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A SIMPLE AND RELIABLE METHOD FOR THE DETERMINATION OF CELLULAR RNA CONTENT.

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SUMMARY.

This communication describes a rapid, simple and reliable method for the determination of the cellular RNA content. In the study of microbial growth and product formation the cellular RNA content is a good measure of the activity of the biomass. Results from applying the method to monitor transient experiments in a chemostat with *Lactococcus cremoris* are presented.

INTRODUCTION.

The intracellular RNA content of microorganisms is well correlated with the size of the protein synthesizing machinery (Maaløe, 1972), and the cellular RNA content is, therefore, an important variable in the study of microbial physiology. In simple structured models for microbial growth and product formation the cellular RNA content has succesfully been used to describe the state of the biomass (Esener et al., 1982; Nielsen et al., 1990). For many different microorganisms the cellular RNA content increases monotonically with the specific growth rate for different growth limiting substrates (sugars, amino acids, etc, Maaløe, 1972). The intracellular RNA content is also an interesting variable during hybridoma cultivations since the antibody production only occurs when the RNA content is high (Dalili and Ollis, 1990). Irrespective of the microorganism, measurement of the cellular RNA content during transients in a chemostat gives fundamental insight into the growth dynamics.

As seen in the literature from the last decade, researchers often resort to the Schneider method of hot perchloric acid hydrolysis of RNA and DNA (Jöbses et al., 1985, Nielsen et al., 1990). Because both RNA and DNA are hydrolysed the orcinol method is used to determine RNA. The orcinol method is quite laborious and relies on a spectrophotometric determination of a derivative of ribose. In this communication we propose a reappraisal of the simple UV-spectrophotometric determination of RNA specifically degraded by alkali and extracted in perchloric acid (Munro and Fleck, 1962).

MATERIALS AND METHODS.

Sampling. Fermentation broth volumes equivalent to approx. 0.4 mg RNA (2.5 mg bacteria) collected during a fermentation are centrifuged in chilled test tubes, the supernatant is discarded and the cells can be frozen for analysis.
Analysis. The cells are washed three times with 3 ml cold 0.7M $HClO_4$ and digested with 3 ml 0.3M KOH for 60 minutes at 37 °C with occasional mixing. The extracts are cooled and neutralized with 1.0 ml 3M $HClO_4$. The supernatant is collected and the precipitate is washed twice with 4 ml cold 0.5M $HClO_4$. Finally, the extracts are made up to 15 ml with 0.5M $HClO_4$ and the solutions are centrifuged to remove any non-visible precipitate of $KClO_4$ that might be in the extracts. The RNA concentration is determined by measuring the absorbance at 260 nm using average nucleotide data for calibration : M_w = 340 g/mol, ϵ = 10800 $M^{-1}cm^{-1}$ (Herbert et al., 1971). One should carefully check whether the absorbance follows Beers law by measurement of diluted samples. Measurement of A_{260} can be postponed for at least 24 hours if the extracts are stored in a refrigerator.
Fermentations. All fermentations are performed in a 2L MBR fermenter with a medium containing yeast extract, casein peptone and glucose.

RESULTS AND DISCUSSION.

The method for RNA quantification described in this paper has been compared with the much more frequently used method of hot perchloric acid hydrolysis and determination of RNA by the orcinol method (Herbert et al., 1971). It was observed that the KOH/UV method results in much lower relative standard deviations (RSD) than the $HClO_4$/orcinol measurements. This difference is not surprising since calibration for both methods has been done using average nucleotide data. If an exact calibration is necessary one should purify RNA from the organism studied to determine the precise extinction coefficient. The reason for the poor precision of the $HClO_4$/orcinol method has not been determined since this method is more laborious than the one described in this paper.

	KOH hydrolysis UV absorbance		$HClO_4$ hydrolysis orcinol method	
D h^{-1}	RNA (%w/w)	RSD (%)	RNA (%w/w)	RSD (%)
0.093	8.93	0.4	10.06	3.9
0.460	17.03	1.1	18.58	8.1
0.096	8.61	0.7	10.70	9.5

Table 1. Comparison of the two methods for RNA quantification of a L. cremoris strain from a chemostat (D = dilution rate, RSD = relative standard deviation).

The results presented in this communication were obtained in a physiological study of *Lactococcus lactis* subsp. *cremoris* FD1 with the aim to describe the growth and product formation by a mathematical model. During transient operating conditions in a chemostat different variables are measured. The analyzers for on-line measurement of glucose and biomass are described elsewhere (Benthin et al., 1990). The samples for RNA analysis are automatically withdrawn from the fermentor to test tubes in an icebath. It has been experimentally verified that both the biomass and the RNA content are constant during 24 hours when stored in the icebath (data not shown).

