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# The effect of water on the crystallization of thin films of poly(hydroxybutyrate)

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The effect of water on the growth rate of thin films of poly(hydroxybutyrate) and its copolymers with hydroxyvalerate has been studied. The presence of water significantly enhanced the growth rate of the polymer over all temperatures up to 90°C. The increased growth rate is coupled with some change in morphology of the crystalline polymer and an apparent reduction in the glass transition temperature of the amorphous polymer from +4 to −3°C when in contact with water. The apparent change in the glass transition is discussed, and the implications for the presence of an atmospheric plasticizer for poly(hydroxybutyrate) are considered. © 1997 Elsevier Science Ltd.

**(Keywords: poly(hydroxybutyrate); water; spherulite growth rates)**

## INTRODUCTION

Poly(hydroxybutyrate) (PHB) and its copolymers with hydroxyvalerate (PHB/HV) are bacterial thermoplastics produced commercially by Monsanto under the trade name Biopol[1,2]. The commercial uses of the inherently biodegradable plastics include thin films and paper coatings. There is, accordingly, an increase of interest in the crystalline morphology of thin films of these polymer systems.

Although PHB has frequently been referred to as hydrophobic, Marchessault *et al.* have pointed out that this is not truly the case[3], and have suggested an active role for water in the amorphous state of the bacterial granules[4]. Lauzier *et al.* have suggested that water may act as a plasticizer for PHB[4], and proposed that a hydrogen-bonded network of PHB and water may be formed during the bacterial synthesis of the polymer. It has, however, been pointed out in a number of papers[5,6] that the addition of a plasticizer should merely shift the maximum in the growth rate curve to lower temperatures, and should not prevent crystallization—it may even be expected to increase the nucleation rate at certain temperatures. An alternative possibility for the amorphous state of the bacterial granules has been suggested by de Koning[6], based on the small size of the granules and the low probability of homogeneous nucleation occurring. However, the nucleation data on which this conclusion is based[7] are taken from dry, thin films, crystallized from the melt, and not necessarily applicable to the bacterial granules.

While studying the thin film crystallization behaviour of PHB[8] we have observed some substantial differences in the growth behaviour of films with and without a free surface and when the surface is in contact with water. In this paper we present evidence that water, when in contact with the surface of the polymer, greatly enhances the crystal growth rate of PHB and its copolymers with HV over the whole range of temperatures attainable. This observation may have implications for the commercial processing of PHB as well as for the role of water in the amorphous state of the native bacterial granules.

## EXPERIMENTAL

The polymers used were PHB homopolymer 'fluff', $\bar{M}_w$ 866000, $\bar{M}_n$ 316000, 8% HV copolymer from batch PB26B, and 24% HV copolymer from batch PBP05. All were free of nucleating agents, etc. The 'fluff' had been cleaned by dissolving in chloroform, filtering and precipitating in ethanol.

Samples of these materials were placed on glass cover-slips and melted on a hot-bench at 200°C. The thickness of the films was controlled by scraping with a razor blade. Using this technique we can prepare films with thicknesses in the range 1–20 μm. The films were kept at the high temperature for a total of not more than 90 s. The cover-slip with an adhering thin polymer layer was then removed from the hot-bench and quenched to the desired temperature. A complete quench to room temperature without any visible crystallization could be attained by placing the cover-slip on a metal plate at room temperature. In order to observe crystallization at temperatures above room temperature, films prepared as above were placed in a Linkam microscope hotstage preheated to the needed temperature. This allowed a more effective quench than was possible by melting and cooling samples in the Linkam hotstage.

Radial spherulite growth rates were measured using an optical microscope with a video attachment. At least two separate samples were used for each temperature, and a large number (10–20) of readings were taken from each sample.

In all cases samples had one surface in contact with a glass slide and the other one free. Bulk growth rates were

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measured by focusing below the surface of the polymer—these were found to be in good agreement with growth rate measurements made when crystallization occurred between two glass slides.

