Predictions of thermodynamic efficiency in a pumped biochemical reaction

(6-phosphofructokinase reaction/chemical gradients/oscillations/periodic perturbations/dissipation)

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ABSTRACT We propose and analyze a possible experimental system for the investigation of the thermodynamic efficiency of generating biochemical gradients. We investigate the efficiency of a model pump that uses 6-phosphofructokinase (EC 2.7.1.11, an enzyme that exhibits highly nonlinear kinetics), chromatophores from Rhodobacter sphaeroides, and light to generate a biochemical gradient. We analyze the experimental system and an equivalent alternative configuration and show that the establishment and maintenance of a concentration gradient across a membrane is thermodynamically equivalent to the establishment and maintenance of a stationary state in a single-phase, isothermal, open, homogeneous reaction system. With a constant input of light, the system can exist in a stable node (a stable steady state), a stable focus (upon perturbation from its steady state, the system returns to its steady state with an oscillatory component), and a stable limit cycle (at steady state the system exhibits stable oscillations). We investigate the efficiency of the system with both steady and oscillatory light input and observe efficiency changes that depend upon the autonomous system of the system and the frequency and amplitude of the periodic light input. When the system is in a stable focus, an efficiency maximum is seen when the system is perturbed at its resonant frequency. When the system is in a stable limit cycle, efficiency increases are seen near the 1:3, 1:2, and 2:1 entrainment regions and an efficiency decrease is seen near the 1:1 entrainment region. We further calculate various contributions to the efficiency: the phase shift of the force and flux, the magnitude of the response to the perturbation, and changes in the average values of the force and flux during a perturbation. We show that all three changes contribute to the overall changes in efficiency, but increases and decreases in the average force make the largest contributions.

We propose and analyze a possible experimental system for a study of the production of gradients of (bio)chemical species. The gradients may be used to do work in the surroundings of the system and the subject is closely related to the issue of efficiency of biological pumps. Prior articles have considered the thermodynamic efficiency of nonlinear model systems (1–3), combustion processes (4–7), the production of ATP in glycolysis (8–13), and the thermodynamic efficiency of utilization of ATP in a simple model of a proton pump (14, 15). In these studies, two modes of operation are investigated. In one mode, the system is in a stable attractor (a stationary state, either a node or a focus; a stable limit cycle) maintained by the constant influx of reactants; in the other mode, there are externally imposed oscillations of constraints such as the influx of ATP in the case of the proton pump, and of fuel influx in combustion. Nonlinearities in these systems far from equilibrium are sufficient to lead to variable dissipation and hence variable thermodynamic effi-

Fig. 1. The proposed experimental system consists of two compartments (A and B) separated by a semipermeable membrane. In A, light and chromatophores (Chr) produce ATP from ADP and inorganic phosphate (Pi). ATP and fructose 6-phosphate (F6P) then react to form fructose 1,6-bisphosphate (FDP) and ADP, catalyzed by 6-phosphofructokinase (PFK). In B, FDP is converted to F6P and Pi by fructose-bisphosphatase (FDPase). At steady state a gradient exists such that FDP_A > FDP_B and F6P_A < F6P_B.

POSSIBLE EXPERIMENTAL SYSTEM

In Fig. 1, we give a schematic representation of a proposed experimental system. White light of a given intensity is made to illuminate the entire subsystem A. Chromatophores (Chr) from Rhodobacter sphaeroides, in A, absorb a fraction of the light in combination with ADP and Pi of concentrations ADP_A and Pi_A, and produce ATP, of concentration ATP_A. This energetic species combines with F6P (concentration, F6P_A) and produces FDP in a reaction catalyzed by the enzyme PFK. If the rates of reactions exceed those of diffusion, then the concentration of FDP in compartment A (with volume V_A), FDP_A, may exceed that in compartment B (volume V_B), FDP_B, and diffusion occurs across the membrane M (volume V_M, thickness e). In B, the regeneration of FDP takes place in the presence of the enzyme FDPase. As the concentration of F6P_B exceeds that of F6P_A, a flow of that substance from A to B takes place. Thus, for a given light intensity, given initial concentrations of all chemical species,

Abbreviations: F6P, fructose 6-phosphate; FDP, fructose 1,6-bisphosphate; PFK, 6-phosphofructokinase; FDPase, fructose-bisphosphatase; Pi, inorganic phosphate.

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and given rate and transport coefficients across the membrane, a stationary state is achieved after a transient period. In that stationary state, there exists a gradient of FDP such that FDP_B > FDP_A and a gradient of F6P such that F6P_A > F6P_B. The light energy absorbed by the system in volume V_A establishes and maintains a chemical potential difference between FDP_A and (F6P_A + Pi_A) that may then be used to do work in the surroundings of the system.

