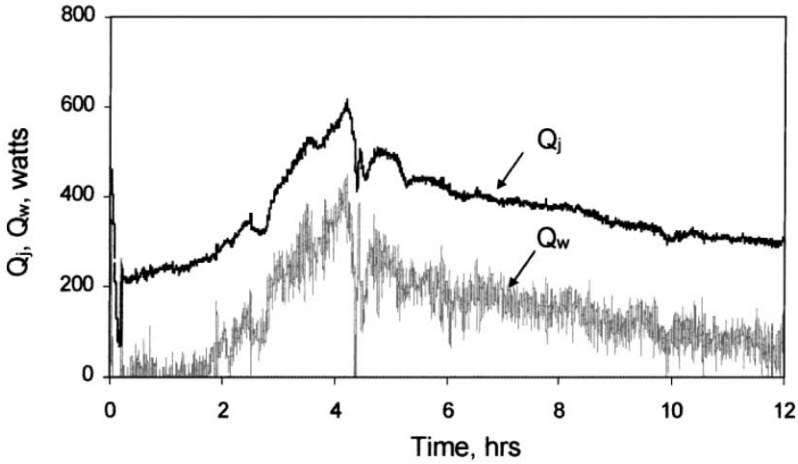

Fig. 6. Profiles of heat flux across the jacket into the cooling water (Q_j) and heat carried away by the cooling water (Q_w) for the maintenance of bioreactor temperature at 30 °C during the batch cultivation of *Btg* in GY medium.

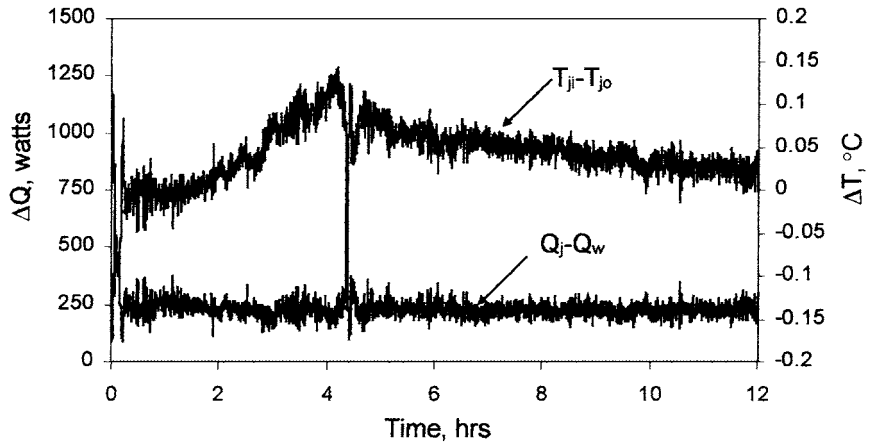

Fig. 7. Profile of the differences in the estimated heat transfer rate (ΔQ) across cooling jacket and heat carried away by the cooling water during the batch cultivation of *Btg* in GY medium. This difference was due to the variation in the estimated UA from the actual value. Also, difference in the jacket inlet and outlet temperature (ΔT) on heat transfer during cultivation is shown in the figure.

the metabolic heat generation rate after incorporating the other heat generation and heat dissipation rates in the baseline of the heat signal. Hence, this could be employed in industrial scale fermentation as an on-line measurement. The heat signal could be used to design fed-batch strategies in industrial scale fermentations for enhancing product yield by the measurements of jacket inlet and outlet temperatures on-line, and used for biological cultivations as it is versatile in the ease of incorporating additional parameters involved in heat transfer, such as changes in rheological properties of culture, manipulation of agitation and aeration for maintenance of dissolved oxygen concentration during cultivation.

4. Conclusions

The metabolic heat produced by cultures was estimated using the overall heat balance taking into account all the possible sources of heat in the pilot scale bioreactor. This measurement could be used as an effective means to optimize processes and understand the on-line status of the culture under cultivation. The correlation between the heat production rate and the temperature profile in the jacket has indicated that the heat transfer across the jacket into the cooling water was the major contributing heat flux. This has allowed the jacket inlet and outlet temperature to be monitored in order to access the microbial heat production rate. With the development of a robust controller for maintaining the bioreactor temperature at the desired value the metabolic status of the microbial culture could be monitored on-line by measuring the temperature profile in the jacket in industrial scale fermentation. This measurement of heat flow from industrial scale fermentation will lead to more economic production of various biological products.

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