Growth rates were also obtained for films crystallized in contact with water. Molecular biology grade 'ultra-pure' water was used. For temperatures from 25 to 45°C, thin films were first quenched to room temperature and then placed in water in a Linkam hotstage preheated to the desired temperature. Sufficient time was allowed for thermal equilibrium to be reached, typically 3 min. For higher temperatures the samples were quenched directly into water preheated to the desired temperature on the hot-bench and then placed in a preheated Linkam hotstage. The growth rate changed as thermal equilibrium was approached. We made our measurements of growth rate once a constant value was reached.

A Perkin Elmer DSC 7 was used to examine the glass transition temperature of the different samples. By using steel, high-pressure pans it was possible to melt the polymer in the presence of water, quench to low temperatures and reheat in order to see the effect of the water on the glass transition temperature.

## RESULTS AND DISCUSSION

### *Variation of growth rates with temperature*

*Figure 1* shows the growth rate of the 8% HV copolymer over a range of temperatures both with and without water in contact with the free surface. It can be seen that at all temperatures there is an increase in growth rate in the presence of water. *Figure 2* shows the ratio between the growth rates with and without water which falls approximately exponentially from about 4 at 30°C to around 1.75 at 55°C. *Figure 3* shows the growth rates of the 24% HV copolymer with and without water over a range of temperatures. It was not possible to measure growth under water at high temperatures as the water evaporated too quickly. However, it appears that the behaviour is similar to that of the 8% HV, with an approximate doubling in the growth rate over the range observed.

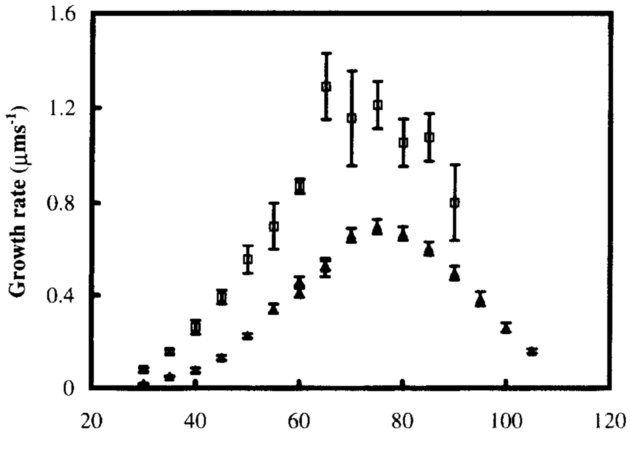

**Figure 1** A graph showing the variation of growth rate with temperature for an 8% HV copolymer: □, under water; ▲, under air

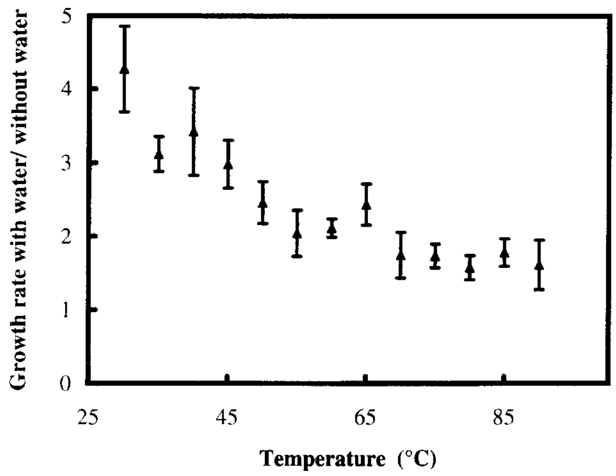

**Figure 2** A graph showing the ratio of growth rates between an 8% HV copolymer with and without water in contact with the surface