For the analysis of the thermodynamics of this system, it is helpful to construct an alternative configuration, shown in Fig. 2. Here the light again illuminates the entire volume V_A. As the reaction of ATP and F6P occurs, the concentration of F6P in volume V_A is kept constant by a flow of F6P from reservoir Y, with constant concentration of F6P_y, and the Pi concentration is kept constant by an influx of Pi from reservoir X. Similarly, the concentration of FDP_A is kept constant by an influx of that substance into reservoir Z. The concentrations in the reservoirs are chosen to be identical to the concentrations of the respective species in volume V_A at the given stationary state. We see from the arrangement given in Fig. 2 that the energy input of the light absorbed produces a transfer of F6P_y and Pi_x to FDP_z according to the stoichiometric equation

$$F6P_A + Pi_A \rightarrow FDP_A,$$

where the symbols denote both the species and the concentrations, and for which $\Delta G_1$ denotes the Gibbs free energy change of reaction 1.

The advantage of the proposed experimental arrangement in Fig. 1 over that in Fig. 2 is related to the fact that the arrangement in Fig. 1 constitutes a closed system of constant mass, open only to light input. The method of using a flux of light to displace a closed chemical system far from equilibrium (16–18) is experimentally simpler than the displacement from equilibrium by mass fluxes. In Fig. 2, the subsystem composed of V_A is clearly open to mass fluxes and light input, but this configuration allows an easier conceptualization of the power output of the system, which will be shown to be the same as that in Fig. 1. The system specified in Fig. 1 is fully determined by the initial concentrations of all chemical species, the light intensity, and the volumes of the subsystems. At stationary state we need to measure the concentrations FDP_A and F6P_A in order to determine the thermodynamic efficiency.

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**Fig. 2.** Alternative but equivalent configuration of proposed experimental system. In A, light and chromatophores (Chr) produce ATP from ADP and Pi. ATP and F6P react to form FDP at concentration FDP_z. Work can be done by the system by the conversion of FDP back to F6P and Pi through an external cycle. F6P and Pi are supplied from reservoirs X and Y.

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**Fig. 3.** Dynamical domains of the system of Eqs. 3, obtained numerically by the use of the AUTO program, as a function of parameter $\sigma_1$ (proportional to the input light intensity, $I_0$) and the total fructose phosphates concentration, FXP (see Eqs. 2), showing domains of stable nodes (sn) and foci (sf) and of unstable foci (uf)—that is, oscillations. Vertical bars correspond to the amplitudes of perturbations imposed on the system (see Fig. 4). AXP = 150, $\rho = 0.2$, $\sigma_2 = 300$, and $\sigma_3 = 150$. Case I: FXP = 400 and $\sigma_1 = 100$. Case II: FXP = 450 and $\sigma_2 = 100$. Case III: FXP = 500 and $\sigma_3 = 80$.

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**THEORETICAL ANALYSIS**

**Kinetic Equations.** By taking into account the mass conservation of the various chemicals, that is,

$$AXP = \text{Const.} = \Sigma (ATP_1 + ADP_1 + Pi_1)$$

and

$$FXP = \text{Const.} = \Sigma (F6P_1 + FDP_1),$$

the whole system shown in Fig. 1 is fully defined by a set of six kinetic equations, set to zero at stationary state,

$$\frac{dATP_A}{dt} = -V_m,PFK \Phi (ATPA, F6PA) + V_m,Chr \Phi (ADPA, I_0)$$

$$\frac{dATP_B}{dt} = \rho \frac{D_M V_M}{e^2 V_A} (ATPB - ATPA)$$

$$\frac{dF6PA}{dt} = -V_m,PFK \Phi (ATPA, F6PA)$$

$$\frac{dF6PB}{dt} = V_{m,FDPase} \Phi (FDPB)$$

$$\frac{dFDPB}{dt} = V_{m,FDPase} \Phi (FDPB)$$

$$\frac{dATPA}{dt} = V_{m,PFK} \Phi (ATPA, F6PA) - V_{m,Chr} \Phi (ADPA, I_0)$$

$$+ \rho \frac{D_M V_M}{e^2 V_B} (ADPB - ADPA),$$

$$\frac{dADPB}{dt} = \rho \frac{D_M V_M}{e^2 V_B} (ADPB - ADPA).$$
where (see ref. 19)

