Transient Response of Continuously Cultured Heterogeneous Populations to Changes in Temperature

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Completely mixed, once-through continuous culture systems of heterogeneous microbial populations of sewage origin were systematically examined for response to changes in reactor temperature. Systems were operated at two dilution rates of 0.125 and 0.25 per h. "Steady state" conditions of the systems were assessed with the reactors operating at 25 C. From this base line, temperature was decreased to as low as 8 C and increased to as high as 57.5 C. Response was assessed in the ensuing transient phase as the system approached a new "steady state." The response was measured by changes in amount and type of carbon source in the reactor effluent as determined by the chemical oxygen demand test, the anthrone test, and gas chromatography. Biological solids concentration and cell composition (protein, carbohydrate, ribonucleic acid and deoxyribonucleic acid) were also determined. These systems responded more favorably to increases than to decreases in temperature. Regardless of the direction of change, the system with the lowest dilution rate (D = 0.125 per h) responded more successfully; i.e., there was less leakage of carbon source in the effluent and less dilute-out of cells during the transient phase.

Although there has been much investiga-
tional interest regarding the effects of tempera-
ture on the growth and composition of chem-
ostatically grown cells under "steady state" con-
ditions, there is scant experimental data in the
literature regarding the transitional response to
step increases or decreases in temperature, or
both. Such aspects are of general interest in the
area of continuous culture of microorganisms
and of considerable applied interest regarding
the understanding and control of microbial
processes such as biological treatment, e.g., by
activated sludge, of organic-laden wastewaters
wherein heterogeneous populations rather than
pure or specific mixtures of species are
employed.

Much of the work on effects of temperature on
microbial growth and physiology through 1966
has been reviewed by Farrel and Rose (5). There
has been recent interest in the caloric values of
cells grown at various temperatures, and it
would appear that the calories per gram of cells
remain essentially unchanged regardless of
growth temperature (12, 14). There is continu-
ing controversy regarding the effect of growth

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temperature from a common base-line temperature, and we were interested in assessing the effect, if any, which initial growth rate or dilution rate might have on the nature and extent of the transient phase between initial and final steady states. These results are presented to provide possible guidelines for limits of tolerance of activated sludge processes to temperature shock and because the experimental picturization of changes in system parameters in the transient phase may have some usefulness in the general area of microbial kinetics and physiology, even though the response involves transient ecological as well as physiological reaction to the environmental stress.

**MATERIALS AND METHODS**

The continuous-flow growth reactors employed were once-through chemostats (2.5-liter Pyrex glass) and have been previously described in detail (8). Prior to receiving a temperature shock, the units were maintained at 25 C ± 0.5 C. Temperature was controlled by a Precision LoTemprot (Precision Scientific) which was connected to the water bath in which the reactors were placed. Aeration through carborundum diffusers was maintained at 5,000 ml/min. The growth medium contained: glucose, 1,000 mg/liter; (NH₄)₂SO₄, 500 mg/liter; MgSO₄·7H₂O, 100 mg/liter; MnSO₄·H₂O, 10 mg/liter; CaCl₂·2H₂O, 7.5 mg/liter; FeCl₃·6H₂O, 0.5 mg/liter; tap water, 100 ml/liter; 1 M phosphate buffer solution (pH, 7.0), 10 ml/liter, and distilled water to volume.

Each experiment was initiated by inoculating the synthetic waste with a sample of sewage obtained from the effluent of the primary clarifier of the municipal sewage treatment plant at Stillwater, Okla. The reactors were run 2 to 4 days under continuous-flow conditions at the “steady state” to establish the base-line condition prior to the change in temperature. Each step change in temperature was applied by adjusting the temperature of the water flowing into the water bath. Thus, the “shock” was not immediate. In all cases, the temperature was closely monitored so that the rate of temperature change was known. Experiments were conducted at two dilution rates: D = 0.125 per h (8-h mean hydraulic retention, t) and 0.25 per h (t = 4 h).