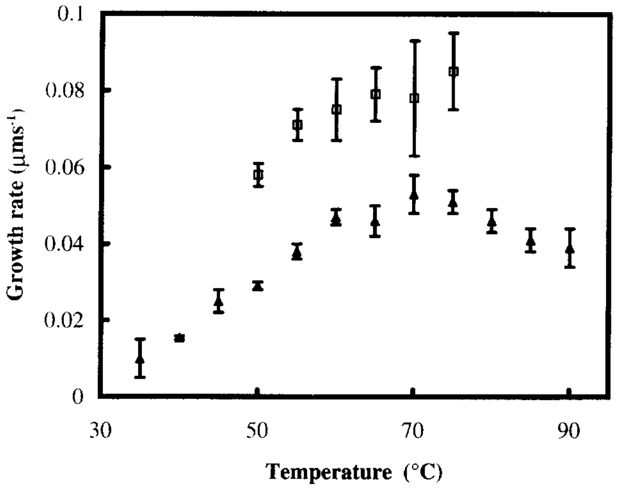

**Figure 3** A graph showing the variation of growth rate with temperature for a 24% HV copolymer: □, under water; ▲, under air

It was not possible to study the effect of water on the growth rates of PHB homopolymer except at room temperature, as the growth was too fast to allow thermal equilibrium to be reached. It would also not be possible to observe the effect of water both above and below the growth rate maximum as the peak growth rate is around 90°C. At room temperature, a 14-fold increase of the growth rate from 0.01 from 0.14$\mu s^{-1}$ was observed on the addition of water.

For both hompolymers and copolymers it is possible, in all cases, to return the polymer to its normal growth rate by removing the water, so chemical change or loss of molecular weight cannot be responsible for the behaviour.

*Figure 4* shows d.s.c. traces for the 8% HV polymer on heating from −50°C at 5°C min$^{-1}$. When water is not in contact with the sample the glass transition is at approximately 4°C, which is apparently reduced to −3°C when water is added, although the exact identification of a glass transition in the presence of water is difficult. By opening the d.s.c. pan we were then able to remove the water and re-run the sample. We found that the glass transition returned to its previous value of 4°C, showing that the change had not been due to degradation and subsequent plasticization by low molecular weight degradation products.

When water is in contact with the surface of amorphous PHB/HV copolymer, a surface roughening effect apparently occurs, as indicated by the presence of dark markings when observed through the optical microscope in bright field. When the polymer crystallizes, this roughening is frozen in but, if the water is removed from the surface before crystallization can be completed, the surface returns to its 'normal' smooth appearance, as shown in *Figure 5*. It is possible that the surface roughening is due to a very low surface energy between PHB and water, and may provide evidence for some partial mixing.

In the case of 'fluff', the high molecular weight homopolymer, the optical texture of the growth under water was very different from that observed when crystallization occurs between two glass slides. Although it has an apparently constant radial growth rate, it does not display a maltese cross or any banded structure as may be expected for a spherulite at this temperature. Unfortunately, the growth rate under water is too fast to take well-focused pictures.

Observations made of crystal growth at room temperature with a free surface but without the addition of water also revealed the frequent appearance of circular crystal growths resembling spherulites but without the maltese cross, which bore a striking resemblance to those grown under water. However, in these cases of growth of 'spherulites' with no maltese cross, it was possible to discern that the growth was occurring preferentially at the surface and subsequently growing down from this surface layer. *Figure 6* shows such a growth with and without crossed polars. The magnitude of birefringence reduces as the growth front is approached, implying that the crystal growth is thinner near its growth front. *Figure 7* shows the variation of 'surface' and bulk growth rates for 'fluff' with temperature. Above about 60°C two distinct types of growth were no longer observed. The chaotic optical texture can be explained in terms of a growth mechanism where the lamellae grow at some angle to the surface, and, since they twist, there is no clearly discernible maltese cross.