\[
\mathcal{F}(\text{ATP}_A, \text{F6P}_A) = \frac{k \text{ATP}_A \text{F6P}_A}{2K_{m,\text{ATP}}} \left[ \frac{1 + \frac{\text{ATP}_A}{K_{m,\text{ATP}}} + \frac{\text{F6P}_A}{2K_{m,\text{ATP}}} + \frac{\text{ATP}_A \text{F6P}_A}{2K_{m,\text{ATP}}^2}}{1 + \frac{\text{ATP}_A}{K_{m,\text{ATP}}} + \frac{\text{F6P}_A}{2K_{m,\text{ATP}}} + \frac{\text{ATP}_A \text{F6P}_A}{2K_{m,\text{ATP}}^2}} \right]^4 + L^* \left[ \frac{1 + \frac{c \text{ATP}_A}{K_{m,\text{ATP}}} + \frac{d \text{F6P}_A}{K_{m,\text{ATP}}} + \frac{c d \text{ATP}_A \text{F6P}_A}{2K_{m,\text{ATP}}^2}}{1 + \frac{c \text{ATP}_A}{K_{m,\text{ATP}}} + \frac{d \text{F6P}_A}{K_{m,\text{ATP}}} + \frac{c d \text{ATP}_A \text{F6P}_A}{2K_{m,\text{ATP}}^2}} \right]^4
\]

with \(L^* = L\)

\[
F(\text{ADP}_A, I_0) = \frac{I_0}{K_f + I_0 K_{m,\text{ADP}} + \text{ADP}_A}
\]

\[
F(\text{FDP}_B) = \frac{\text{FDP}_B}{K_{m,\text{FDP}} + \text{FDP}_B}
\]

In the above expressions, \(V_A, V_B,\) and \(V_M\) represent respectively the volumes of reservoirs A and B and the volume of the inactive membrane (of thickness \(e\)) that separates A and B. The parameter \(\rho\) is the ratio \(D_A/D_B\), where \(D_A\) and \(D_B\) are respectively the diffusion coefficients of the AXP and FXP species across the membrane. \(V_m\) and \(K_m\) refer respectively to the maximal activities and half-saturation concentrations (and/or intensity) of enzymes PFK (\(K_{m,\text{ATP}}\)) and FDPase (\(K_{m,\text{FDP}}\)) and of chromatophores (\(K_f\) and \(K_{m,\text{ADP}}\)). \(r, c, d, L, k_1,\) and \(k_2\) are constants (AUTO program package, produced by E. J. Doedel of the Department of Applied Mathematics at the California Institute of Technology; a monograph describing the latest version is available from him).

The system of Eqs. 3 is solved numerically with the use of the AUTO program. A nondimensional form of the equations is used for all calculations (21). Some of the dynamic features of the system are shown in Fig. 3, in which we indicate the regions of stable states, which are stable nodes (on perturbation from such a stable state the system decays monotonically back to the stationary state) or stable foci (in which after a perturbation the system returns to the stationary state with an oscillatory component), and the regions of stable oscillations.

**Power Input.** The power input, \(P_{in}\), into the system is defined to be the incident light intensity, i.e., the light energy supplied to the system per unit time:

\[
P_{in} = I_0.
\]

[4]

Other definitions of \(P_{in}\) are possible (see ref. 22).

**Power Output.** The power output, \(P_{out}\), of the alternative system shown in Fig. 2 is

\[
P_{out} = \Delta G_1 J_{\text{FKK}}.
\]

[5]

with \(\Delta G_1 = \Delta G_{o,1} + RT \ln \left( \frac{\text{FDP}_A}{\text{F6P}_A \text{P}_A} \right)\)

[6]

and \(J_{\text{FKK}} = V_m J_{\text{FKK}} F(\text{ATP}_A, \text{F6P}_A)\).

[7]

In the system shown in Fig. 2, the power output produces work in unit time, the work of transferring F6P and P1 to FDP at the indicated concentrations. In the proposed experimental system, the power output of the subsystem A is the same, but that power output is dissipated in the transference of FDP from A to B, the chemical reaction of FDPB to (F6PB + P1B), and the dissipation due to the transport of F6P and P1 from concentration B to A. The sum of the dissipation in these three steps equals the power output of subsystem A. This analysis shows that the establishment and maintenance of a concentration gradient across a membrane is thermodynamically equivalent to the establishment and maintenance of a stationary state in a single-phase, isothermal, open, homogeneous reaction system. For the sake of the experimental convenience of a system closed to mass flow, we dissipate the power output instead of using it in the surroundings.

