Solution of some equations in Biochemistry

J.P Bennett and J.H. Davenport
School of Mathematical Sciences
University of Bath
Claverton Down
Bath BA2 7AY
England

and

H.M. Sauro
Institue of Grassland and Animal Production
Poultry Division
Roslin,
and Midlothian
Scotland

Abstract It is possible to write down the equations governing a one-stage enzyme-catalysed reaction (according to Michaelis-Menton kinetics) quite easily, and deduce information about the steady-state flow in such a system. The situation is somewhat more complicated if several such reactions form a linear chain. We have applied Gröber-basis techniques to solve such systems.

Introduction.

If we consider an enzyme-catalysed reaction in steady-state, such as

$$X_0 \xrightarrow{v} X_1$$

(with the underlying first-order mechanism

$$X_0 + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X_0E \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X_1 + E$$

in which we have written

$$\begin{aligned} K_{eq} &= \frac{k_1 k_3}{k_2 k_4} && \text{(overall equilibrium constant)} \\ K_{m,f} &= \frac{k_2 + k_3}{k_1} && \text{(forward Michaelis constant)} \\ K_{m,r} &= \frac{k_2 + k_3}{k_4} && \text{(reverse Michaelis constant)} \\ V_{max} &= [E_{TOT}]k_3 && \text{(maximum flux)} \\ [E_{TOT}] &= [E] + [X_0E] && \text{(conservation of enzyme)} \end{aligned}$$

as is standard) the rate of conversion of X_0 to X_1 (flux) can be written as

$$v = \frac{\frac{V_m}{K_m}\left([X_0] - [X_1]K_{eq}\right)}{1 + \frac{[X_0]}{K_{m,f}} + \frac{[X_1]}{K_{m,r}}}. \tag{1}$$

When the reactions are more complex, e.g.

$$X_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} X_2,$$

it becomes harder to analyse the situation, even under the steady-state assumption that $[S_1]$ is constant. We would like to eliminate $[S_1]$ from the two equations (analogous to (1)) which determine the fluxes in the two stages. This has been done [...], and the result is that $[S_1]$ satisfies a quadratic equation. As far as the authors are aware, the case of three-stage linear chains has not been solved.

Our solution.

The three-stage chain is

$$X_0 \xrightarrow{v_1} S_1 \xrightarrow{v_2} S_2 \xrightarrow{v_3} X_3.$$

From the point of view of Gröbner-bases, the problem is, in principle, trivial. We have the equation $v_1 = v_2 = v_3$ at steady state, and all that is required is to eliminate the variables $[S_1]$ and $[S_2]$ from this. We used REDUCE-3 [Hearn, 1983] running on a Sun 3/160 and a Gröbner-basis package written by the second author. The only delicate point is the ring in which the Gröbner-basis is to be calculated: this is $L[s_1, s_2]$ where s_1 and s_2 are $[S_1]$ and $[S_2]$ (trying to mix biochemical notation with that of modern algebra is confusing at best!), where L is the field generated over the rational by all the other indeterminates that appear in the equations. The REDUCE input to solve this problem is surprisingly simple:

```
v1:=(v1m/k1mf)*(x0-s1*k1eq)/(1+x0/k1mf+s1/k1mr);
grobner!-constant v1m,k1mf,k1mr,k1eq;
v2:=(v2m/k2mf)*(s1-s2*k2eq)/(1+s1/k2mf+s2/k2mr);
grobner!-constant v2m,k2mf,k2mr,k2eq;
v3:=(v3m/k3mf)*(s2-x3*k3eq)/(1+s2/k3mf+x3/k3mr);
grobner!-constant v3m,k3mf,k3mr,k3eq;
grobner!-constant x0,x3;
grobner(num(v1-v2),num(v1-v3));
```

The calls to `grobner!-constant` declare that the indeterminates mentioned belong in L, rather than to the polynomial extension of L. The call to `grobner` passes in two polynomials (the numerators of $v_1 - v_2$ and $v_1 - v_3$), and computes a lexicographical-order Gröbner-basis for the resulting ideal of $L[s_1, s_2]$. The calculation took about five seconds, compared with one minute if all three equations are given to `grobner` (if only $v_1 - v_2$ and $v_2 - v_3$ are given to `grobner`, the calculation runs for about 15 minutes before exceeding the 2Mb heap available in our version of REDUCE). It turns out that s_1 satisfies a cubic equation, reproduced in the appendix, and s_2 is linear in s_1 (the details are also in the appendix). REDUCE is capable of generating equations in FORTRAN form, and machine-readable copies of these equations are obtainable from the first author.