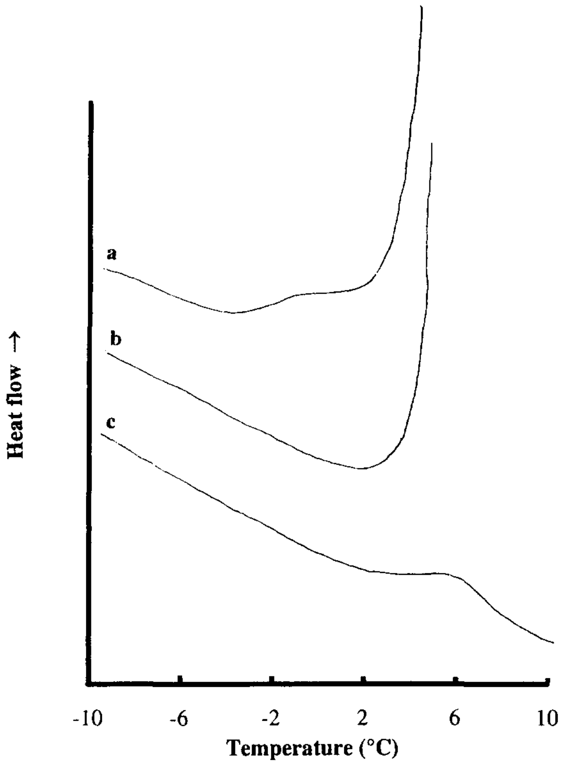

**Figure 4** D.s.c. traces for an 8% HV copolymer heated at 5°C min$^{-1}$ from −50°C after rapid quenching from the melt: (a) 8% HV with an approximately equal mass of water; (b) water only; and (c) 8% HV only

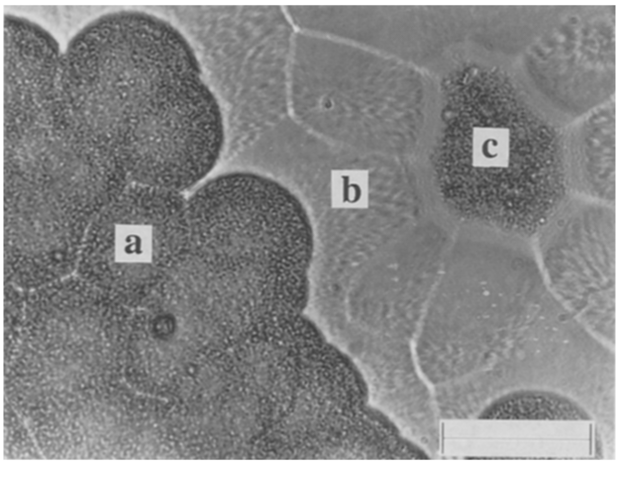

**Figure 5** An optical micrograph, unpolarized light, showing the surface roughening effect of water on an 8% HV copolymer. Water was added while area 'a' was crystallizing, poured off before crystallization of area 'b', and then water was added again before crystallization of area 'c'. Scale bar represents 30 $\mu$m