**Efficiency of Energy Transduction.** The experimental system proposed in Fig. 1 can be considered either as an energy-transduction or a power-transduction engine, in which light energy per unit time is converted to Gibbs free energy difference per unit time. We define the efficiency, \(\eta\), of the power transduction as the ratio of the power output to the power input (23, 24),

\[
\eta = \frac{P_{out}}{P_{in}}.
\]

[8]

where the angle brackets extend over one period of oscillation of the input of light.

We wish to compare this efficiency under stationary-state conditions, in which the power input (light intensity) is constant in time, to that under conditions in which the light intensity varies sinusoidally with a given amplitude and frequency. The efficiency in the oscillatory mode of operation of the system can be altered from that in the stationary state mode in different ways: for example, it can be increased by increasing the numerator on the average, or by decreasing the denominator on the average, or a combination of both.

In Fig. 4 we show a calculation of the ratio of the efficiency with an oscillatory input of light to that with a constant input of light \((E/E_o)\) plotted against the ratio of the frequency of

![Fig. 4. Relative efficiency (\(\eta/\eta_{0}\) of the perturbed system divided by the efficiency of the autonomous system) plotted against the relative frequency (\(W/W_o\) of perturbation divided by the autonomous frequency) for the three cases shown in Fig. 3. Case I (\(\bullet\)); FXP = 400 and 75 < \(\alpha_1\) < 125. Case II (\(\bigcirc\)); FXP = 450 and 90 < \(\alpha_1\) < 110. Case III (\(\diamond\)); FXP = 500 and 72 < \(\alpha_1\) < 88. All other parameter values are as in Fig. 3.](image-url)
alteration of light intensity to the inherent frequency of the system in a stable focus with constant input of light ($W/W_0$). We use the definition of power input given by Eq. 4, and Eq. 5 for power output, and an amplitude of perturbation indicated by bars in Fig. 3. The conditions for the three curves are given in the caption of Fig. 4. For two cases (I and II) the system under steady illumination is in a stable focus, and for the third case (III) the system is in a limit cycle. There are modest changes in the efficiency ratio for all cases, depending on frequency and amplitude of the imposed variation in light intensity. If the system under steady illumination is in a stable focus, then a maximum in the ratio of efficiencies occurs near the natural frequency of relaxation to the stationary state. However, if the system is in a limit cycle when under steady illumination, then upon application of an oscillatory light input there are slight increases in the efficiency ratio near the so-called 1:3, 1:2, and 2:1 entrainment bands, and a decrease near the 1:1 (or fundamental) entrainment band. Similar variations have been observed in other cases (2, 13).

We have made further calculations in an attempt to define the sources of the changes in efficiency that occur upon the imposition of an oscillatory constraint. Previous studies have shown that phase shifting between the forces and fluxes in a driven nonlinear system may be responsible for efficiency changes (3, 10, 25). We have analyzed the phase relationship of $\Delta G_1$ (Eq. 6) and $J_{\text{PFK}}$ (Eq. 7) in the time series of cases I and III. Because the waveshape of the response is not always symmetric, we define the centers of the peaks by locating two minima for each peak defining the first moment of the time between the two minima. Signals that are totally in phase have the same time first moment. The phase difference between out-of-phase signals is determined by finding the difference between the centers of the peaks and dividing by the period. The resulting phase difference is measured in units of $2\pi$ or divided by the phase difference in the unperturbed (but oscillatory) mode. We present the result of these calculations in Fig. 5a and b for cases I and III.

For case I, a perturbed stable focus, the phase difference between $\Delta G_1$ and $J_{\text{PFK}}$ shows a sharp increase near the 1:2 entrainment band and a more modest maximum at the 1:1 entrainment band. For case III, a perturbed limit cycle, the phase difference rapidly decreases for perturbations of very low frequencies. As the frequency of the perturbation increases, the magnitude of the phase difference increases and then levels off at a frequency ratio near 1.75. At this perturbation frequency, the response of the system period doubles, and a consequent sharp drop in the phase difference between $\Delta G_1$ and $J_{\text{PFK}}$ is seen. As the frequency is increased through the 2:1 entrainment region, the phase difference again grows until the period again doubles and the phase difference abruptly decreases. A minimum phase difference between $\Delta G_1$ and $J_{\text{PFK}}$ should have a positive effect on the power output of the system. In both case I and case III, phase shifting between the force, $\Delta G_1$, and the flux, $J_{\text{PFK}}$, occurs as a result of a perturbation to the light intensity; however, phase shifting as a result of a periodic perturbation is not the only factor affecting the efficiency.

