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Batch Fermentation of Bioethanol from the Residues of Elaeis Guineensis: Optimisation using Response Surface Methodology

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In oil palm industry, large quantity of oil palm trunk (OPT) and palm oil mill effluent (POME) are generated. These residues are not fully utilised. They are served as wastes, which lead to serious environmental pollution. Realising that OPT sap contains high glucose concentration while POME contains essential nutrients required by microorganisms for growth, in this study, bioethanol was produced from OPT sap and POME by Saccharomyces cerevisiae in shake flasks culture. OPT sap was used as carbon source, and POME was utilised as nutrient supplier for the fermentation process. To obtain the optimum ratio of OPT sap to POME, inoculum size, initial pH, and incubation time for maximum bioethanol yield, response surface methodology (RSM) via face centred central composite design (FCCCD) was employed. The experimental data for glucose consumption, bioethanol yield, and biomass growth were fitted to polynomial equations and analysed. The quadratic models were employed to fit the data. The optimum bioethanol fermentation conditions were as follows: OPT sap to POME ratio of 63 : 37, inoculum size of 4.3 vol%, initial pH of 8.0, and incubation time of 118 h. Under these conditions, the expected bioethanol yield is 0.453 g/g. To evaluate the accuracy and validate the results, five additional runs were carried out at the optimum point. It was found that the range of bioethanol yield within 95 % confidence level was 0.448 - 0.457. It can be concluded that there is no difference between the experimental values and the predicted data within 95 % confidence level since the predicted data were within the range.

1. Introduction

In recent years, biofuels including bioethanol have gained increasing interest. Bioethanol is produced through fermentation process of simple sugar by microorganisms (usually yeast or bacteria) (Oyeleke et al., 2012). Oil palm (Elaeis guineensis) is planted in 42 countries worldwide with total planted areas of 1.09 x 10^5 km^2 (Khalil et al., 2010). Oil palms are replanted every 25 to 30 y. The annual availability of oil palm trunk (OPT) is expected to be around 13.6 million logs per 100,000 hectares replanted (Azhar et al. 2011). Most of OPT were left abandoned or used as the mulch (Ahmad et al., 2007). During the process of palm oil production, palm oil mill effluent (POME) is generated in the mill at the final stage. 67 wt% of POME is produced for every fresh fruit bunch (FFB) processed (Sulaiman et al., 2011). POME has very high BOD and COD and needs to be treated before being released into the water streams. These situations may lead to serious environmental problem. It has been reported that OPT sap contains relatively high glucose content, while POME contains almost all essential nutrients required by microorganisms to grow (Samsudin and Mat Don, 2015). In this study, bioethanol was produced by Saccharomyces cerevisiae from OPT sap with nutrient supplemented by POME. To obtain the optimum process condition for bioethanol production, response surface methodology (RSM) was performed. RSM is an established technique to measure the association between the input parameters and the response (Delavari and Amin, 2014). The purpose of the present study was to optimise the process for bioethanol production from oil palm residues by S. cerevisiae using RSM, by employing face

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centred central composite design (FCCCD) (4 factors and 3 levels) to study the effect of OPT to POME ratio, inoculum size, initial pH, and incubation time on the bioethanol yield.

2. Materials and method

2.1 Preparation of OPT sap, POME and inoculum

OPTs were obtained from Felda Trolak Selatan Development Land, Sungkai, Perak, Malaysia. The trunks were chipped by a wood chipper and squeezed by a hydraulic squeezer at the Forest Research Institute of Malaysia (FRIM), Kepong, Selangor. OPT sap obtained was then sieved to remove the fibres. POME was used as nutrient supplements for the growth of yeast. It was collected from Serting Hilir Palm Oil Mill, Felda Palm Industries Sdn. Bhd, Negeri Sembilan directly after being discharged. Due to the fast degradation, OPT sap and POME were stored in a freezer at −20 °C, and defrosted to room temperature when needed. The yeast (S. cerevisiae Type II) used was purchased from Sigma–Aldrich. Inoculum was prepared by mixing 10 g of yeast with 100 mL of sterile deionised water. The mixture was then vortexed for 2 min so that the mixture was well mixed, and homogeneously distributed. The cell counting was carried out with a haemocytometer under microscope. Cell concentration is then adjusted to be around 1 × 109 cell number/mL.