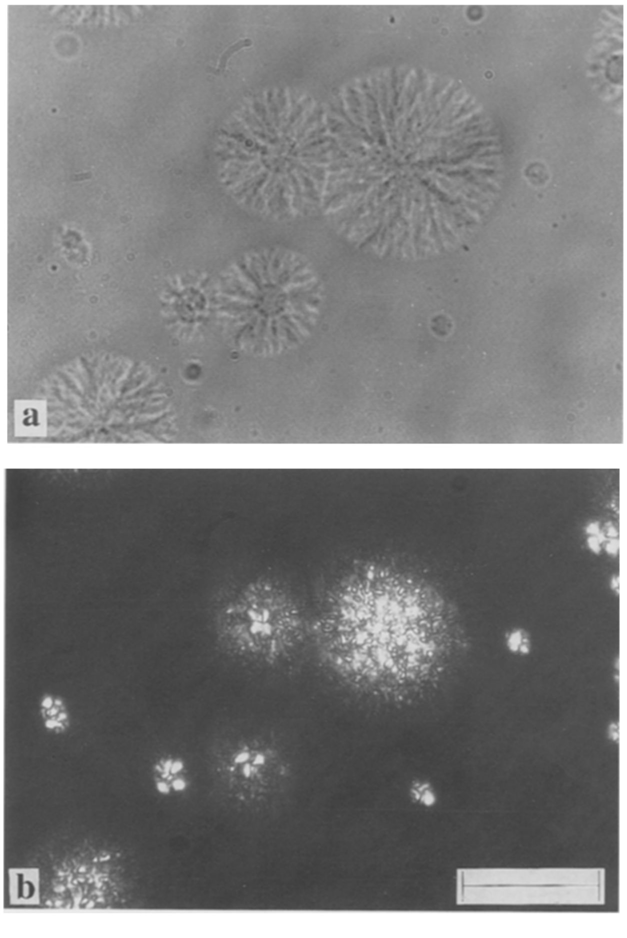

**Figure 6** A series of optical micrographs showing the crystallization of PHB at room temperature with one free surface. (a) Unpolarized light, showing the extent of surface 'spherulite' growth. (b) The same area, taken immediately afterwards but between crossed polars, showing the decrease in magnitude of birefringence as the growth front is approached. Scale bar represents 30 $\mu$m

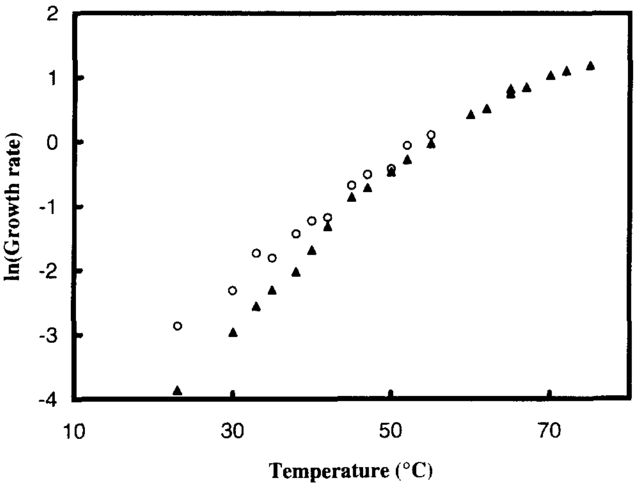

**Figure 7** A graph showing the variation of growth rate of PHB with temperature: ○, surface growth; ▲, bulk growth

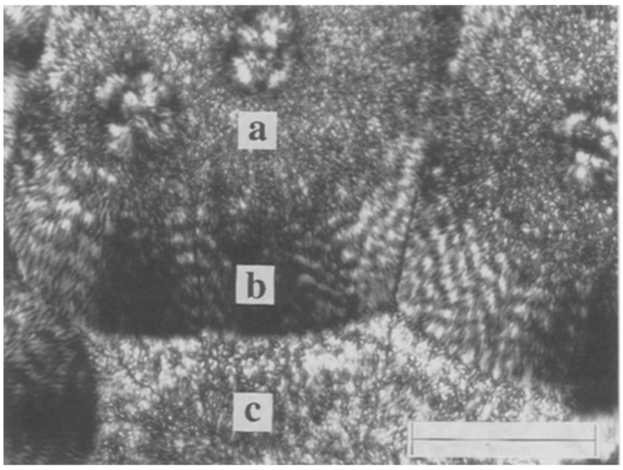

**Figure 8** An optical micrograph (crossed polarizers) showing the effect of phosphorus pentoxide on the optical texture of a thin film of PHB at room temperature. (a) Area crystallized under air, showing chaotic optical texture. (b) Area crystallized in a sealed container under phosphorus pentoxide, showing regularly banded texture. (c) Area crystallized on removal from phosphorus pentoxide into air, showing return to chaotic texture. Scale bar represents 30 $\mu$m

For the homopolymer the appearance of crystal growth under water was similar to that of those crystals which appear to grow preferentially at a free surface. It seems reasonable to suggest that there may also be surface growth under water which is responsible for this optical texture. Alternatively, this different morphology could be evidence of a different growth mechanism at temperatures less than 60°C when crystallization occurs in the presence of water, and the presence of this morphology when no water is added is merely evidence of sufficient atmospheric moisture to access this different mechanism.

