BISUBEK User's Manual

Bi-Substrate Enzyme Kinetic Modeling

The simplest SAS program for using BISUBEK is

```
%include 'bisubek.sas';
proc import datafile='example.xls' out=s2 replace;
run;
%bisubek(s2,absorbance_dhf, absorbance_nadph, rate);
```

The first assumption of this program is that you have the macro file BISUBEK.SAS in the current working directory of SAS. If it is located elsewhere, specify the complete path as illustrated here.

```
%include 'c:\macros\bisubek.sas';
```

The %include statement simply makes the macro available to current program.

The second assumption is that the data are available in an Excel spreadsheet, with filename EXAMPLE.XLS in the current directory. Again, if it is located elsewhere, specify the complete path name. The Import procedure reads the data and converts it to a SAS dataset called S2 (out=s2). The replace option allows S2 to be replaced if the program is rerun. The spreadsheet should be structured as follows, with variable names in row 1. The file can contain additional variables variables, but the minimum requirements are concentrations for the two substrates and the reaction rate. If blanks are included in the variable name, then SAS will replace those with the underscore character. In this example ABSORBANCE DHF will be recognized in SAS as ABSORBANCE_DHF.

ABSORBANCE DHF	DHF µM	ABSORBANCE NADPH	NADPH µM	RAW RATE	Rate
0.575	74.2	0.539	86.5	0.047825	45.2
0.575	74.2	0.539	86.5	0.047812	45.2
0.585	75.5	0.539	86.5	0.047465	44.9
0.57	73.5	0.539	86.5	0.044892	42.4
0.209	27.0	0.539	86.5	0.037625	35.6
0.211	27.2	0.539	86.5	0.036153	34.2
0.212	27.4	0.539	86.5	0.035830	33.9
0.111	14.3	0.539	86.5	0.023862	22.5
0.107	13.8	0.539	86.5	0.023623	22.3
0.107	13.8	0.539	86.5	0.023876	22.6
0.074	9.5	0.539	86.5	0.018066	17.1
0.075	9.7	0.539	86.5	0.019401	18.3

If data are not in Excel, it is recommended that it be converted to that form, using the equivalent of "Save As". An alternative is to include the data directly in the SAS program. This would look as follows, where the data name is given by "DATA S2;",

the variable names are listed in the INPUT statement (one variable name per column of data), and the data follow the DATALINES statement (columns separated by at least one space). The BISUBEK macro is then applied to the data, and note the variable names must match those created in the INPUT statement. Details on BISUBEK use are discussed next.

```
data s2;  input  dhf nadph rate;
datalines;
0.575  0.539  45.2
0.575  0.539  45.2
0.585  0.539  44.9
0.57    0.539   42.4
0.209  0.539  35.6
0.211  0.539  34.2
0.212  0.539  33.9
0.111  0.539  22.5
0.107  0.539  22.3
0.107  0.539  22.6
0.074  0.539  17.1
0.075  0.539  18.3
;
%include 'bisubek.sas';
%bisubek(s2,dhf, nadph, rate);
```

The final step is actually running the macro on the data. This is done with a statement of the form

```
%bisubek( datasetname, substrate1, substrate2, reactionrate);
```

where SAS needs to be told what dataset and what variable names to use in the analysis. Variable names must match the spelling used by SAS. You can print the data, using

```
proc print data=s2; run;
```

and the variable names will be printed as column labels. Printing and checking the data for accuracy is recommended as a routine step.

Transforming variables

Occasionally the recorded data needs to be converted to different units, or transformed for other reasons. There are three ways to do this.

1. Make the conversions in Excel or other spreadsheet, creating columns with the desired transformation.
2. Make the conversions in SAS, as illustrated here. Using a so-called data step (data s2; set s2;), each variable is set equal to the desired mathematical function of itself. Always put semicolons at the end of each statement.

```
%include 'bisubek.sas';
proc import datafile='example.xls' out=s2 replace;
run;
data s2; set s2;
 absorbance_dhf = absorbance_dhf*1000/7.75;
 absorbance_nadph = absorbance_nadph*1000/6.23;
 rate = rate*63*15;
run;
%bisubek(s2,absorbance_dhf, absorbance_nadph, rate);
```

3. Mathematical expressions can be used as variable names in running BISUBEK, as shown here.

```
%include 'bisubek.sas';
proc import datafile='example.xls' out=s2 replace;
run;
%bisubek(s2,absorbance_dhf*1000/7.75 , absorbance_nadph*1000/6.23,
rate*63*15);
```

Identifying Groups

BISUBEK is designed for use on data where one substrate is held at various constant levels, while the other substrate is varied. These constant levels form groups that are initially analyzed separately, so groups must be identified. BISUBEK attempts to create these groups automatically. However, if differences between substrate levels are not distinct enough, automatic grouping may be done incorrectly. This will be apparent by the number of regression lines in the initial regression graphs.

There are several options for controlling the grouping. One possibility is to tell BISUBEK how many groups there are, and let it decide which observations belong to each group. Again, this should be checked for accuracy. The SAS statement

```
%bisubek(s2,absorbance_dhf , absorbance_nadph, rate,
            ncluster1=5,ncluster2=5);
```

sets the number of clusters to 5 for each substrate. This would be the case for an experiment in which a 5 by 5 array of substrate levels was used, or 25 observations per replicate.

If this fails, then the user is responsible for identifying groups. Variables must be added to the data, as illustrated in this (partial) spreadsheet.

ABSORBANCE DHF	Group1	ABSORBANCE NADPH	Group2	RAW RATE	Rate
0.575	1	0.539	1	0.047825	45.2
0.575	1	0.539	1	0.047812	45.2
0.585	1	0.539	1	0.047465	44.9
0.57	1	0.539	1	0.044892	42.4
0.209	2	0.539	1	0.037625	35.6
0.211	2	0.539	1	0.036153	34.2
0.212	2	0.539	1	0.035830	33.9
0.111	3	0.539	1	0.023862	22.5
0.107	3	0.539	1	0.023623	22.3
0.107	3	0.539	1	0.023876	22.6
0.074	4	0.539	1	0.018066	17.1
0.075	4	0.539	1	0.019401	18.3

These variables are then given to BISUBEK using the GROUP1= and GROUP2= options, grouping for substrates 1 and 2, respectively.

```
%bisubek(s2,absorbance_dhf , absorbance_nadph, rate,
            group1=group1, group2=group2);
```

Other Options

Two other options are available. By default the SAS/GRAPH product is used to produce high quality graphs. If the user prefers character-based text plots, then the PLOT=GPLOT option should be specifed, changing GPLOT to anything else, such as PLOT=NO, or PLOT=OFF, or PLOT=TEXT.

By default BISUBEK does a weighted analysis, incorporating the variance of the initial regression into the analysis of the secondary plots. An unweighted analysis can be requested by changing the option WEIGHT=YES to some other value, like WEIGHT=NO.

Use of both options is illustrated by this BISUBEK analysis.

```
%bisubek(s2,absorbance_dhf , absorbance_nadph, rate,
             plot=no, weight=no );
```

Availability

This manual, the example data spreadsheet, and the BISUBEK program are freely available on the Internet at
http://www.agriculture.utk.edu/ansci/faculty/saxton/software.htm

For suggestions or problems, contact
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Reference

Smiley, RD, Hicks, SN, Stinnett, LG, Howell, EE and Saxton, AM. 2002. Bi-substrate kinetics using SAS computer software. Analytical Biochemistry 301(1): 153-156.