2.2 Analytical method and statistical analysis

The glucose concentration was determined using DNS colorimetric method (Das et al., 2013), with slight modification. 3 mL DNS reagent solution (dinitrosalicylic acid: 10 g, phenol: 2 g, sodium sulfite: 0.5 g, sodium hydroxide: 10 g, deionised water: 1 L was added to 3 mL sample in a capped test tube. The samples were then heated at 90 °C for 15 min to form red-brown colour. 1 mL 40 % w/v potassium sodium tartarate solution (Rochelle salt) was then added to the sample to stabilise the colour. After cooling to room temperature in a cold water bath, the absorbance with spectrophotometer at wavelength 575 nm was recorded and compared to the standard calibration curve. The standard calibration curve was obtained using glucose anhydrous. Bioethanol was measured using a gas chromatography as described by Negro et al. (2014), equipped with flame ionisation detector, and a column of Carbowax 20M (Supelco, USA) at 85 °C. Injector and detector temperature was maintained at 150 °C. Ethanol standard was used for quantification of bioethanol in the sample. Sodium hydrochloride, sulphuric acid, acetonitrile, phenol, sodium sulfite, potassium sodium tartarate, glucose, ethanol, and sodium hydroxide were purchased from Merck. Dinitrosalicylic acid was purchased from Sigma-Aldrich. All the chemicals were of analytical grade. All samples were run in triplicate. The bioethanol yield was calculated and expressed as fraction by Eq(1):

$$\text{Bioethanol yield, } Y_{P/S}\ (\text{g/g}) = \frac{\text{Final concentration of bioethanol (g/L)}}{\text{Initial concentration of sugar (g/L)}} \quad (1)$$

2.3 Design of experiment

In this study, bioethanol was produced by S. cerevisiae using OPT sap and POME as substrate. Fermentations were run in 250 mL cotton plugged shake flasks. 100 mL of substrate was prepared at different variables. Response surface methodology (RSM) was used to model the effect of independent variables (A: OPT sap to POME ratio, B: inoculum size, C: pH, and D: incubation time) on the bioethanol yield (%). A face centred central composite design (FCCCD) at three levels was employed for designing the experimental data. A commercial statistical package, Design-Expert version 6.0.6 (Stat-Ease Inc., 2003) was used to apply the RSM to the experimental data. Our previous study (Samsudin and Don, 2014) was done as the preliminary work using one-factor-at-a-time (OFAT) method to choose the levels of the four independent variables. The levels of each independent variable are shown in Table 1.

Table 1: List of range and levels of the experimental independent variables

Factor	Coding	Unit	Level		
			-1	0	1
OPT sap : POME ratio	A	vol% OPT sap	0.4	0.6	0.8
Inoculum size	B	vol%	3.0	4.0	5.0
Initial pH	C		7.0	8.0	9.0
Incubation time	D	h	72	96	120

3. Result and Discussion

3.1 Development of regression model equation and statistical analysis

The complete design matrix, consisting 30 experimental runs is shown in Table 2 together with the variations of the four factors. The corresponding bioethanol yield was also reported in Table 2. The experimental runs were divided into three blocks. In each block, two centre points were replicated (Standard order no.: 9, 10, 19, 20, 29, and 30) to evaluate the experimental error and the reproducibility of the data. From the table, the bioethanol yields were in the range of 0.095 to 0.440. All the responses are reported as their mean from three replicated run.