Conclusions.

Acknowledgements. The Gröbner-basis package used was written while the second author was visiting the Royal Technical Highschool, Stockholm, and the author would like to acknowledge the hospitality and helpful suggestions of Stefan Arnborg and Ian Cohen.

References

Appendix.

The cubic satisfied by s_1 can be written as

$$as_1^3 + bs_1^2 + cs_1 + d = 0,$$

where the coefficients are (in the order a, b, c, d)

$$-v_{max,1}^3 k_{eq,1}^3 k_{m,1,r}^3 k_{m,2,r} k_{m,3,r} - v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 v_{max,2} k_{m,2,r} k_{m,3,r}$$
$$-2v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,r} v_{max,3} k_{m,3,r} - 2v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$-v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} k_{m,2,r} v_{max,3}^2 k_{m,3,r} - k_{m,1,f}^3 v_{max,2} k_{m,2,r} v_{max,3}^2 k_{m,3,r},$$

$$x_3 v_{max,1}^3 k_{eq,1}^3 k_{m,1,r}^3 k_{m,2,f} k_{m,3,f} - x_3 v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f}$$
$$+x_3 v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} v_{max,3} k_{m,3,f} - x_3 v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{m,3,f}$$
$$+x_3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} k_{m,2,f} v_{max,3}^2 k_{eq,3} k_{m,3,r} + x_3 k_{m,1,f}^3 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3}^2 k_{eq,3} k_{m,3,r}$$
$$+2 v_{max,1}^3 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,r} k_{m,3,r} - v_{max,1}^3 k_{eq,1}^3 k_{m,1,r}^3 k_{m,2,f} k_{m,2,r} k_{m,3,r} + v_{max,1}^3 k_{eq,1}^3 k_{m,1,r}^3 k_{m,2,f} k_{m,3,f} k_{m,3,r}$$
$$+v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{m,2,r} k_{m,3,r} + 3 v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$-v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^3 v_{max,2} k_{m,2,r} k_{m,3,r} - v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$-v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} k_{m,3,r} - 2 v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$+v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} v_{max,3} k_{m,3,f} k_{m,3,r} - v_{max,1}^2 x_0 k_{eq,1}^2 k_{m,1,r}^3 v_{max,2} k_{m,2,r} k_{m,3,r}$$
$$-v_{max,1}^2 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,r} v_{max,3} k_{m,3,r} + v_{max,1} k_{m,1,f}^2 x_0 k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$+v_{max,1} k_{m,1,f}^2 x_0 k_{m,1,r} k_{m,2,r} v_{max,3}^2 k_{m,3,r} - 3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$-v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r}^2 k_{m,2,r} v_{max,3}^2 k_{m,3,r} - v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{m,3,f} k_{m,3,r}$$
$$-v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r} k_{m,2,f} k_{m,2,r} v_{max,3}^2 k_{m,3,r} - 3 v_{max,1} k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$-v_{max,1} k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,r} v_{max,3}^2 k_{m,3,r} - 2 k_{m,1,f}^3 k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3}^2 k_{m,3,r}$$
$$-2 k_{m,1,f}^2 x_0 k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3}^2 k_{m,3,r},$$