The second calculated result is a measurement of the magnitude of the response of the system to the perturbation. If phase shifting occurs between the forces and fluxes, the magnitude of the response of the system to the oscillatory perturbation determines how much the phase shifting will affect the efficiency. We have measured the magnitude of the response of the system to the oscillatory perturbation by calculating the standard deviation of the signal from its average over one period. In Fig. 6a, we present our results for case I. The response of the system is expressed as the standard deviation divided by the average value of $\Delta G_1$ or $J_{\text{PFK}}$ over a period. In both cases, the response of the system is greatest at the 1:1 resonant frequency. The response of $J_{\text{PFK}}$, however, is of a much larger magnitude than that for $\Delta G_1$. Our results for case III are presented in Fig. 6b. In this figure the response is calculated as the standard deviation of $\Delta G_1$ or $J_{\text{PFK}}$ over a period in the perturbed mode divided by the standard deviation of $\Delta G_1$ or $J_{\text{PFK}}$ in the unperturbed oscillatory mode. Both the response of $\Delta G_1$ and that of $J_{\text{PFK}}$ show maxima near frequency ratios of 0.75 and 2.0, and again the response of $J_{\text{PFK}}$ is greater than the response of $\Delta G_1$.

The average values of the forces and fluxes of a driven nonlinear system may also change upon the imposition of an external periodic perturbation, and these changes may have consequent effects on the efficiency. We have calculated the average $\Delta G_1$ and $J_{\text{PFK}}$ over one period of the system's

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![Fig. 5](attachment:image5.png)

**Fig. 5.** Phase difference between $\Delta G_1$ and $J_{\text{PFK}}$ in units of $2\pi$ for case I (\(\triangleright\)) and relative phase difference (phase difference of perturbed system divided by the autonomous phase difference) for Case III (\(\bigtriangleup\)) plotted against the relative frequency ($W/W_0$).

![Fig. 6](attachment:image6.png)

**Fig. 6.** (a) Responses of $\Delta G_1$ (\(\triangleright\)) and $J_{\text{PFK}}$ (\(\bigtriangleup\)) to a periodic perturbation in light intensity expressed as the standard deviation of variation about the average over one period divided by the average values, plotted against relative frequency ($W/W_0$) for case I in Fig. 3. (b) Responses of $\Delta G_1$ (\(\triangleright\)) and $J_{\text{PFK}}$ (\(\bigtriangleup\)) expressed as a ratio of the standard deviation over a period in the perturbed mode to the standard deviation over a period in the unperturbed (but oscillatory) mode, plotted against relative frequency ($W/W_0$) for case III in Fig. 3.
greater divided than the increase decreased.

response, or over a large number of periods if the response of the system is quasiperiodic. Our results for cases I and III are shown in Fig. 7a and b, respectively. For case I, the average $\Delta G_1$ and $J_{\text{PK}}$ are maximal at the resonant frequency and decrease as the frequency of the perturbation is increased. At low frequencies, the averages may be slightly greater than in the unperturbed case, while at high frequencies, the averages approach the same value as in the unperturbed case. The increase in $\Delta G_1$ (7%) is significantly larger than the increase in the average value of $J_{\text{PK}}$ (4%). For case III (Fig. 7b), the change in average $\Delta G_1$ closely resembles the efficiency changes. The change in average $J_{\text{PK}}$ shows a small maximum near the 1:2 entrainment region, a sharp decrease to a minimum near the 1:1 entrainment region, and a very broad but small maximum at the 2:1 entrainment region. Again, the changes in average $\Delta G_1$ are of much greater magnitude than the changes in the average value of $J_{\text{PK}}$.

**DISCUSSION**

We analyze the thermodynamic efficiency of the formation of a chemical potential difference between chemical species by a nonlinear reaction far from equilibrium, as in biological pumps, and discuss that efficiency for conditions of constant and oscillatory input of one of the reactants. Three factors interact to produce efficiency changes in driven nonlinear systems far from equilibrium. Phase shifting may occur between the forces and the fluxes of the system, influencing the efficiency of the system through variable overlap of the periodic signals. The effect of the phase shifting, however, is modulated by the magnitude of the response of the system. A large phase shift in a system that shows only a small response to a perturbation has only a small effect on the efficiency. A third effect resulting from an external periodic perturbation is changes in the average values of the forces and fluxes. In the present system, all three effects contribute to the efficiency changes, although these changes follow most closely the changes in the average $\Delta G_1$, especially for case III. We may postulate, then, that a large percentage of the efficiency changes occurs because FDP is pumped through a larger $\Delta G_1$, on average, during a perturbation of the proper frequency. The proposed experimental system provides a method of experimental verification for efficiency changes in a driven biochemical pump (20). Moreover, the sources for those efficiency changes can be analyzed and compared to the calculated results.

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