Table 2: Experimental design matrix

Standard order	Block	Factors				Response
		A	B	C	D	Y: bioethanol yield (% g/g)
1	1	0.4	3	7	120	0.150
2	1	0.8	3	7	72	0.106
3	1	0.4	5	7	72	0.271
4	1	0.8	5	7	120	0.385
5	1	0.4	3	9	72	0.135
6	1	0.8	3	9	120	0.190
7	1	0.4	5	9	120	0.360
8	1	0.8	5	9	72	0.284
9	1	0.6	4	8	96	0.438
10	1	0.6	4	8	96	0.438
11	2	0.4	3	7	72	0.095
12	2	0.8	3	7	120	0.180
13	2	0.4	5	7	120	0.343
14	2	0.8	5	7	72	0.265
15	2	0.4	3	9	120	0.167
16	2	0.8	3	9	72	0.150
17	2	0.4	5	9	72	0.280
18	2	0.8	5	9	120	0.358
19	2	0.6	4	8	96	0.439
20	2	0.6	4	8	96	0.430
21	3	0.4	4	8	96	0.372
22	3	0.8	4	8	96	0.400
23	3	0.6	3	8	96	0.196
24	3	0.6	5	8	96	0.370
25	3	0.6	4	7	96	0.414
26	3	0.6	4	9	96	0.416
27	3	0.6	4	8	72	0.360
28	3	0.6	4	8	120	0.440
29	3	0.6	4	8	96	0.428
30	3	0.6	4	8	96	0.428

The experimental data was fitted to four types of model, namely linear, two-factor interaction (2FI), quadratic, and cubic polynomials. The summary of the probability values of sequential model sum of squares and lack-of-fit test relative to the pure error, and the model summary statistics for every type of model are shown in Table 3. The probability value ("Prob > F") of smaller than 0.0001 indicates the statistical significance (Feng et al., 2013). The best model is the highest order polynomial model where the sequential model sum of squares is significant. Based on the Table 3, the quadratic model is the best model to represent the relationship between the factors and the response where the P value was lower than 0.0001. Another criterion that also needs to be considered is the lack-of-fit test. The adequacy of the model is rejected if the model has significant lack-of-fit. Based on Table 3, the highest order polynomial model with insignificant lack-of-fit is cubic model. The cubic model could not be used for further modelling of the experimental data because the model was found to be aliased. Aliased model occurs due to lack of experimental run to independently estimate all the terms for that model (Tarangini et al., 2009). The second highest order polynomial model with insignificant lack-of-fit must be selected which was the quadratic model. A good model should have a low standard deviation (SD), high coefficient of determination R-squared (R^2) (raw, adjusted, and predicted) and low

PRESS (predicted residual sum of squares) (Feng et al., 2013). Based on Table 3, quadratic model is the best model to describe the relationship of the factors to the response since it has the lowest SD, highest R^2 (raw, adjusted, and predicted), and lowest PRESS.
It can be concluded that quadratic model is the best polynomial model to describe the relationship between the independent variables and the response. The model was analysed via analysis of variances (ANOVA) and presented in Table 4. The model terms were selected based on the P value with 95 % confidence level. Model terms with *P* value of more than 0.05 were considered insignificant.

Table 3: Summary of probability values and model summary statistics

Source	Prob > F		SD	R^2	Adjusted-R^2	Predicted-R^2	PRESS
	a	b					
Linear	0.0029	< 0.0001	0.0842	0.4902	0.4016	0.2707	0.23
2FI	0.9993	< 0.0001	0.0971	0.4994	0.2050	-0.4455	0.46
Quadratic (Suggested)	< 0.0001	0.1143	0.0072	0.9979	0.9956	0.9824	0.01
Cubic (Aliased)	0.0723	0.2929	0.0043	0.9997	0.9985	0.9327	0.02

a = Sequential model sum of squares; b = lack-of-fit test relative to the pure error

Table 4: Analysis of variances (ANOVA) for the quadratic model

Model Terms	Sum of Square	DF	Mean Square	F value	Prob > F
A	0.0012	1	0.0012	22.43	0.0004
B	0.1329	1	0.1329	2,552.32	< 0.0001
C	0.0010	1	0.0010	18.45	0.0009
D	0.0218	1	0.0218	419.03	< 0.0001
A^2	0.0033	1	0.0033	62.98	< 0.0001
B^2	0.0491	1	0.0491	943.13	< 0.0001
C^2	0.0001	1	0.0001	2.66	0.1266
D^2	0.0012	1	0.0012	23.79	0.0003
AB	0.0001	1	0.0001	2.01	0.1800
AC	0.0001	1	0.0001	1.71	0.2142
AD	0.0003	1	0.0003	5.93	0.0300
BC	0.0005	1	0.0005	10.40	0.0066
BD	0.0013	1	0.0013	25.48	0.0002
CD	0.0006	1	0.0006	10.86	0.0058