$$-2 x_3 v_{max,1}^3 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} k_{m,3,f} + x_3 v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f}$$
$$-2 x_3 v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,f} v_{max,3} k_{m,3,f} + x_3 v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^3 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} - x_3 v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1}^2 x_0 k_{eq,1}^2 k_{m,1,r}^3 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} - x_3 v_{max,1}^2 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$+x_3 v_{max,1} k_{m,1,f}^2 x_0 k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{m,3,f} + x_3 v_{max,1} k_{m,1,f}^2 x_0 k_{m,1,r} k_{m,2,f} v_{max,3}^2 k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{m,3,f}$$
$$+2 x_3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1} k_{m,1,f}^2 k_{eq,1} k_{m,1,r}^2 k_{m,2,f} v_{max,3}^2 k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1} k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{m,3,f}$$
$$+2 x_3 v_{max,1} k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{eq,3} k_{m,3,r}$$
$$-x_3 v_{max,1} k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,f} v_{max,3}^2 k_{eq,3} k_{m,3,r}$$
$$+2 x_3 k_{m,1,f}^3 k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3}^2 k_{eq,3} k_{m,3,r} + 2 x_3 k_{m,1,f}^2 x_0 k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3}^2 k_{eq,3} k_{m,3,r}$$
$$-v_{max,1}^3 x_0^2 k_{eq,1} k_{m,1,r}^3 k_{m,2,r} k_{m,3,r} + 2 v_{max,1}^3 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} k_{m,2,r} k_{m,3,r}$$
$$-2 v_{max,1}^3 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} k_{m,3,f} k_{m,3,r} - v_{max,1}^2 k_{m,1,f} x_0^2 k_{m,1,r}^2 k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$+v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^3 v_{max,2} k_{m,2,r} k_{m,3,r} + v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^3 k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$+v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} k_{m,3,r} + 3 v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,f} k_{m,2,r} v_{max,3} k_{m,3,r}$$
$$-2 v_{max,1}^2 k_{m,1,f} x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,f} v_{max,3} k_{m,3,f} k_{m,3,r} - v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^3 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} k_{m,3,r}$$
$$-v_{max,1}^2 k_{m,1,f} k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} k_{m,2,r} v_{max,3} k_{m,3,r} + v_{max,1}^2 x_0^2 k_{eq,1} k_{m,1,r}^3 v_{max,2} k_{m,2,r} k_{m,3,r}$$
$$+v_{max,1}^2 x_0^2 k_{eq,1} k_{m,1,r}^3 k_{m,2,r} v_{max,3} k_{m,3,r} - v_{max,1}^2 x_0 k_{eq,1}^2 k_{m,1,r}^3 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} k_{m,3,r}$$
$$-v_{max,1}^2 x_0 k_{eq,1}^2 k_{m,1,r}^3 k_{m,2,f} k_{m,2,r} v_{max,3} k_{m,3,r} + v_{max,1} k_{m,1,f}^2 x_0 k_{m,1,r}^2 v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}$$

$$
\begin{gathered}
+v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}^2k_{m,2,r}v_{max,3}^2k_{m,3,r}+v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f}k_{m,3,r} \\
+v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}k_{m,2,f}k_{m,2,r}v_{max,3}^2k_{m,3,r}-v_{max,1}k_{m,1,f}^2k_{eq,1}k_{m,1,r}^3v_{max,2}k_{m,2,r}v_{max,3}k_{m,3,r} \\
-v_{max,1}k_{m,1,f}^2k_{eq,1}k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f}k_{m,3,r} \\
-v_{max,1}k_{m,1,f}^2k_{eq,1}k_{m,1,r}^2k_{m,2,f}k_{m,2,r}v_{max,3}^2k_{m,3,r} \\
+v_{max,1}k_{m,1,f}x_0^2k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}k_{m,3,r}+v_{max,1}k_{m,1,f}x_0^2k_{m,1,r}^2k_{m,2,r}v_{max,3}^2k_{m,3,r} \\
-2v_{max,1}k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^3v_{max,2}k_{m,2,r}v_{max,3}k_{m,3,r} \\
-v_{max,1}k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f}k_{m,3,r} \\
-v_{max,1}k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^2k_{m,2,f}k_{m,2,r}v_{max,3}^2k_{m,3,r}-v_{max,1}x_0^2k_{eq,1}k_{m,1,r}^3v_{max,2}k_{m,2,r}v_{max,3}k_{m,3,r} \\
-k_{m,1,f}^3k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r}-2k_{m,1,f}^2x_0k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r} \\
-k_{m,1,f}x_0^2k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r}
\end{gathered}
$$

