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()JU":~JII'X"::l.Pt>·Il:~Il: IllI 0
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l\1ethane Production from Formate by Syntrophic Association of MethanobacteriLl/n bryantii and Desulfovibrio vLllgaris JJ
J E A N · P I ~ R R E GUYOTt· AND ALAIN BRAUMAN

Lah"raroir/! de Microbiologie and insrirllf Français de Ru'herche Scienrifique pour le Df!velnppemenr en Coopirarion.
U n i ~ ' u s i r i de Provence. IJJJI Marseille Ced/!.'C. Frunce

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Coculture of a suJrate·reducing bacterium, when grown in the absence of added sulfate. wlth
JI erhunobaclerium br)'anrii, which uses only Hl an'd CO, for methanogenesis. de~r:1ded formate to CIL. A pure
culture of Desu/fo\'ibrio vu/garis JJ was able to produce small amounts of Hl' Such a syntrophic relalionship
might provide an addilionaJ way to avoid rormatc: accumulation in anaerobic en\'ironments.

Il was previoL!sly shown that fonnate can inhibit the
acetoclastic reaction in the presence of Merhanosarcina burkaj '227 or lvJerhanosarcina barkeri subsp. rhermophi/a ' ()1. ln the same pape:r. a hypothesis was postulated thaf formale could be lr.olnsforme:d to CfL in sulfate.depleted environmems through interspecies hydrogen transfer. Sulfate·reducing bacteria (SRB) cannot use: formate as an energy source without electron Ilcceptors. as for ethano) or laClal'. The use of ethan\.ll or lactale bv SRB in sulfate· de:;>kled environmenls is possible only th~ou~h an inlerspe· CIe:5 hyàrogen Iransc'er 10 prol1uce methane: 13. lOI. This paper repons thc: proJuclion oC methane: in the: absence of adued 'iulfale by coupling an SRB with Il hydrogenophilic baclc:rium unable to use: formale.

Techniques describe:ù by Hung:lle 'c7) and B:lIch et al. Il.
~ l were: used throughOUI this study. De.w{flll·ihrio YU/Karis
JJ. Isolal,d from e's-tUarine sediments. was a girl' of W. J. Jone:.;. L' ni versny of Ge:\.lrgia.."'1 f!rJllInubtlcrl:riwn bryunrii DSM Sb) was purchased l'rom the DSM Collection. Goningc:n. Federal Republic of Germany.
D, l'lIlguris JJ was cultivateù nt 37·C in n previously
describeJ medium /ln. except that formate was used as sUbStrale l:!0 m:-'O with 5 mM sulfate. D. "Il/garis was im\culaled at the end \.lI' the: e.~pone:ntial phase to obtain the h.>weSI residual conct:ntration of sulfnte in the inoculum. .\1t!lhnflllb{/crniil11l brYWllii DSM 863 was cultivaled at 37·C in medium 1 of Balch e:t al. Cl). in the presence of H~.CO~
(80:20l. .
Ali e.~periments we:re canied out in lriplicate in 6Q·ml sc:rum boules. each containing 20 ml of the: B:ilch et al. Il) mc:uium 1 prepareJ wilhout sulfate:. Il W:IS che:cked that
. \ll'rlwl/ohucieri/lm bryal/lii wa's unable: to p,r'yuuce me\hane
from l'ormaIe in,pure culture unde:r the: expl:rime:OIal condi.
li\.lns usc:J. Formate: was determint:d,;:olorime:trically" 1I.'i
Jescribc:d by Lang and Lang (9). Liquiù sample:s 10.5 mil for
'formate 牥㩭潶攺搀慳攺灴楣慬汹✮㩩摤楦楥搀睩瑨

䤭汩 ㄵ 〢 慮䨀䤺敮瑲楦畧攺搀慴氺℮住伀 ≦潲 浩渀

睡 㨱 灲 敶 楯 畳 汹摃❉捲楢攺搀ㄶ 䡹杣㩮 屖慳 尬尬楬栀 䝩牤敬捨牯洺ㅴ潧爺ㅰ栀攺煵楰灥㩤

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湩 瘮 㩮 楊 慬 椀 䵥 䥬氻䥉∺䩉㩬灡⸀伮㩰慲瑡浥㩮瑵 ILlap;llapa. lN3.w l'tIc li 0
Il:ù OF.