On crystallizing a thin film in a sealed container containing phosphorus pentoxide, a powerful desiccant, the growth slowly reverted to the 'normal' type observed without a free surface, i.e. a banded spherulite with a maltese cross. Simply removing the slide from the desiccant before growth was complete was sufficient to revert further growth to the surface type, as shown in *Figure 8*. We therefore infer that the variation in growth rate is related to the relative humidity around the sample. From this we can infer that there would be a range of different growth rates depending on the humidity at which they are taken, lying between the growth rate measured for the sample with water and without. It may be that this humidity effect is partly responsible for the large variation in recorded growth rates for PHB reported in the literature[9,10] as the levels of humidity in different laboratories are unlikely to be the same. It should be noted that this pronounced effect has only been observed for the homopolymer—in the case of the copolymers it is generally necessary to add water to the surface to see a significant increase in growth rate.

The above observations perhaps imply that the water is not fully penetrating and mixing with the polymer, as does the fact that it is possible to simply pour the water off to remove it and return the growth rate to 'normal'. Also, we were not able to grow single crystals in water. This is in contrast to the d.s.c. results, which show a clear glass transition when water is in the d.s.c. pan at a lower temperature than without the water. Either the magnitude of the glass transition in the material at the surface is sufficiently large to cause a noticeable transition or the PHB and water have been able to mix when the PHB was molten.

If we analyse the growth rate data using Hoffman's equations for growth rates[11], we can introduce the change in the glass transition observed using the d.s.c. and look at its theoretical effect on the growth rates. The relevant equation is

$$G = G_0 \exp[-U^*/R(T - T_\infty)] \times \exp[-K\sigma\sigma_e T_m^0/\Delta H\,\Delta T\,kT] \qquad (1)$$

where $G$ is the growth rate, $G_0$ is a constant, $U^*$ is an activation energy for transport of molecules to the growth front, $R$ is the gas constant, $T$ is the crystallization temperature, $T_\infty$ is the temperature below which molecules become immobile, $\Delta T$ is the supercooling, $k$ is Boltzmann's constant, $\sigma$ and $\sigma_e$ are the side and end surface free energies, respectively, and $K$ may take a value of 2 or 4 depending on the growth mechanism.

By plotting $\ln G + U^*/R(T - T_\infty)$ against $1/T\Delta T$ we may gain information about the growth regime in which growth is occurring as well as the surface energies of the polymer crystal. *Figure 9* shows such a plot for growth with and without water, taking $U^*$ as 10 250 kJ mol$^{-1}$ and $T_\infty$ as $T_g - 50$°C from Organ *et al.*[9] and Barham *et al.*[10], with $T_g$ as 4°C in both cases. Using this analysis there appears to be a change in slope for growth under water at about 55°C, possibly implying a change in growth regime. This analysis would give a very large change in $G_0$, and in nucleation rates, a subject which will be discussed below.

An alternative analysis is again to assume that $U^*$ stays constant but to input the changed value of $T_g$ into the water data and again plot both sets of data. We have made such a plot in *Figure 10*, taking $T_g$ with and without water to be −3°C and 4°C, respectively. This appears to collapse the two data sets onto the same line remarkably well. However, we note that the d.s.c. data, from which we deduced the reduction in glass transition temperature, is difficult to interpret as the presence of the ice–water transition interferes with the presence of the glass transition temperature in this polymer.

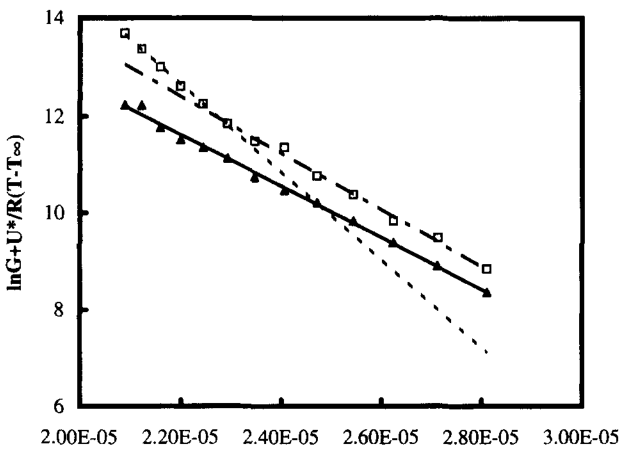

**Figure 9** A graph of $\ln G + U^*/R(T - T_\infty)$ *versus* $1/T\Delta T$ for an 8% HV copolymer with and without water, showing a change in slope at low temperatures when under water: □, under water; ▲, no water