and

$$
\begin{gathered}
x_3v_{max,1}^3x_0^2k_{eq,1}k_{m,1,r}^3k_{m,2,f}k_{m,3,f}+x_3v_{max,1}^2k_{m,1,f}x_0^2k_{m,1,r}^2k_{m,2,f}v_{max,3}k_{m,3,f} \\
+x_3v_{max,1}^2k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}+x_3v_{max,1}^2k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^3k_{m,2,f}v_{max,3}k_{eq,3}k_{m,3,r} \\
+x_3v_{max,1}^2x_0^2k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}+x_3v_{max,1}^2x_0^2k_{eq,1}k_{m,1,r}^3k_{m,2,f}v_{max,3}k_{eq,3}k_{m,3,r} \\
+x_3v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f}+x_3v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}^2k_{m,2,f}v_{max,3}^2k_{eq,3}k_{m,3,r} \\
+x_3v_{max,1}k_{m,1,f}^2k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{eq,3}k_{m,3,r} \\
+x_3v_{max,1}k_{m,1,f}x_0^2k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f} \\
+x_3v_{max,1}k_{m,1,f}x_0^2k_{m,1,r}^2k_{m,2,f}v_{max,3}^2k_{eq,3}k_{m,3,r} \\
+2x_3v_{max,1}k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{eq,3}k_{m,3,r} \\
+x_3v_{max,1}x_0^2k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{eq,3}k_{m,3,r}+x_3k_{m,1,f}^3k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}^2k_{eq,3}k_{m,3,r} \\
+2x_3k_{m,1,f}^2x_0k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}^2k_{eq,3}k_{m,3,r}+x_3k_{m,1,f}x_0^2k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}^2k_{eq,3}k_{m,3,r} \\
-v_{max,1}^3x_0^2k_{eq,1}k_{m,1,r}^3k_{m,2,f}k_{m,2,r}k_{m,3,r}+v_{max,1}^3x_0^2k_{eq,1}k_{m,1,r}^3k_{m,2,f}k_{m,3,f}k_{m,3,r} \\
-v_{max,1}^2k_{m,1,f}x_0^2k_{m,1,r}^2k_{m,2,f}k_{m,2,r}v_{max,3}k_{m,3,r} \\
+v_{max,1}^2k_{m,1,f}x_0^2k_{m,1,r}^2k_{m,2,f}v_{max,3}k_{m,3,f}k_{m,3,r}+v_{max,1}^2k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}k_{m,3,r} \\
+v_{max,1}^2k_{m,1,f}x_0k_{eq,1}k_{m,1,r}^3k_{m,2,f}k_{m,2,r}v_{max,3}k_{m,3,r}+v_{max,1}^2x_0^2k_{eq,1}k_{m,1,r}^3v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}k_{m,3,r} \\
+v_{max,1}^2x_0^2k_{eq,1}k_{m,1,r}^3k_{m,2,f}k_{m,2,r}v_{max,3}k_{m,3,r}+v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f}k_{m,3,r} \\
+v_{max,1}k_{m,1,f}^2x_0k_{m,1,r}^2k_{m,2,f}k_{m,2,r}v_{max,3}^2k_{m,3,r}+v_{max,1}k_{m,1,f}x_0^2k_{m,1,r}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{m,3,f}k_{m,3,r} \\
+v_{max,1}k_{m,1,f}x_0^2k_{m,1,r}^2k_{m,2,f}k_{m,2,r}v_{max,3}^2k_{m,3,r}.
\end{gathered}
$$

When REDUCE is asked to factorise these expressions as much as possible (`on factor`), the results become somewhat more manageable, with a, b, c and d becoming:

$$
\begin{gathered}
-(2v_{max,2}+v_{max,3})v_{max,1}k_{m,1,f}^2k_{eq,1}k_{m,1,r}k_{m,2,r}v_{max,3}k_{m,3,r} \\
-(v_{max,2}+2v_{max,3})v_{max,1}^2k_{m,1,f}k_{eq,1}^2k_{m,1,r}^2k_{m,2,r}k_{m,3,r}-v_{max,1}^3k_{eq,1}^3k_{m,1,r}^3k_{m,2,r}k_{m,3,r} \\
-k_{m,1,f}^3v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r},
\end{gathered}
$$

$$
-\Bigg(\Bigg(\Bigg(\Big(((2k_{m,2,r}-k_{m,3,f})k_{m,2,f}v_{max,3}+v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}\Big)+(v_{max,2}+v_{max,3})k_{m,1,r}k_{m,2,r}\Bigg)k_{eq,1}
$$