Frequent samples were taken to assess the response of the biomass. The concentration of biological solids was determined by the membrane filter technique (15) by using 0.45-μm pore size filters. The filtrate was analyzed for chemical oxygen demand (COD) (15) and carbohydrates by the anthrone test (7). The filtrate was also analyzed by gas-liquid chromatography for volatile (acetic) acids by using a PolyCap-2 column (model BB Hewlett-Packard Co., Avondale, Pa.). Protein and carbohydrate contents of the biological solids were determined, respectively, by the biuret and anthrone analyses (7). RNA and DNA contents of the biological solids were determined, respectively, by the orcinol (13) and the diphenylamine (3) reactions, by using a trichloroacetic acid extract of the cells. Frequent checks on the pH of the reaction liquor were made, and the reactors were also checked frequently for complete mixing (11).

**RESULTS**

Five long-term continuous-flow experiments were conducted in which like changes in temperature, increases and decreases from the base temperature at 25 C, were administered to chemostat systems operating at dilution rates of 0.125 and 0.25 per h. The temperature shock range was from 8 to 57.5 C.

In Fig. 1, as in all succeeding figures, data to the left of the dashed vertical line (negative time scale) depict the preshock “steady state” condition. The temperature change was initiated at time zero, and the responses are shown to the right (positive values on the time scale). In all cases, the graph on the left depicts response at D = 0.125 per h; response at D = 0.25 per h is shown on the right. The curve identified as “substrate dilute-in curve” in Fig. 1 represents the calculated value of the reactor (or effluent) COD in the absence of metabolism, i.e., if metabolism had stopped at the time of changing the temperature. The curve labeled “T-COD” depicts the total chemical oxygen demand of the filtrate. The curve labeled “A-COD” is the carbohydrate concentration in the filtrate calculated to its COD value as hexose sugar (e.g., COD glucose = mg of glucose per liter × 192/180). The difference between T-COD and A-COD may be taken as a measure of noncarbohydrate metabolic intermediates or end products produced by the organisms, or both. The curve labeled “acetic acid COD” results from gas-liquid chromatographic analysis. In the experiment shown in Fig. 1, the only chromatographic peak detected corresponded to acetic acid, and the amount was very small (± 30 mg/liter) in the system operating at D = 0.25 per h; none was detected at the lower dilution rate. Prior to the change in temperature, both systems provided excellent substrate removal efficiency. The cell yield was somewhat higher for the higher dilution rate. The change from 25 to 8 C was effected in 12 h. It is apparent that neither system responded successfully as the temperature was decreased to the psychrophilic range; there was no indication of impending recovery after 200 h of operation at the post-shock temperature. It appears that the lower dilution rate permitted a greater degree of dissimilation of substrate (compare A-COD curves) and slightly greater utilization of the
organic carbon (compare T-COD curves). There was some indication of "overshoot" with regard to A-COD in the system with lower D, whereas there was a smoother transition at the higher dilution rate.

When a less severe decrease in temperature, from 25 to 17.5 °C, was applied (see Fig. 2), the response of the system growing more slowly in the preshock state was much more successful than that of the more rapidly growing one. The change in temperature was effected in 6 h, i.e., 0.75 and 1.5 mean hydraulic retention times, respectively, for the two systems. There was essentially no leakage of anthrone-reactive material in the system of lower D, and there was only a short-lived transient rise in T-COD which corresponded to a transient decrease in biological solids concentration. During the tran-

Fig. 1. Response of a heterogeneous microbial population growing in a chemostat at 25 °C to a decrease in temperature to 8 °C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.

Fig. 2. Response of a heterogeneous microbial population growing in a chemostat at 25 °C to a decrease in temperature to 17.5 °C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.
sient stage, there was an increase in protein and RNA content of the biomass and a slight decrease in carbohydrate content. Although this system successfully accommodated the decrease in temperature, the one at D = 0.25 per h could not accommodate the change. During the first 20 h, the effluent COD rose sharply but there was essentially no leakage of carbohydrate. During this period, acetic acid concentration rose to 114 mg/liter. The concentration of biological solids decreased from 510 to 240 mg/liter. The system appeared to be on the verge of recovery after changing the temperature, but both T-COD and A-COD rose to nearly 600 mg/liter 100 h after initiating the shock. There was an unexpected recovery of the concentration of biological solids to nearly 390 mg/liter concomitant with the rise in effluent COD, leading to a cell yield of approximately 90% as compared to one of approximately 50% in the preshock condition. The high cell concentration was not due to incomplete mixing, i.e., retention of cells in the reactor, since the concentrations of cells in the reactor and the effluent were the same. As in the case of the system with lower D there was, during the transient stage, an increase in RNA and protein content and a decrease in carbohydrate content of the biomass.