Based on Table 4, A, B, C, D, A^2, B^2, D^2, AD, BC, BD, and CD are significant model terms. The quadratic regression model bioethanol yield of bioethanol fermentation from palm oil residues by S. cerevisiae obtained from FCCCD design in terms of actual factors are presented in Eq(2).

$$\text{Bioethanol yield, (g/g)} = -3.774 + 1.176\,(\text{OPT sap : POME ratio}) + 1.216\,(\text{inoculum size}) + 0.180\,(\text{pH}) + 0.210\,(\text{incubation time}) - 0.899\,(\text{OPT sap : POME ratio})^2 - 0.139\,(\text{inoculum size})^2 - 0.022\,(\text{incubation time})^2 + 0.022\,(\text{OPT sap : POME ratio})(\text{incubation time}) - 0.006\,(\text{inoculum size})(\text{pH}) + 0.009\,(\text{inoculum size})(\text{incubation time}) - 0.005\,(\text{pH})(\text{incubation time}) \quad (2)$$

3.2 Optimum conditions and effect of process variables

In order to get the optimum process condition, numerical optimisation was applied. It was found that the highest possible bioethanol yield could be obtained when the process conditions are at OPT sap to POME ratio of 63 : 37, inoculum size of 4.3 vol%, initial pH of 8.0, and at 118 h of incubation time. By performing the fermentation at the suggested condition, 0.453 g/g bioethanol yield could be expected. From the ANOVA test (Table 4), only four interaction terms between the factors were significant on influencing the bioethanol yield during the fermentation. They were coded as AD, BC, BD, and CD, which represented the interaction between the OPT sap to POME ratio and incubation time, the inoculum size and initial pH, the inoculum size and incubation time, and the initial pH and incubation time. The visualisation of the predicted models (Eq(2)) on the bioethanol yield for each significant interaction terms is shown in Figure 1 in the form of contour plots where the line of constant response are drawn on the plane of independent variables. The contour plots of interaction between OPT sap to POME ratio and incubation time (Figure 1 (a)), inoculum size and incubation time (Figure 1 (b)), and initial pH and incubation time (Figure 1 (c)) on the bioethanol yield are part of parabolic cylinder.

These show that all those interaction showed a maximum ridge in the experimental domain (Amari et al., 2008). The contour plot of interaction between inoculum size and initial pH on bioethanol yield (Figure 1 (d)) displayed a part of ellipses pattern. An elliptical contour plot means that the centre of the plot showed a maximum response as described by Bas and Boyacı (2007). It can be seen that higher bioethanol was achieved as the OPT sap to POME ratio, inoculum size, and initial medium pH approach at around 63 %, 4.3 %, and 8.0 and it declined beyond these values.