$$-(v_{max,2}+3v_{max,3})x_0k_{m,2,r}\Big)k_{m,1,f}+(v_{max,2}+v_{max,3})x_0k_{eq,1}k_{m,1,r}k_{m,2,r}\Bigg)v_{max,1}^2k_{eq,1}k_{m,1,r}^2k_{m,3,r}$$
$$-\Bigg(\Big(\big((v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})+(3v_{max,2}+v_{max,3})k_{m,1,r}\big)k_{eq,1}-(v_{max,2}+v_{max,3})x_0\Big)k_{m,1,f}$$
$$+(3v_{max,2}+v_{max,3})x_0k_{eq,1}k_{m,1,r}\Bigg)v_{max,1}k_{m,1,f}k_{m,1,r}k_{m,2,r}v_{max,3}k_{m,3,r}$$
$$-\big((k_{m,2,r}-k_{m,3,f})k_{eq,1}k_{m,2,f}-2x_0k_{m,2,r}\big)v_{max,1}^3k_{eq,1}^2k_{m,1,r}^3k_{m,3,r}$$
$$+(v_{max,1}k_{eq,1}k_{m,1,r}k_{m,2,f}-k_{m,1,f}v_{max,2}k_{eq,2}k_{m,2,r})(v_{max,1}k_{eq,1}k_{m,1,r}k_{m,3,f}-k_{m,1,f}v_{max,3}k_{eq,3}k_{m,3,r})\times$$
$$(v_{max,1}k_{eq,1}k_{m,1,r}+k_{m,1,f}v_{max,3})x_3$$
$$-2k_{m,1,f}^3k_{m,1,r}v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r}-2k_{m,1,f}^2x_0k_{m,1,r}v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r},$$

$$\Bigg(\Bigg(\Big(\big((3k_{m,2,r}-2k_{m,3,f})k_{m,2,f}v_{max,3}+v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}\big)+(v_{max,2}+v_{max,3})k_{m,1,r}k_{m,2,r}\Big)x_0k_{eq,1}$$
$$-(v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})k_{eq,1}^2k_{m,1,r}k_{m,2,r}-x_0^2k_{m,2,r}v_{max,3}\Bigg)k_{m,1,f}$$
$$-(v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})x_0k_{eq,1}^2k_{m,1,r}k_{m,2,r}$$
$$+(v_{max,2}+v_{max,3})x_0^2k_{eq,1}k_{m,1,r}k_{m,2,r}\Bigg)v_{max,1}^2k_{m,1,r}^2k_{m,3,r}$$
$$+\Bigg(\Big(\big((v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})+(v_{max,2}+v_{max,3})k_{m,1,r}\big)x_0$$
$$-\big((v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})+k_{m,1,r}v_{max,2}\big)k_{eq,1}k_{m,1,r}\Big)k_{m,1,f}^2$$
$$-\Big(\big((v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})+2k_{m,1,r}v_{max,2}\big)k_{eq,1}-(v_{max,2}+v_{max,3})x_0\Big)k_{m,1,f}x_0k_{m,1,r}$$
$$-x_0^2k_{eq,1}k_{m,1,r}^2v_{max,2}\Bigg)v_{max,1}k_{m,1,r}k_{m,2,r}v_{max,3}k_{m,3,r}$$
$$+\big(2(k_{m,2,r}-k_{m,3,f})k_{eq,1}k_{m,2,f}-x_0k_{m,2,r}\big)v_{max,1}^3x_0k_{eq,1}k_{m,1,r}^3k_{m,3,r}$$
$$-(2v_{max,1}^2x_0k_{eq,1}k_{m,1,r}k_{m,2,f}k_{m,3,f}-v_{max,1}k_{m,1,f}x_0v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}$$
$$-v_{max,1}k_{m,1,f}x_0k_{m,2,f}v_{max,3}k_{eq,3}k_{m,3,r}+v_{max,1}k_{m,1,f}k_{eq,1}k_{m,1,r}v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}$$
$$+v_{max,1}k_{m,1,f}k_{eq,1}k_{m,1,r}k_{m,2,f}v_{max,3}k_{eq,3}k_{m,3,r}+v_{max,1}x_0k_{eq,1}k_{m,1,r}v_{max,2}k_{eq,2}k_{m,2,r}k_{m,3,f}$$
$$+v_{max,1}x_0k_{eq,1}k_{m,1,r}k_{m,2,f}v_{max,3}k_{eq,3}k_{m,3,r}-2k_{m,1,f}^2v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{eq,3}k_{m,3,r}$$
$$-2k_{m,1,f}x_0v_{max,2}k_{eq,2}k_{m,2,r}v_{max,3}k_{eq,3}k_{m,3,r})(v_{max,1}k_{eq,1}k_{m,1,r}+k_{m,1,f}v_{max,3})x_3k_{m,1,r}$$
$$-k_{m,1,f}^3k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r}-2k_{m,1,f}^2x_0k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r}-$$
$$k_{m,1,f}x_0^2k_{m,1,r}^2v_{max,2}k_{m,2,r}v_{max,3}^2k_{m,3,r}$$