with a thermal conductivity detector. using a 1.8Q..m
Carbosphere /60/80 me:sh: Interchim. Francel column oper·
ated at 85·C. Gas sampling was done with a gastight pressure lock svringe,
Af t ~r 90 1'1 of incubation. D.,·u/garis. when inoculaled in:l medium without sulfale but in the presence,of formate. produced 3.;....mol of H! per ml of liquid phase without any deu:cuble growth. This value was very low compared with the theoretical amounl,)i hvdroRen (F?5 mmoll which could be evolved from the adde'd fo~ate. The coculture of D. "U/IlIITi,{ and ''''I~rllanohucl~rium b"'anrii produced methan~ from formate (Fig. 11: in pan 1 of Fig. 1. 17.5 mmol of formate was convene:d tO ~.S....mol of CH~ per ml of liquid ph:l,se al'ler 40 h of incubation. The yield. compared with the expected methane production 14HCO~- - lCH~). was 579'. The hvdrollen needed to"prodUce this amount or meth:ln~

was Hi I-lm~lImJ of liqulU ph:l..~e. calculated from the equation 4H! - HCO) -.;- H· - CH~ + 3H~O (BI. This W:lS muc.h higher than the amounl of hydrogen produced by D. ",,/guru alone. After 80 h of im:ubation. 17.5 mmol of formate was

added lFig. 1. pan Il). and ~O h later ail fonnate had been

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FIG. 1. Me:lhano: proJuction from formale: in Ihe ab~ence "r :llll.leJ sulfale by Ihe c,)cuhure of D. "ù/I{uris JJ anJ.\1c'rllllnv• but'/&'rilllll b~·I/llIii. III CH. proJuclion from 17.5 mmol of f.,r~ale aJJeJ al Ihe: t>':lIinning.,.th.: e:l.pe:riment: III. CH. pruJuclion Irllm 17.' mmol of formaI': aJJ.:J hll h l:lIo:r. S) mbùl~: •. CH.: j.. form.alc.

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SOTES

T, \ BLE l. Dilfere:!! rC;''::h'n~ t>y \\'hieh f'lrmalc:.:an bc Ir:..n,r,':meJ in.,n;IC:rl,~ic c:n\'inlnOlcnl~

ulilized. produeing 3.5 I-Lmol of methane per ml of Ii'luid
p h a ~ ~ . dcmonstrating that formate can be /Jsed to produce
melh:Jne through interspecies hydrogen transfer. The level or CH~ produced. comp:..red with the expected value. was ï SC:C. The fact lhat this value is higher than the former perCe!llage of 57% might be due to residu:u sulfate. which mig.hl divcn a small pan of formate toward sulfate reduction al lhe beginning of the ex periment.
The results indicale that formate could be convened ta melhane. by intcrspecies hydrogen lransfer berween SRB and hvdrogenoohilic 'meth:mol!ens. as fol1ows: (iJ half reac·
lion complëted by SRB. 4HeO:- + 4H~O - 4HeO) - 4H~
UC'" = +5.2 kJ) 113): Iii) half reaclion completed by M"llwnohacterillm bryanrii. 4H! + HeQ)- + Hf - eH. + 3H,O (tl,C'" = -135.6 kJ): and Cm) sum of these reactions.
4HCO:-.;. H20;- H- - eH.;- 3Heo)- ('~G''' = -130.4
kJl. 5ince formate. Iike hydrogen. can be produced by vurious mieroorganism~in anaerobic en\·Îronments. then besidc:s the strategies already known (Table 1). a new one to prevenl formate buildup would be available. In sulfate-depieted environm'ents. conversion of formate might be aehic:ved through interspecies hydrogen transfer between SRB and methanogens-...as for lactate or ethanol (3. 10). ln such.:n\'iro'nments. methanogens using formate and SRB coupied 10 methanogens using H: could aet as an efficient butiering system to pre\'ent formate accumulation. since formate cun inhibit some aeeticlastic melhanogens (5,. From :his ~oint of view. the e:({reme spedalization 'of the .TIH.:rortorii in an anaerobic digestor is remarkabl~: the hydro-een· and formute-using methanogens which could be coupled lO SRB from one part and the acetielastic methanogens una!:'k [0 use formate from another part. Sueh a spc:daliza-lÎ\"'n is of great imerest. sinee acetate is the major methane pn:;;ursor in such cnvironments 1121.
Funhermore. the results described above cauld suppan a

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hvllrucc:n-cvdinc mc:~h;,"ism for f~lrm.lle: mC:la!-ll!i,m ~y SRB. ';S prOPl)~~U ~y Ollom:Jnu Pe:..:k Ill/. Sinee: h~ Jw~c:n from formale can he: c\'lllvc:lI by D. "lIit:lIriJ for use thwugh interspecies hyur~lgen lransfcr 10 pn'uuce mc:lh:me:. lInc might think. that in the presence of sulf;lle. hydruge:n toO could be evol\'~d by D. "lIigClris for sulfate reduelion und
lhen. accordinc to this mechanism. rnuuch enerR\' WllUld he available throu-gh a proton gradient for Ih~ 'iYnlh~~is ~If.~TP.

Jlt'IIIu/luhU('ltrium 13
furmidcum

SRB 13

:~(""I"",'cl('ri/lm ...,Iodii

. lHC(): - - 50.;' - H-
- .lHCO,- - HS'
HCO;- + H:O - H;
- HCO,_
. lHCO:-.,.·H -- CH,.CO:-
- :HCO)·
. lHCO,- + H.O + Ht
- C'H.... J'HCOl -