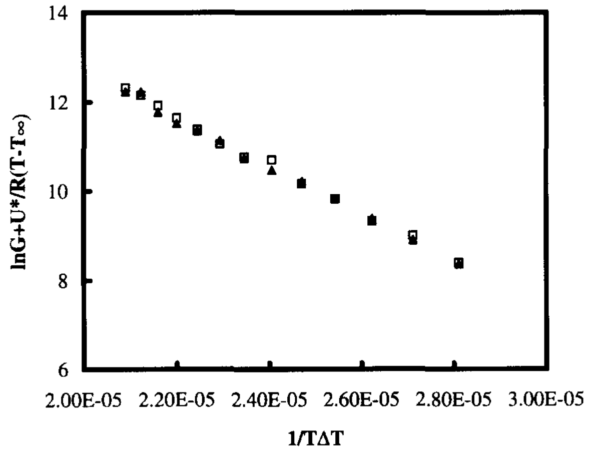

**Figure 10** A graph of $\ln G + U^*/R(T - T_\infty)$ *versus* $1/T\Delta T$ for an 8% HV copolymer with and without water, but with the value of $T_g$ for crystallization under water changed from +4 to −3°C: □, under water; ▲, no water

Both of the above analyses have evidence to support them. The difference in morphology of the homopolymer at temperatures below about 60°C could be evidence of a different crystal phase—possibly of the existence of a liquid crystalline semi-ordered phase before crystallization occurs in the normal orthorhombic structure. This would explain the increased growth rate and the change in morphology, but would also change a number of other terms in the analysis given, particularly the melting point used in the value of $1/T\Delta T$. This explanation would give the pronounced change in slope at the low temperature end of the crystallization curve, which would then lead to a large change in $G_0$. Making the reasonable assumption of a direct relationship between $G_0$ and $I_0$ from nucleation theory[12], and assuming the change in slope comes from a change in $\sigma_e/\Delta T$, this would lead to a massive increase in the nucleation rates for PHB at low temperatures when in the presence of water. At 30°C there will be a maximum increase from $4 \times 10^{-7}$ to $5 \times 10^{-3}$ events per cubic micrometre per second, taking nucleation data from Barham[7]. This could have implications for the de Koning argument[6] about the amorphous state of the bacterial granules, based on a low rate of homogeneous nucleation.

The alternative explanation—that water causes a change in $T_g$—is supported by the d.s.c. data and the remarkable coincidence of the two growth rate data sets when this analysis is used. If this explanation is correct, the nucleation rates would increase by a similar factor to the growth rates—a factor of about 4 at 30°C, which would be of little significance to the de Koning argument about the state of the amorphous granules. We shall be conducting a full study of the nucleation rates of PHB under water in order to answer these questions.

Although this change in $T_g$ is quite significant and produces a relatively large increase in growth rate, it is not sufficiently large to explain some of the effects observed by Marchessault *et al.*[13]. It is clear that the presence in the atmosphere of a weak plasticizer for PHB may have some bearing on its mechanical properties and on the well-documented ageing process[6], and a further study of these processes is being carried out.

## CONCLUSIONS

Water affects the spherulite growth rates in PHB and its copolymers with HV, giving a significant increase over all temperatures at which rates can be measured. Two possible mechanisms for this increase have been suggested. Either there is a change in growth mechanism on contact with water at temperatures below about 60°C, coupled with some change in the activation energy for transport of molecules to the growth front, or the water may be acting as a weak plasticizer for PHB, changing the glass transition, in the case of the 8% HV copolymer, from 4°C without water to −3°C with water. At present, the available data support the second mechanism more strongly.

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