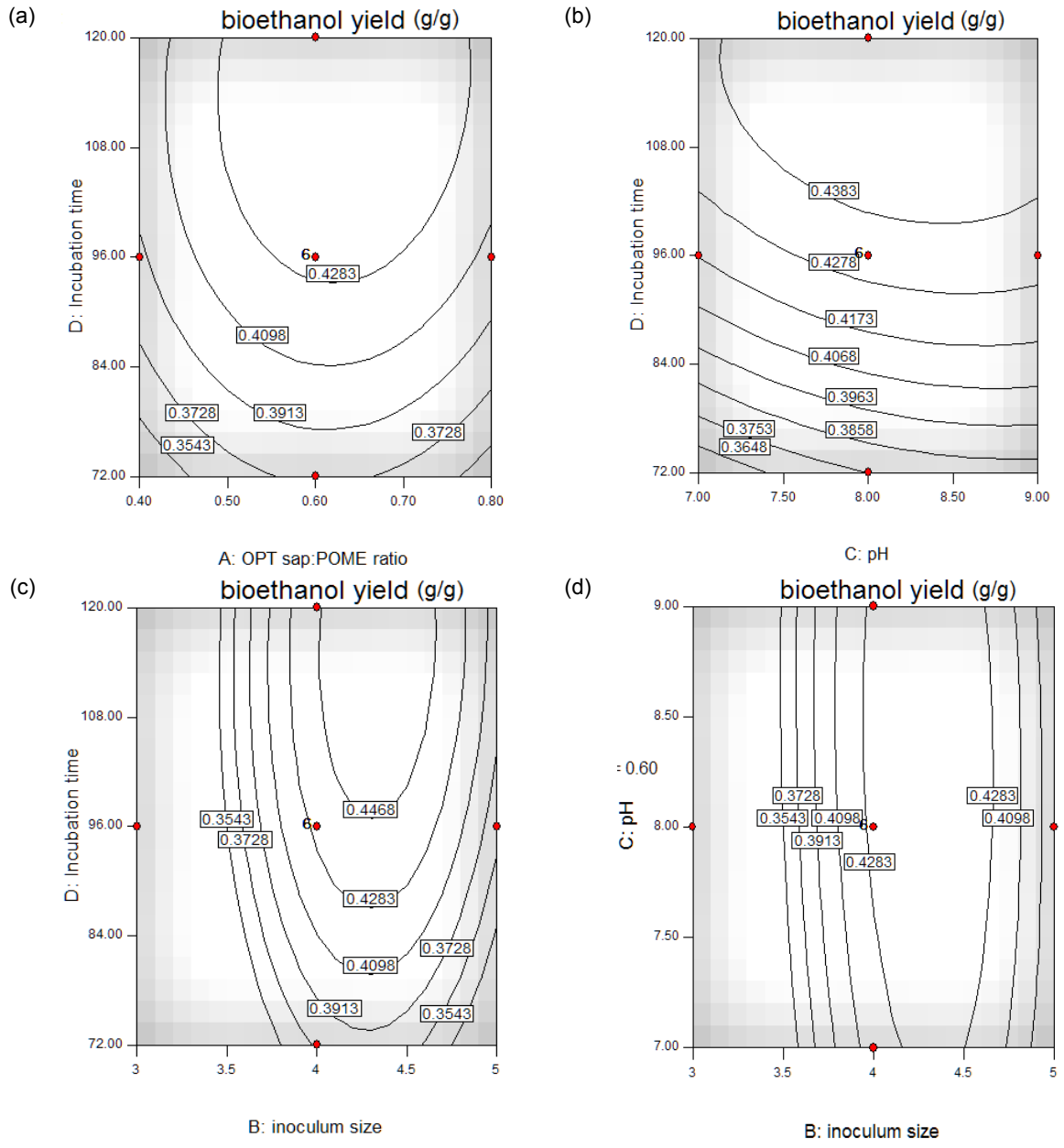

Figure 1: Contour plot of bioethanol yield as a function of interaction between variables (OPT sap to POME ratio, incubation time, initial pH, and inoculum size)

3.3 Validation of the model

The result suggested that fermentation at the optimum condition would produce bioethanol yield at 0.453 g/g. In order to evaluate the accuracy and validate the suggested optimum point, five additional experimental runs were carried out at that point. In order to compare the experimental and predicted data, the ranges of 95 % confidence level were also determined. All the statistical data were calculated using IBM SPSS Statistics Version 21 software (IBM, 2012). It was found that the range of bioethanol yield within 95 % confidence level

was 0.448 – 0.457 g/g. Since the predicted data were within the tested ranges, it can be concluded that there is no difference between the experimental values and the predicted data within 95 % confidence level and that the optimum condition as suggested by RSM is valid (Amari et al., 2008). As comparison, from our previous study (Samsudin and Don 2014), after optimisation using OFAT method, on average, 0.428 g/g bioethanol yield was achieved, while after this RSM optimization, on average, bioethanol yield was around 0.453 g/g, an improvement of 5.77 %.