The mildest increasing temperature shock studied was one from 25 to 36°C. The change was effected over a period of approximately 40 h (5 × t at D = 0.125 per h and 10 × t at D = 0.25 per h). Successful responses occurred at both dilution rates (Fig. 3). At the lower dilution rate, there was only slight fluctuation in effluent quality and a slow, but completely reversible, decrease in the concentration of biological solids. There was a decrease in protein content and a concomitant increase in carbohydrate content, but these parameters, along with the concentrations of effluent COD (S) and biological solids (X), returned to the preshock level. At the higher dilution rate, there was initially a rapid loss of biological solids but the biomass concentration recovered rapidly, followed by a slow decrease to the new “steady state” level. The fluctuations and decreasing trend in X did not result in any deterioration in purification efficiency. The most noticeable effects were the decrease in cell yield and increase in protein content of the biomass.

When the systems were subjected to a more severe increase in temperature, from 25 to 47°C over a period of 26 h, a severe transient leakage of substrate ensued at both dilution rates. The effect of lower dilution rate in attenuating the severity of dilute-out in X and leakage in S in the transient phase is amply demonstrated in these experiments. It is also seen that, for the system with lower D, dissimilation of the substrate proceeded without interruption, whereas in the system of higher D, the leakage of anthrone-reactive material paralleled the T-COD concentration. Both systems recovered the preshock level of treatment efficiency, and the higher operating temperature led to a lower cell yield (see Fig. 4).

An increase in temperature to the thermophilic range, i.e., from 25 to 57.5°C, led not only to severe transient disruption of the system but to inability to recover treatment efficiency within 200 h after changing the temperature. Again the system with lower D evidenced the more successful response. Analyses for sludge composition were not performed, since the cell concentration was extremely low in the transient phase. It is important to note that the deleterious response could not be attributable to deficiency of dissolved oxygen. The lowest dissolved oxygen concentration recorded was 3 mg/liter, a value much in excess of oxygen concentration usually found to limit metabolism of microorganisms (see Fig. 5).

**DISCUSSION**

In these studies, the temperature shocks, either increases or decreases, were applied at equal rates of change to each of two comparable systems which were growing at specific growth rates of 0.25 and 0.125 per h in the initial steady state, and it is amply apparent that, regardless of the direction of temperature change, the system with lower D exhibited a greater degree of accommodation to the shock. The same effect has been observed in other shock-loaded systems for which the carbon source was the growth-limiting nutrient. For example, systems with lower D have been observed to leak less substrate during transient response to qualitative shock, i.e., changes in the type of compounds comprising the carbon source in multicomponent substrate systems, e.g., carbohydrate-amino acid systems (9) and carbohydrate-alcohol systems (unpublished data, Komolrit and Gaudy). We have also observed in other studies (Krishnan and Gaudy, unpublished data) that systems with lower D respond more favorably to quantitative shock, i.e., changes in the concentration of the inflowing carbon source.

Similar trends for various types of system perturbations by no means imply similar mechanisms of metabolic or ecological response, but
the general trend does tend to explain the rather good ability of activated sludge systems to successfully withstand, without serious metabolic malfunction, the various environmental stresses which are imposed upon them. The specific growth rate, $\mu$, for such systems is naturally rather low because of cell feedback, i.e., $\mu = D(1 + \alpha - \alpha X_S/X)$, wherein $\alpha$ is the hydraulic feedback ratio, $X_S$ is the cell concentration in the feedback, and $X$ is the concentration of cells in the aeration tank. In addition, cell feedback provides a much greater concentration of biomass in the reactor than could exist without feedback, and we have observed in some experiments that a higher cell concentration also attenuates the transient leakage of substrate. Both results of operation with cell feedback (lower $\mu$ and higher $X$) can combine to provide apparent greater protection against various types of environmental stress. Indeed, there may be some doubt as to which factor most affects the resistance to environmental shock. For this reason, studies in once-through systems are apropos to activated sludge process research because they provide the investigator with a tool with which to separate the effects. The present study on temperature shock, as well as other shock load study results mentioned above, would seem to leave little doubt that the specific growth rate at which the cells were growing in the preshock steady state has a separate and a rather significant influence on the response. It is also quite possible that the response is more greatly influenced by the hydraulics of the system than by the preshock physiological condition of the cells as influenced by $\mu$ or $X$ at the time of applying the stress. A longer mean hydraulic retention time (i.e., $1/D = \bar{t}$) may, simply by retaining cells in the reactor longer, provide more time for adjusting to the new conditions.