and

$$\Big(\big((v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})k_{eq,1}k_{m,1,r}k_{m,2,r}-(k_{m,2,r}-k_{m,3,f})x_0k_{m,2,f}v_{max,3}\big)k_{m,1,f}$$
$$+(v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})x_0k_{eq,1}k_{m,1,r}k_{m,2,r}\Big)v_{max,1}^2x_0k_{m,1,r}^2k_{m,3,r}$$
$$+(v_{max,1}x_0k_{m,2,f}+k_{m,1,f}v_{max,2}k_{eq,2}k_{m,2,r}+x_0v_{max,2}k_{eq,2}k_{m,2,r})$$
$$(v_{max,1}x_0k_{m,3,f}+k_{m,1,f}v_{max,3}k_{eq,3}k_{m,3,r}+x_0v_{max,3}k_{eq,3}k_{m,3,r})(v_{max,1}k_{eq,1}k_{m,1,r}+k_{m,1,f}v_{max,3})x_3k_{m,1,r}^2$$
$$+(k_{m,1,f}+x_0)(v_{max,2}k_{eq,2}k_{m,3,f}+k_{m,2,f}v_{max,3})v_{max,1}k_{m,1,f}x_0k_{m,1,r}^2k_{m,2,r}v_{max,3}k_{m,3,r}$$
$$-(k_{m,2,r}-k_{m,3,f})v_{max,1}^3x_0^2k_{eq,1}k_{m,1,r}^3k_{m,2,f}k_{m,3,r}.$$

Once s_1 is determined, s_2 satisfies a linear equation in s_1, so we have $s_2 = e/f$ where e and f (in that order) are:

$$\begin{aligned}
&s_1^2 k_{m,2,r} k_{m,3,r} (v_{max,1}^2 k_{eq,1}^2 k_{m,1,r}^2 + v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} v_{max,2} \\
&\qquad + v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} v_{max,3} + k_{m,1,f}^2 v_{max,2} v_{max,3}) \\
&+s_1(- x_3 v_{max,1}^2 k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} k_{m,3,f} + x_3 v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} \\
&\quad + x_3 v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r} - x_3 k_{m,1,f}^2 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{eq,3} k_{m,3,r} \\
&\quad - v_{max,1}^2 x_0 k_{eq,1} k_{m,1,r}^2 k_{m,2,r} k_{m,3,r} + v_{max,1}^2 k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} k_{m,2,r} k_{m,3,r} \\
&\quad - v_{max,1}^2 k_{eq,1}^2 k_{m,1,r}^2 k_{m,2,f} k_{m,3,f} k_{m,3,r} - v_{max,1} k_{m,1,f} x_0 k_{m,1,r} k_{m,2,r} v_{max,3} k_{m,3,r} \\
&\quad + v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{m,2,r} k_{m,3,r} + v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} k_{m,3,r} \\
&\quad + v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} k_{m,2,f} k_{m,2,r} v_{max,3} k_{m,3,r} + v_{max,1} x_0 k_{eq,1} k_{m,1,r}^2 v_{max,2} k_{m,2,r} k_{m,3,r} \\
&\quad + k_{m,1,f}^2 k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r} + k_{m,1,f} x_0 k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}) \\
&+k_{m,1,r}(x_3 v_{max,1}^2 x_0 k_{eq,1} k_{m,1,r} k_{m,2,f} k_{m,3,f} - x_3 v_{max,1} k_{m,1,f} x_0 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} \\
&\qquad + x_3 v_{max,1} k_{m,1,f} k_{eq,1} k_{m,1,r} k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r} + x_3 v_{max,1} x_0 k_{eq,1} k_{m,1,r} k_{m,2,f} v_{max,3} k_{eq,3} k_{m,3,r} \\
&\qquad - x_3 k_{m,1,f}^2 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{eq,3} k_{m,3,r} - x_3 k_{m,1,f} x_0 v_{max,2} k_{eq,2} k_{m,2,r} v_{max,3} k_{eq,3} k_{m,3,r} \\
&\qquad - v_{max,1}^2 x_0 k_{eq,1} k_{m,1,r} k_{m,2,f} k_{m,2,r} k_{m,3,r} + v_{max,1}^2 x_0 k_{eq,1} k_{m,1,r} k_{m,2,f} k_{m,3,f} k_{m,3,r} \\
&\qquad - v_{max,1} k_{m,1,f} x_0 v_{max,2} k_{eq,2} k_{m,2,r} k_{m,3,f} k_{m,3,r} - v_{max,1} k_{m,1,f} x_0 k_{m,2,f} k_{m,2,r} v_{max,3} k_{m,3,r})
\end{aligned}$$