In figure 1 the intracellular RNA concentration is depicted during a step-down change of the dilution rate. After the shift of the feed rate the RNA concentration (g/l broth) increases for two hours and then decreases. The reason for this transient accumulation is the increasing biomass concentration. The specific RNA concentration (g/g cells, %w/w) decreases monotonically towards the new steady state level.
From the same experiment, figure 2 shows the specific RNA concentration together with measurements of the glucose concentration and the specific lactic acid formation rate (r_p, g lactic acid/g cell dry mass/h). Immediately after the shift, the specific lactic acid formation rate r_p decreases simultaneously with the decreasing RNA content. This is due to some component in the complex N-source that is already limiting before the step change. Glucose is only limiting at very low concentrations (below 30 mg/l) and glucose limitation therefore occurs after 3.8 hours.

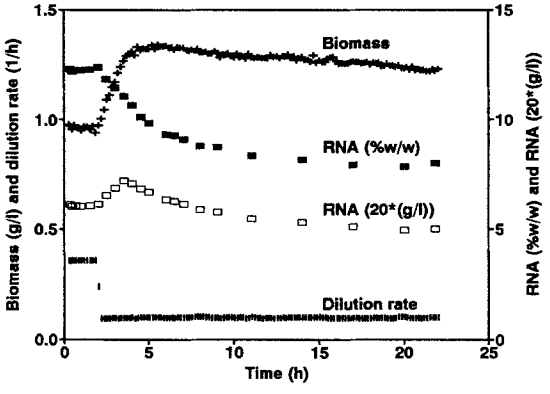

Figure 1. Measurements of the biomass concentration (OD_{565}) and the intracellular RNA concentration (g RNA/g cells and g RNA/l broth multiplied by 20) during a step change of the dilution rate from 0.356 to 0.097 h^{-1}.

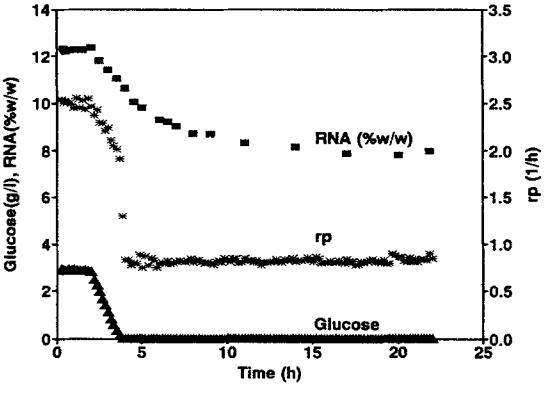

Figure 2. For the same experiment as in figure 1 the cellular RNA content is depicted together with the glucose concentration and the specific lactic acid formation rate, r_p.

Figure 3 shows the results from a step-up change of the dilution rate for a situation where glucose is limiting at both steady states due to a large excess of the N-source. Prior to the step change the cellular RNA content is low (7%) but increases after the step change as a result of the increased glucose concentration leading to synthesis of

active cellular machinery (of which RNA is a major component). There is an incipient wash-out of the biomass and accompanying transient accumulation of glucose until the size of the active machinery of the cells becomes large enough to metabolise the glucose. The temporary high glucose concentration causes the overshoot in the cellular RNA content as the specific growth rate for a while exceeds the new dilution rate. This experiment is a good illustration of the importance of the RNA measurement for interpreting the behavior observed in the other measured quantities (biomass, glucose and r_p).

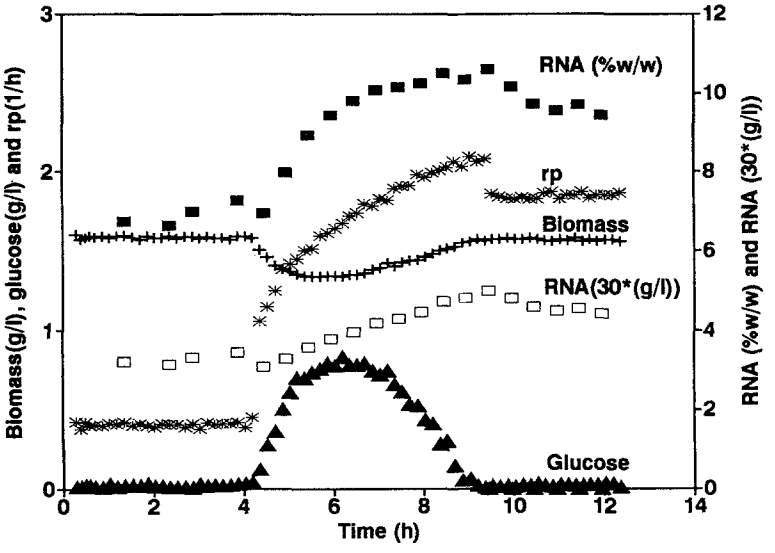

Figure 3. Measurements during a step change of the dilution rate from 0.075 to 0.409 h^{-1} where glucose is the limiting substrate. The feed rate is changed at 4.3 hours.

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