In studies cited above on changes in type and concentration of substrate, the imposed change was usually administered at a rate governed by the hydraulic feed rate, $D$. Thus, the change in concentration or type of substrate, or both, would be administered more slowly for lower values of $D$ in accordance with the calculatable dilute-in curves. However, for the present study, the rate of temperature change in each system was the same, since the temperature shock was not administered via a change in the inflowing medium, and the difference in response can be attributed solely to the different dilution rates (with allowance for possible differences in the populations in the reactors prior
Fig. 4. Response of a heterogeneous microbial population growing in a chemostat at 25 C to an increase in temperature to 47 C. Left, dilution rate = 0.125 per h; right, dilution rate = 0.25 per h.

to the shock. It would appear that the lower dilution rate provides a greater intra- or intercellular response time which permits the more favorable metabolic response.

With regard to the composition of the biomass, the lowering of temperature (see Fig. 2) caused an early decrease in carbohydrate and an increase in RNA and protein contents during
the transient stage. The cell composition in the final "steady state" was approximately the same as in the preshock state, and there was a slight rise in cell yield. The increase in protein and RNA accompanied by a decrease in carbohydrate is indicative of rapid growth. Since the lower temperature would be expected to decrease the growth rate of the population predominating prior to the shock, the change in biomass composition may be indicative of an adaptive response in which cells which could grow rapidly at the cooler temperature were being selected, while cells incapacitated at the lower temperature were diluting out of the system. The higher carbon source concentration in the reaction liquor during the transient stage would also tend to increase the specific growth rate of the cells which were able to grow. It is noted, however, that if such a change in predominance took place it was not reflected in a noticeable change in the morphological appearance of the biomass as adjudged by frequent microscope examination during the experiment.

Dilution rate apparently affected the change in protein content in response to an increase in temperature (Fig. 3, 4). In both cases, the population which was growing more slowly in the preshock condition responded with a decrease in protein content, whereas in the more rapidly growing system protein content increased during the transient phase. There was, in general, a decreased cell yield at the elevated temperature. During the period of cell dilute-out and recovery, there was some evidence for changes in species predominance for all three temperature increases as indicated by changes in morphology; however, an ecological (or, in any event, a morphological) shift was particularly evident at the 47 and 57.5 C temperatures. Prior to the shock, short, thick rods predominated in the biomass. These began to dilute out as the concentration of biological solids decreased, and they were replaced in the recovery phase by thin, elongated cells.

Although there was, in these experiments, a general pattern of cell dilute-out and substrate leakage followed by recovery, and although the severity and duration of dilute-out were greater with greater changes in temperature from the base of 25 C, it is somewhat difficult to determine if these responses can be modeled mathematically. The ultimate utility of a model for the transient stage depends upon the adequacy of its physiological (mechanistic) basis. Although there have been attempts, by using a systems approach, to devise such predictive models for single species systems without demonstration of a mechanistic basis for them, the utility of such approaches for heterogeneous population systems would seem rather minimal. For natural populations, the problem is much more complicated, and successful modeling will, in any event, depend upon the availability of experimental results obtained in controlled experiments which provide a record of the response as measured by a number of significant parameters. The results herein presented are intended to help satisfy this need. Also, they provide some guidelines regarding the magnitude of change which a natural system can accommodate, and it may be tentatively concluded that systems operating at reasonably moderate temperatures, e.g., ± 25 C, can more readily accommodate increases than decreases in temperature. This may be due in part to the fact that the most general effect of a nonlethal rise in temperature is an increase in growth rate, either of the existing predominants or of cells selected by the higher temperature.

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