and

$$\begin{aligned}
&v_{max,1} k_{m,1,r} k_{m,3,r} (k_{m,1,f} x_0 v_{max,2} k_{eq,2} k_{m,2,r} + k_{m,1,f} x_0 k_{m,2,f} v_{max,3} + k_{m,1,f} k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} \\
&\quad + k_{m,1,f} k_{eq,1} k_{m,1,r} k_{m,2,f} v_{max,3} + x_0 k_{eq,1} k_{m,1,r} v_{max,2} k_{eq,2} k_{m,2,r} + x_0 k_{eq,1} k_{m,1,r} k_{m,2,f} v_{max,3}).
\end{aligned}$$

The results with `on factor` this time are (for $-e$ and f)

$$\begin{aligned}
&\Bigg(\bigg(\Big(\big((v_{max,2} k_{eq,2} k_{m,3,f} + k_{m,2,f} v_{max,3}) + s_1 v_{max,3}\big) x_0 \\
&\quad - \big((v_{max,2} k_{eq,2} k_{m,3,f} + k_{m,2,f} v_{max,3}) + k_{m,1,r} v_{max,2}\big) s_1 k_{eq,1} \\
&\quad - (v_{max,2} + v_{max,3}) s_1^2 k_{eq,1}\Big) k_{m,1,f} - x_0 s_1 k_{eq,1} k_{m,1,r} v_{max,2}\bigg) v_{max,1} k_{m,1,r} k_{m,2,r} k_{m,3,r} \\
&\quad - (v_{max,1} x_0 k_{m,1,r} k_{m,3,f} - v_{max,1} s_1 k_{eq,1} k_{m,1,r} k_{m,3,f} + k_{m,1,f} s_1 v_{max,3} k_{eq,3} k_{m,3,r} \\
&\qquad + k_{m,1,f} k_{m,1,r} v_{max,3} k_{eq,3} k_{m,3,r} + x_0 k_{m,1,r} v_{max,3} k_{eq,3} k_{m,3,r}) \times \\
&\quad (v_{max,1} k_{eq,1} k_{m,1,r} k_{m,2,f} - k_{m,1,f} v_{max,2} k_{eq,2} k_{m,2,r}) x_3 \\
&\quad + (x_0 - s_1 k_{eq,1})(s_1 k_{m,2,r} + k_{m,2,f} k_{m,2,r} - k_{m,2,f} k_{m,3,f}) v_{max,1}^2 k_{eq,1} k_{m,1,r}^2 k_{m,3,r} \\
&\quad - (s_1 + k_{m,1,r}) k_{m,1,f}^2 s_1 v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r} - k_{m,1,f} x_0 s_1 k_{m,1,r} v_{max,2} k_{m,2,r} v_{max,3} k_{m,3,r}
\end{aligned}$$

and

$$\big((x_0 + k_{eq,1} k_{m,1,r}) k_{m,1,f} + x_0 k_{eq,1} k_{m,1,r}\big)(v_{max,2} k_{eq,2} k_{m,2,r} + k_{m,2,f} v_{max,3}) v_{max,1} k_{m,1,r} k_{m,3,r}.$$

The advantages gained by partial factoring are quite evident in this formulation.