4. Conclusion

The present study revealed that the bioethanol fermentation condition significantly influenced the bioethanol yield, particularly the interaction effect of OPT sap to POME ratio and incubation time, inoculum size and initial pH, inoculum size and incubation time, and initial pH and incubation time. The present work revealed that RSM can be utilised to optimise the process condition of batch bioethanol fermentation in shake flasks by S. cerevisiae from oil palm residues particularly oil palm trunk (OPT) sap and palm oil mill effluent (POME). It was found that the highest possible bioethanol yield could be obtained when the process conditions are at OPT sap to POME ratio of 63 : 37, inoculum size of 4.3 vol%, initial pH of 8.0, and at 118 h of incubation time. At this condition, 0.453 g/g bioethanol yield could be expected.

References

Ahmad A.L., Loh M.M., Aziz J.A., 2007, Preparation and characterization of activated carbon from oil palm wood and its evaluation on methylene blue adsorption, Dyes and Pigments 75 (2), 263-272.

Amari A., Gannouni A., Chlendi M., Bellagi A., 2008, Optimization by response surface methodology (RSM) for toluene adsorption onto prepared acid activated clay, The Canadian journal of chemical engineering 86 (6), 1093-1102.

Azhar B., Lindenmayer D.B., Wood J., Fischer J., Manning A., McElhinny C., Zakaria M., 2011, The conservation value of oil palm plantation estates, smallholdings and logged peat swamp forest for birds, Forest Ecology and Management 262 (12), 2306-2315.

Bas D., Boyaci I.H., 2007, Modeling and optimization I: Usability of response surface methodology, Journal of Food Engineering 78, 836-845.

Das B.K., Gaur S.S., Bhattacharya J., 2013, Sweetmeat waste fractions as suitable organic carbon source for biological sulfate reduction, International Biodeterioration & Biodegradation 82, 215-223.

Delavari S., Amin N.A.S., 2014, Response Surface Methodology Analysis of CO2 Photocatalytic Conversion in Presence of CH4 over Nitrogen-Doped Titania Nanotube Arrays, Chemical Engineering Transaction 39, 235-240.

Feng Y.,Cai Z., Li H., Du Z., Liu X., 2013, Response surface optimization of fluidized roasting reduction of low-grade pyrolusite coupling with pretreatment of stone coal, Journal of Mining and Metallurgy B: Metallurgy 49 (1), 33-41.

Khalil H.P.S.A., Nurul Fazita M.R., Bhat A.H., Jawaid M., Nik Fuad N.A., 2010, Development and material properties of new hybrid plywood from oil palm biomass, Materials & Design 31 (1), 417-424.

IBM Corporation, 2012, IBM SPSS Statistics Version 21 software, New York, United States.

Negro M.J., Alvarez C., Ballesteros I., Romero I., Ballesteros M., Castro E., Manzanares P., Moya M., Oliva J.M., 2014, Ethanol production from glucose and xylose obtained from steam exploded water-extracted olive tree pruning using phosphoric acid as catalyst, Bioresource Technology 153 (0), 101-07.

Oyeleke S.B., Dauda B.E.N., Oyewole O.A., Okoliegbe I.N., Ojebode T., 2012, Production of bioethanol from cassava and sweet potato peels, Advances in Environmental Biology, 241-246.

Samsudin M.D.M., Mat Don M., 2014, Batch fermentation of bioethanol from the residues of Elaeis guineensis: optimization and kinetics evaluation, Iranica Journal of Energy and Environment 5 (1), 1-7.

Samsudin M.D.M., Mat Don M., 2015, Assessment of bioethanol yield by S. cerevisiae grown on oil palm residues: Monte Carlo simulation and sensitivity analysis, Bioresource Technology 175, 417-423.

Stat-Ease Inc., 2003, Design-Expert version 6.0.6, Minnesota, United States.

Sulaiman F., Abdullah N., Gerhauser H., Shariff A., 2011, An outlook of Malaysian energy, oil palm industry and its utilization of wastes as useful resources, Biomass and bioenergy 35 (9), 3775-3786.

Tarangini K., Kumar A., Satpathy G.R., Sangal V.K., 2009, Statistical optimization of process parameters for Cr (VI) biosorption onto mixed cultures of Pseudomonas aeruginosa and Bacillus subtilis, CLEAN – Soil, Air, Water 37, 319-327.